INTEGRITY OF SPINAL AUTONOMIC PATHWAYS

IN SUB-ACUTE AND CHRONIC SPINAL CORD INJURIES

by

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Abstract

The outcome of spinal cord injury (SCI) is still commonly described in terms of motor and sensory function, despite increasing awareness that there is also damage to the autonomic nervous system (ANS). The purpose of this thesis was to examine the integrity of spinal autonomic pathways among individuals with sub-acute and chronic SCI. The selection of appropriate tests to assess autonomic function is challenging since the ANS is complex. Examining reliability and comparability among measures provides a comprehensive understanding of the validity of specific tools. This doctoral thesis is comprised of three separate investigations that focus on determining the integrity of spinal autonomic pathways following SCI. In the first study (Chapter 2), the focus was on the reliability of heart rate variability (HRV), sympathetic skin responses (SSRs) and an orthostatic challenge (sit-up test). Measures of HRV, SSRs and blood pressure changes during the sit-up test were found to be reliable. Additionally, the sit-up test was compared with the gold standard orthostatic challenge (tilt-table test), which revealed that the sit-up test provokes an orthostatic response comparable to the tilt-table test. In the last two studies (Chapters 3 and 4) HRV and changes in blood pressure (BP) during an orthostatic challenge, SSRs and the Valsalva manoeuvre (VM) were used to examine spinal autonomic integrity. The novel focus on integrity of spinal autonomic pathways revealed that it is affected by lesion level, neurologic severity of injury, and time post-injury. As expected based on extensive existing research on cardiovascular autonomic function following SCI, higher lesion levels produced greater cardiovascular impairments. That is, there is greater compromise to spinal autonomic integrity in high-level compared to low-level SCI. However, the association between neurologic and autonomic "completeness" of injury is unclear. Our findings suggest that time post-injury may affect the latter. During the sub-acute stage, autonomic tests revealed cardiovascular changes in patients in a one-month follow-up after admission to a rehabilitation hospital. The exact time course of alterations to integrity is unknown. Not acknowledging change to spinal autonomic integrity is inherently problematic since it is unclear what neurologic severity of injury infers about autonomic dysfunction.

Preface

The project presented in Chapter 2 received ethical approval from the UBC Clinical Research Ethics Board (Certificates # H10-0014 and # H07-00102). I identified the research question, designed the study, and analyzed the data. Data collection was shared between Andrei Krassioukov and myself.

The project presented in Chapter 3 received ethical approval from the UBC Clinical Research Ethics Board (Certificate # H10-0014). I identified the research question, designed the study, and analyzed the data. Data collection was shared between Andrei Krassioukov and myself.

The project presented in Chapter 4 received ethical approval from the UBC Clinical Research Ethics Board (Certificate # H04-70374). I, along with Andrei Krassioukov, and members of the Autonomic Research Laboratory identified the research question, designed the study, and analyzed the data. Data collection was shared between Andrei Krassioukov, Patricia Mills, Christopher West, Amira Tawashy, Dmitri Krassioukov-Enns, David Mikhail, and myself.

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List of Abbreviations

AB	Able-bodied individuals
AD	Autonomic dysreflexia
AIS	American Spinal Injury Association Impairment Scale
ANOVA	Analysis of variance
ANS	Autonomic nervous system
BP	Blood pressure
BRSa	Adrenergic baroreflex sensitivity
CI	Confidence interval
cm	Centimetre(s)
DBP	Diastolic blood pressure
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
Hz	Hertz
ICC	Intraclass correlation coefficient
IPC	International Paralympic Committee
ISNCSCI	International Standards for the Neurological Classification of Spinal Cord Injury
ISAFSCI	International Standards on the documentation of remaining Autonomic Function after Spinal Cord Injury
kg	kilogram(s)
LF	Low frequency
MAP	Mean arterial blood pressure
mmHg	Millimetres of mercury
ms^2	Milliseconds squared
nu	Normalized units
OH	Orthostatic hypotension
RRI	RR interval
s	Second (s)
SBP	Systolic blood pressure

SCI	Spinal cord injury
SEM	Standard error of the mean
SD	Standard deviation
SPN	Sympathetic preganglionic neuron
SpO_2	Oxyhaemoglobin saturation with pulse oximetry
SSR	Sympathetic skin response
VLF	Very low frequency
VM	Valsalva manoeuvre
wk	Week(s)
yr	Year(s)

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Dedication

I would like to dedicate this thesis to my parents, Ann and Peter Wong. Your unwavering love, support and encouragement have inspired me and given me the strength to be the adventurer and explorer that I am. I love you both.

Chapter 1: Introduction

Spinal cord injury (SCI) is a life altering event. Currently, the outcome of SCI is commonly described in terms of motor and sensory function. The International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI)[1] is used to determine the score of the American Spinal Injury Association Impairment Scale (AIS) by examining motor function (integrity of corticospinal tracts) along with pinprick (integrity of spinothalamic tract) and light touch sensation (integrity of dorsal columns). Based on this assessment, individuals are classified into one of five categories: motor and sensory complete (AIS A), motor complete and sensory incomplete (AIS B), motor and sensory incomplete (AIS C and D), and normal motor and sensory function (AIS E). In addition to the ISNCSCI[1] used to document neurologic impairment, international standards to document remaining autonomic function following SCI have been introduced by a group of international experts commissioned by the American Spinal Injury Association and the International Spinal Cord Society to develop a common strategy to document remaining autonomic function[2]. When the spinal cord is injured, there is also damage to the autonomic nervous system (ANS). The two components of the ANS, the parasympathetic and sympathetic nervous systems are complex and involved in the control of almost every system in the body. The cardiovascular system is one of these systems and associated dysfunction may be problematic following SCI. Previous studies have found that cardiovascular autonomic impairment as indicated by severity of dysfunctions such as orthostatic hypotension (OH) and autonomic dysreflexia (AD) correlate with the completeness of SCI as assessed by the AIS[1, 3]. However, contrasting data demonstrate that autonomic function is not reliably predicted by the degree of residual motor or sensory function[4, 5]. Since the concept of autonomic completeness has only been presented in the literature, the majority of studies that have examined physiological responses of patients or individuals living in the community have not considered autonomic completeness of injury[6, 7]. As such, there is relatively little information on the relationship between the neurologic completeness of injury and the degree of impairment to spinal autonomic pathways[8].

Neurologic and autonomic impairment are dependent upon the level of the lesion, whereby function is reduced as the level of SCI moves cranially[9, 10]. Individuals with SCI, especially those with cervical or high-thoracic injuries are faced with unstable blood pressure (BP) control for the rest of their lives[11, 12]. Previous studies in humans and animals have shown that descending vasomotor pathways are primarily located in the dorsal aspect of the lateral funiculus[13-16]. Accordingly, a critical determinant of cardiovascular dysfunction and spinal autonomic integrity after SCI is the degree of impairment to descending vasomotor pathways following injury[17]. It is possible that the dorsolateral aspects of the spinal cord remain intact since compression and contusion of the spinal cord are the main causes of injury[18] rather than complete transection of the cord,. Thus, the ability to provide descending vasomotor control even following neurologically complete injury to the spinal cord may remain.

Managing unstable BP on a daily basis may manifest as OH[5, 19-21] and AD[22-26] in individuals with SCI. The latter may result in catastrophic consequences including myocardial ischemia, intracranial hemorrhage, seizures, and death[27-30]. Furthermore, cardiovascular diseases are the leading causes of morbidity and mortality in individuals with SCI[31, 32], making proper identification and management of individuals predisposed to cardiovascular dysfunctions priorities in this population. The clinical benefits of understanding the integrity of spinal autonomic pathways after injury include the ability to identify individuals who are predisposed to autonomic dysfunction such as cardiovascular impairment to ensure appropriate and timely treatment and management. As such, since compromised integrity of spinal autonomic pathways following injury leads to cardiovascular dysfunction, investigating cardiovascular autonomic function contributes to understanding changes in spinal autonomic integrity after injury[5].

The selection of a specific battery of tests to assess autonomic function that do not require cumbersome research equipment and can be easily performed by a trained clinician at the bedside after injury is crucial. This is important in to help identify individuals at risk for autonomic dysfunction. Primary causes of morbidity and mortality following SCI are related to autonomic dysfunctions[31, 33], and recovery of autonomic functions is rated as a priority by

individuals living with SCI[34]. Of various autonomic assessments that are available, non-invasive tests that are easy to facilitate include the sit-up test (orthostatic challenge), analysis of heart rate variability (HRV) and sympathetic skin responses (SSRs). Evaluating sudomotor (via SSRs) in addition to cardiovascular pathways is recommended when examining spinal autonomic integrity[7]. The sit-up test has been previously validated in individuals with SCI[5], HRV reproducibility has been demonstrated previously[35], and SSRs are an established technique for the study of sympathetic neuropathies[36-38]. Establishing the reliability and comparability of these assessments will contribute to the development of a battery of tests that are appropriate to use in examining autonomic function in individuals with SCI.

A more comprehensive understanding of integrity of spinal autonomic pathways is warranted to improve knowledge about consequential dysfunction associated with varying degrees of autonomic impairment. Extensive focus on changes to cardiovascular control after injury has provided a foundation upon which to explore integrity of spinal autonomic pathways. This introduction will present a brief overview of the impact that changes to the integrity of spinal autonomic pathways has on changes to cardiovascular autonomic control following SCI, methods used to assess autonomic function, and finally, the objectives of the thesis.

1.1 The autonomic nervous system and cardiovascular control

Since the cardiovascular system is under control of the ANS, investigating components of the spinal cord involved in cardiovascular function lends itself to understanding the integrity of spinal autonomic pathways[5, 7, 9]. The medulla oblongata is primarily responsible for cardiovascular control and its various nuclei receive excitatory and inhibitory input from the cerebral cortex and the hypothalamus. Medullary neurons located in the rostroventrolateral medulla provide descending sympathoexcitatory pathways with tonic input to the sympathetic preganglionic neurons (SPNs) located within the lateral horn of the spinal gray matter and around the central canal of the thoracic and upper lumbar spinal segments (T1 to L2)[39, 40]. Sympathetic preganglionic neurons send efferent tonic signals from the central nervous system to

different target organs such as blood vessels and the heart. They reside in the spinal gray matter in the thoracic (T1 to T12) and upper lumbar segments (L1 to L2) of the spinal cord[41-43]. The majority of SPNs are located within the lateral horns or intermediolateral nucleus, and a small proportion are found near the central canal of the spinal cord. Sympathetic preganglionic neurons from the upper thoracic segments of the spinal cord from T1 to T5 provide sympathetic innervation to the heart and the majority of the vasculature in the upper extremities[39, 40]. The SPNs contained in the more caudal segments from T5 to L2 innervate the major vasculature in the splanchnic region and lower extremities[39, 40]. Axons of SPNs exit the spinal cord via the ventral root and synapse on postganglionic neurons located in the sympathetic chain (paravertebral ganglia). It is these postganglionic neurons that synapse with the heart and blood vessels. The sympathetic nervous system innervates both cardiac and smooth muscle within peripheral vasculature. This system is predominant in the "fight or flight" response and acts to increase BP and heart rate (HR) as appropriate[40]. Evidently impairment to spinal sympathetic control resulting from injury at different locations along the spinal cord may alter integrity of spinal autonomic pathways. The vagus nerve (cranial nerve X) provides the most widespread preganglionic parasympathetic output and innervates the heart. Parasympathetic preganglionic neurons synapse with postganglionic neurons in ganglia near or within the target organs. The vagus nerve is prominent during restful states and acts to decrease HR. In combination, the sympathetic and parasympathetic components of the ANS act to maintain cardiovascular homeostasis.

The baroreflex is a negative feedback system that has a dominant role in preventing short-term fluctuations in BP[44]. Arterial stretch receptors provide the central nervous system (specifically the nucleus tractus solitarius) with information on current changes in BP which are sensed by the stretch receptors in the wall of the carotid sinus and aortic arch. This information subsequently influences efferent autonomic nerve traffic[45]. The baroreflex regulates BP by changing HR (vagal component) and total peripheral resistance (sympathetic adrenergic component)[46]. The baroreflex system employs both the sympathetic and parasympathetic components of the ANS to regulate BP, within a narrow range, over a wide variety of environmental conditions and body positions[47]. When the arterial baroreceptors are activated by a rise in systemic BP, there is an increase in the

discharge of vagal cardioinhibitory neurons and a decrease in the discharge of sympathetic neurons to the heart and the peripheral vasculature. This results in a decrease in HR (bradycardia), decreased cardiac contractility and decreased peripheral resistance and venous return[48, 49]. In contrast, a decrease in systemic BP deactivates the baroreceptors causing an enhancement of sympathetic activity and vagal inhibition, leading to an increase in HR (tachycardia) and cardiac contractility, vascular resistance and venous return[48, 49]. The baroreflex is also integrated with chemoreflex and pulmonary afferent information to produce an appropriate efferent response such that respiration and arterial blood gases influence the baroreflex. Respiration continuously interacts with baroreflex modulation of HR whereby inspiration decreases and expiration increases baroreceptor stimulation of vagal motoneurons in a phenomenon known as respiratory gating[50]. Studies have shown that cardiovagal baroreflex sensitivity holds prognostic value for cardiovascular events in several clinical populations including patients with heart failure and diabetic patients[51, 52]. On the other hand, there is limited value in examining only the cardiovagal branch of the baroreflex in relation to orthostatic intolerance since the vasomotor branch of the baroreflex is much more important for the maintenance of mean BP. This has been illustrated by studies that have shown no differences in BP responses in individuals with complete autonomic failure to an orthostatic challenge before and after complete vagal blockade[8, 53]. Examining both branches of the baroreflex is required to provide insight on autonomic integrity since alterations in baroreflex control reflect changes in cardiovascular autonomic function[54].

1.2 Effect of spinal cord injury on cardiovascular autonomic function and spinal autonomic pathways

Cardiovascular dysfunction varies dramatically with the level of SCI[9, 55] with a very strong correlation between the level and severity of autonomic dysfunction. Vagal cardiac control originates from the medulla and is intact following injury[39]. High-level SCI alters the ability of the medullary vasomotor centre to maintain sympathetically mediated efferent control of vasomotor tone and HR[56]. As such, the higher the level of SCI, the greater the clinical manifestations of sympathetic dysfunction[55-57] and loss of spinal autonomic integrity. The

diminished integrity manifests as autonomic dysfunction that is evident from cardiovascular conditions[5]. Individuals with cervical or high-thoracic SCI must deal with unstable BP control which manifests as resting BP that is generally lower than that of able-bodied individuals (AB)[57]. Furthermore, individuals with neurologically complete cervical and high-thoracic SCI are likely to experience orthostatic intolerance and AD more severely than those with neurologically incomplete injuries at the same level[11, 58]. Regardless of neurological completeness of injury, the degree of cardiovascular impairment and remaining spinal autonomic integrity is determined by the relative loss of supraspinal control over sympathetic outflow. The loss of supraspinal control and reduced sympathetic activity, morphological changes in SPNs, axonal sprouting and potential formation of inappropriate synapses with spinal interneurons and altered baroreflex function contribute to cardiovascular impairment and altered spinal autonomic integrity following injury.

Spinal cord injury disrupts the descending sympathoexcitatory pathways and affects tonic input to the SPNs located in the lateral horns of the spinal gray matter and around the central canal. Individuals with cervical SCI may have disruption of supraspinal connections to SPNs that innervate the heart and vasculature, which removes tonic excitatory input to these organs. Generally, injuries at or above T5 that affect the ANS result in low resting sympathetic tone below the level of SCI. Low resting catecholamine levels in individuals with cervical SCI have helped to explain the reduced sympathetic activity following injury[59, 60]. Cervical and high-thoracic injuries also result in loss of supraspinal sympathetic control of the splanchnic vascular bed which leads to a lowering of BP[61]. Furthermore, with injuries at or above T5, loss of descending tonic inhibition is also crucial to the development of AD since there is a marked reduction in supraspinal control to the splanchnic bed and vasculature of the lower extremities. With SCI below T5, there is generally sufficient sympathetically innervated vasculature under sympathetic control, especially in the important splanchnic blood vessels to limit significant cardiovascular autonomic dysfunction. These individuals are more likely to have resting BP within the normal range[11, 55] and experience episodes of AD less frequently[58]. Based on findings reported on cardiovascular function, individuals with lower lesion levels appear to have better integrity of spinal autonomic pathways which is observed as better cardiovascular control.

Studies on animals and humans have demonstrated that there are morphological changes in SPNs caudal to the injury site. Along with the potential formation of new synapses resulting from the sprouting of axons local to the area of injury, these changes may be partially responsible for cardiovascular instability and loss of spinal autonomic integrity [62, 63]. Significant atrophy of SPNs has been observed in rats (examination at 7 days post-injury)[62] and in humans (examination at 2 weeks post-injury)[63]. It is speculated that the atrophy of these neurons may contribute to the initial hypotension, associated with neurogenic shock, that is seen in humans and animals[55, 64-66]. Neurogenic shock manifests as severe cardiovascular dysfunctions whereby individuals present with severe hypotension and bradycardia[67]. In addition to the loss of important excitatory input from the brainstem[68-70], the atrophy of SPNs may also have a role in the decrease in sympathetic activity. The morphology of these neurons appears to return to normal at a later stage of SCI in humans and animals[62, 63], which may help to explain the presence of large and sustained excitatory sympathetic responses that develop[55] and cause episodic hypertension (AD)[13, 22, 32, 55]. Consequently, different mechanisms may contribute to impaired cardiovascular control and varying degrees of integrity of spinal autonomic pathways at different stages following injury. The sprouting of new afferent inputs could explain the observation of a re-established morphology of SPNs and supports the notion of long-term, stable and intense AD in the chronic stage of SCI in rats[64, 66] and humans[55, 63].

It has been documented that changes in baroreflex function reflect alterations in autonomic control of the cardiovascular system[54]. Although vagal influence on HR is maintained following SCI[56], studies have reported that the sensitivity of the cardiovagal baroreflex may be affected[71-76]. Since the cardiovagal component of the baroreflex has been linked to predicting future cardiovascular events in AB, it can be postulated that it reflects cardiovascular risk in individuals with SCI as well[51, 52, 77]. As mentioned previously, the cardiovagal baroreflex is not likely to influence the overall absolute mean arterial BP response to an orthostatic challenge. However, abnormal cardiovagal baroreflex may still contribute to reduced orthostatic tolerance seen following SCI[78]. Research on AB has demonstrated that the vagal influence on HR plays a dominant role in the short-term regulation of BP (100% in the first 2 to 3 seconds after a stimulus), but only a minor role (23%) after that[53]. The sympathetic branch of the baroreflex is

far more important to BP regulation. This is evident following SCI since the descending pathway becomes disrupted and results in significant reductions in the control of vasomotor tone below the level of injury[12, 13, 40]. There are several methods used to evaluate baroreflex function as well as two non-invasive and simple indices derived from the Valsalva manoeuvre (VM) including the Valsalva ratio (indicative of cardiovagal baroreflex) and the index of adrenergic baroreflex sensitivity (BRSa) (calculated by dividing the decline in systolic BP (SBP) during Phase III by pressure recovery time[79] which is the time, in seconds, that it takes for SBP to return to baseline from its lowest value during Phase III). These two measures allow for the determination of both the cardiovagal and cardiac sympathetic components of the baroreflex.

Examining spinal autonomic pathways may also be accomplished by assessing spinal sympathetic sudomotor pathways via SSRs. Since SSRs provide information on sympathetic cholinergic pathways and descending autonomic pathways[80], evaluating their preservation in combination with measures of cardiovascular autonomic function provides a comprehensive overview of spinal autonomic integrity. Understanding the extent of autonomic impairment is helpful in navigating the approach to treating and managing conditions associated with autonomic dysfunction. Existing and ongoing research about cardiovascular impairment should be used to enhance understanding about integrity of spinal autonomic pathways after injury, an area of research following SCI that is still sparse[7].

1.3 Investigating the integrity of spinal autonomic pathways

There are various methods available to assess autonomic function in SCI that are simple and validated for use in this population. Understanding changes in autonomic function is a priority[2, 67, 81-83], so it is imperative to ensure that assessments being used are appropriate and may be used consistently. Tests used to assess autonomic function that subsequently provide information on the integrity of spinal autonomic pathways include cardiovascular responses to orthostatic challenges (sit-up and tilt-table tests), HRV, the VM, and SSRs. The orthostatic challenges, HRV

and the VM assess cardiovascular autonomic control and the SSRs evaluate the integrity of spinal autonomic pathways and these are used throughout the studies conducted for this thesis.

Over the past three decades, the number of studies investigating SCI has increased dramatically, however, the those with motor outcomes is overwhelmingly higher than those with sensory or autonomic outcomes[84]. Fortunately, there is increased acknowledgement about the importance of examining and documenting changes in autonomic function following SCI since the ANS is involved in the control of almost every bodily system. Therefore, the overarching objectives of this doctoral thesis were to examine the integrity of spinal autonomic pathways in sub-populations of individuals with SCI, and investigate the reliability and agreement of various methods of autonomic testing used in this population. These objectives were accomplished by evaluating tests of autonomic function and assessing individuals with SCI in the following studies:

- Reliability of the sit-up test, HRV and SSRs and agreement between the sit-up and tilt-table test (Chapter 2)
- Assessment of individuals in the sub-acute stage of SCI who are undergoing rehabilitation (Chapter 3)
- Assessment of individuals with chronic SCI (Chapter 4)

Each research chapter begins with a focused introduction and accompanying hypotheses. The introductions are subsequently followed by detailed explanations of the experimental approaches, experimental data, and discussion sections that interpret the findings. The final chapter (Chapter 5) integrates the findings with the literature by providing overall conclusions and perspectives.

Chapter 2: Test-retest Reliability and Agreement of Autonomic Measures

2.1 Introduction

The complexity of the ANS lends itself to difficult decision-making with regards to selecting appropriate tests to assess autonomic function. This decision can be made more easily if the tests being considered for use are understood to be simple, preferably non-invasive and if they are known to be reliable and valid. Four methods to assess autonomic function were used in the studies throughout this thesis and these included two types of orthostatic challenges (sit-up and tilt-table), HRV and SSRs. In the first study, an investigation of the reliability of the sit-up test, HRV and SSRs was conducted and the agreement between the sit-up and the tilt-table tests was assessed. This investigation is warranted since currently there are no studies on the reliability of orthostatic testing or SSRs in individuals with SCI. It is also unknown if the sit-up test is a suitable alternative to the gold standard tilt-table test.

The orthostatic challenges alter BP as a result of change in position. Orthostatic hypotension is characterized by a decrease in SBP of $\geq$ 20 mmHg or diastolic BP (DBP) of $\geq$ 10 mmHg when in an upright position, in the presence or absence of symptoms (e.g., lightheadedness, dizziness, fatigue, etc.) within three minutes of a change in posture[85]. Upon assumption of the seated posture (sit-up test) or when being gradually tilted vertically (tilt-table test), a substantial amount of blood is redistributed to the lower extremities[86]. During the sit-up test, individuals with cervical SCI have been found to experience a decrease in SBP, and individuals with low-thoracic SCI and AB have experienced an increase in SBP[5]. Similarly, the tilt-table test has demonstrated that individuals with tetraplegia (cervical SCI) exhibit a greater reduction in SBP in comparison to individuals with paraplegia (thoracic SCI or lower) and AB[87]. Both of these tests are able to elicit responses that consistently demonstrate that individuals with higher lesion levels have greater orthostatic intolerance. The sit-up test has been validated previously[5], however, agreement in the ability of these two tests to elicit an orthostatic response of the same magnitude is unknown. The tilt-table test is also considered the gold standard against which other orthostatic tests are compared for testing clinical orthostatic BP[88]. Generally, the reliability of

cardiovascular responses during an orthostatic challenge in AB have been inconsistent with some studies observing reliability[89, 90] and others finding varying results on repeated challenges[91-98].

Heart rate variability is spectral analysis of cardiovascular parameters used to evaluate autonomic tone from HR and it has been shown to be reproducible in individuals with SCI by Ditor et al.[99] and in AB by Marks and Lightfoot[100]. The analysis of HRV is based on the observation that basal RR intervals (RRIs) (the time between two successive R waves of an electrocardiogram) continually fluctuate. Oscillations from 0.04 to 0.15 Hz are designated as low frequency (LF), and oscillations from 0.15 to 0.4 Hz are designated as high frequency (HF). For analysis, it is important to understand that HRV may be affected by ectopic beats. Ectopic heartbeats are a disturbance of the cardiac rhythm and are caused by premature heartbeats and other arrhythmias[101]. They lead to extra or skipped heartbeats and often occur without a clear cause and are most often harmless. The two most common types of ectopic heartbeats are premature ventricular contractions and premature atrial contractions. The electrocardiogram recordings for HRV analysis generally include normal RRIs that are modulated by the sinus node, but these may be disturbed by ectopic heartbeats that do not originate from the sinus node. Ectopic heartbeats need to be removed for proper HRV analysis because they distort the electrocardiogram recording with inappropriate frequencies and random spectra in the LF and HF components[102]. High frequency power is related to the respiratory sinus arrhythmia and is an index of cardiac vagal control[86, 103]. On the other hand, what LF power represents is less clear. Previous findings support that LF power is indicative of cardiac sympathetic tone[104] and/or parasympathetic outflow[105, 106]. With the use of atropine, an anticholinergic drug, in the supine position and with paced breathing, LF power has been shown to be reduced by more than 80%[104]. Akselrod et al.[105] showed similar findings with the use of the parasympatholytic agent glycopyrrolate that eliminated HRV above 0.06 Hz and decreased spectral power below this value, and sympathetic blockade with propranolol would inconsistently reduce HRV around 0.04 Hz. It was evident from these findings that LF power (below 0.01 Hz) is mediated by both sympathetic and parasympathetic influences, whereas HF power is controlled solely by the parasympathetic system. In addition to representing parasympathetic and sympathetic outflows, other recent studies demonstrate that HRV represents modulation of cardiac autonomic outflows

by baroreflexes[107-109] suggesting that HRV is a means to evaluate the ability to modulate autonomic outflows via baroreflexes rather than a means to evaluate autonomic tone. There is evidence discussed by Goldstein et al.[109] that argues against the validity of LF power as an index of sympathetic outflow to the heart. A lack of association has been found between levels of noradrenaline, whether altered by drugs, imaging agents or disease, and LF power.

The integrity of sympathetic sudomotor function can be evaluated via the clinical assessment of SSRs[110, 111]. Sympathetic skin responses provide information about autonomic sympathetic function by examining the common efferent pathways of the sympathetic nervous system from the spinal cord to the sweat glands of the hands (palmar) and feet (plantar) relayed by preganglionic and postganglionic sympathetic nerve fibres[112]. This electrophysiological test records a change in potential from the surface of the skin generated by sweat in response to a stimulus and provides an electrophysiological assessment of sympathetic fibres[113]. Sympathetic skin responses provide information on the integrity of the sympathetic cholinergic and descending autonomic pathways[80]. A preserved spinal autonomic pathway is qualified by the absence or presence of response to stimulation. Four small studies: 1) the reliability of the sit-up test; 2) the reliability of HRV; 3) the reliability of SSRs; and 4) agreement between the sit-up and the tilt-table tests were conducted in the first chapter of this thesis. It was hypothesized that the sit-up test, HRV and SSRs are reliable, and the sit-up test is an appropriate alternative to the tilt-table test for assessing orthostatic intolerance in individuals with SCI.

2.2 Materials and methods

Participant characteristics. There were slight variances in the number of participants in each of the four smaller studies: 1) and 2) Nine participants with chronic SCI (10 $\pm$ 2 yrs) (C4 to T11; AIS A and B) (32 $\pm$ 2 yrs; 3 females) and 11 AB participants (29 $\pm$ 2 yrs; 5 females) participated in the sit-up and HRV reliability studies (Table 2.1). All participants had normal height (180 $\pm$ 3 cm and 170 $\pm$ 3 cm, SCI and AB, respectively) and weight (71 $\pm$ 4 and 69 $\pm$ 4, SCI and AB, respectively); 3) there were 16 participants (43 $\pm$ 23; 4 females) with sub-acute and chronic (4 $\pm$

2 yrs) SCI (C2 to T11; 7 with AIS A and 9 with AIS B to D) with normal height (177 $\pm$ 10 cm) and weight (72 $\pm$ 14 kg) for which testing for the reliability of SSRs was performed; and 4) nine participants (40 $\pm$ 21 yrs; 2 females) with chronic and sub-acute (51 $\pm$ 39 wks) SCI (C4 to T7; AIS A and B) of normal height (174 $\pm$ 4 cm) and weight (68 $\pm$ 4 kg) participated in the study comparing the sit-up test to the tilt-table test. Participants with SCI were recruited from an in-patient unit and outpatient clinic located at the G.F. Strong Rehabilitation Centre in Vancouver, British Columbia and must have sustained a traumatic SCI. The neurological exam was performed by a qualified physician. Participants were only included if they were free of any coincident cardiac or pulmonary diseases or active medical issues such as hypertension, pressure sores or urinary tract infections. Able-bodied participants were recruited from the University of British Columbia in Vancouver, British Columbia. On an initial visit, written informed consent was obtained and participants underwent the first of two consecutive and identical testing days for each study except for the study comparing the tilt and sit-up test, in which case these two tests were performed and compared on the same day. All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia which conforms to the Declaration of Helsinki.

Experimental protocol. 1) Sit-up test reliability. Tests were performed in the morning if possible and participants with SCI withheld their medication and were instructed to abstain from caffeine and alcohol from the night before, and to consume only a light breakfast. Upon arrival to the laboratory, they were asked to empty their bladders to minimize the influence of reflex sympathetic activation on peripheral vascular tone. They transferred to the chair used for orthostatic testing (sit-up test). There was an additional period of 10 minutes of supine rest when we instrumented research participants with electrocardiogram electrodes, and arm and finger blood pressure cuffs prior to the initiation of data collection. Following instrumentation, the experimental session began with a minimum of 10 minutes in the supine position during which time resting data were recorded. Participants were then passively moved to a seated position by raising the head of the chair by 90^{o} and dropping the base of the chair by 90^{o} at the knee. They were questioned for the presence of symptoms of hypotension (i.e., dizziness, fatigue, blurred vision, syncope, and/or lightheadedness) when in the seated position.

2) Heart rate variability reliability. Single-lead electrocardiogram was continuously recorded throughout the sit-up test to determine HR. Time series of successive beats were extracted from the electrocardiogram recordings for RRIs. Power spectral analysis was performed using an autoregressive model fitted to each time series (aHRV, Nevrokard, Slovenia)[114]. Occasional ectopic beats were "corrected" by the linear interpolation of adjacent normal beats. Powers were normalized by dividing the power by the total variance minus very low frequency (VLF, <0.03 Hz) and multiplied by 100[115]. Automated BP was measured at the brachial artery (Dinamap, GE Pro 300V2, Tampa, FL). Oxyhaemoglobin saturation (SpO_2) was measured with a pulse oximeter (Dinamap, GE Pro 300V2, Tampa, FL).

3) Sympathetic skin response reliability. Over two consecutive days, SSRs were assessed to examine reliability of this technique in individuals with SCI. Self-adhesive recording electrodes were placed on sites with maximum eccrine sweat gland density (palms of the hands and soles of the feet; left hand, LH; right hand, RH; left foot, LF; right foot, RF). Sympathetic skin responses were recorded simultaneously from both hands and feet following a single electrical pulse (duration 0.2 ms; intensity 8-10 mA) applied to the median nerve. Five consecutive stimuli were applied to the median nerve at the wrist and for a second stimulus participants were instructed to take five consecutive deep breaths. Data were continuously recorded using an analog-to-digital converter (Keypoint, Alpine Biomed, California, USA). To minimize habituation, stimuli were applied in random order and with variable time delays (minimum delay of 90 s). Sympathetic skin responses were deemed present when there was a clear positive deflection from baseline. Any potential that coincided with muscle spasm, limb movement, or cough was excluded from the analysis. Responses were qualified by the number of reproducible SSRs elicited[113]. A response indicated a preserved spinal autonomic pathway.

4) Sit-up test and tilt-table test agreement. The order of performance of these tests was not randomized with each participant undergoing the tilt-table test first. Before undergoing the sit-up test, we ensured that BP returned to baseline values. The preparation and experimental protocol for the sit-up test is identical to that previously described. For the tilt-table test, participants

transferred to the tilt table which was padded and motorized and restraining straps were used on the lower extremities and trunk to ensure participant safety. The restraining straps were padded to avoid stimulation of sympathetic spinal reflexes during testing. Tilting was performed progressively beginning with five minutes of supine rest and five minutes at each progressive angle of tilt (30^o, 45^0, 60^o). The rate of tilt at each angle was such that assumption of tilt at each new angle was immediate and within 3 seconds. The same measures (HR, BP, SpO_2) were collected during the tilt-table test as the sit-up test. The following is a flow chart for the time line of reliability testing.

Day 1 and 2 repeat assessments included: sit-up test, heart rate variability, sympathetic skin responses

Figure 2.1 – Timeline of reliability testing.

Data and statistical analysis. All data were acquired using an analog to digital converter (Powerlab/16SP model ML795; ADInstruments, Colorado Springs, CO) interfaced with a computer and sampled at 1 kHz. Data were stored on a personal computer for subsequent offline analysis (Powerlab version 7.2, ADInstruments). Cardiovascular measures during the sit-up and tilt-table tests were compared in the supine position and at the 5, 10, and 15 minute time points during each test, corresponding to 0^o, 30^o, 45^o, and 60^o angles of tilt. To examine the test-retest reliability for HRV, the sit-up test and SSRs, the intraclass correlation coefficient (ICC) and 95% confidence intervals (CIs) were used according to Shrout and Fleiss[116]. To assess agreement between the sit-up and tilt-table tests, limits of agreement and 95% CIs were calculated. The level of significance was set at $P < 0.05$ for all statistical calculations. Group data are presented as means $\pm$ standard error of the mean (SEM).

2.3 Results

Participant characteristics.

Table 2.1 – Characteristics of participants in sit-up test and heart rate variability reliability studies.

Participant	SCI/ AB	SCI Level	AIS	Time since SCI (yrs)	Age (yrs)	Height (cm)	Weight (cm)
1	SCI	C4	B	13	32	180	72.5
2	SCI	C5	B	7	34	188	72.2
3	SCI	C6	A	14	39	183	61.0
4	SCI	C6	A	12	36	180	61.5
5	SCI	C6	B	8	39	158	63.9
6	SCI	T4	A	17	32	178	57.9
7	SCI	T4	A	11	33	183	88.2
8	SCI	T6	B	11	33	185	84.5
9	SCI	T11	A	1	19	180	73.2
10	AB	N/A	N/A	N/A	24	159	47.0
11	AB	N/A	N/A	N/A	23	181	73.0
12	AB	N/A	N/A	N/A	27	165	60.6
13	AB	N/A	N/A	N/A	23	174	74.9
14	AB	N/A	N/A	N/A	23	164	61.0
15	AB	N/A	N/A	N/A	23	160	68.4
16	AB	N/A	N/A	N/A	30	183	79.5
17	AB	N/A	N/A	N/A	31	190	97.9
18	AB	N/A	N/A	N/A	39	162	66.1
19	AB	N/A	N/A	N/A	38	162	61.8
20	AB	N/A	N/A	N/A	33	169	68.2
Mean ± SD	N/A	N/A	N/A	10 ± 2	32 ± 2 (SCI); 29 ± 2 (AB)	180 ± 3 (SCI); 170 ± 3 (AB)	71 ± 4 (SCI); 69 ± 4 (AB)
Minimum	N/A	N/A	N/A	1	19	158	47.0
Maximum	N/A	N/A	N/A	17	39	190	97.9

SCI, spinal cord injury; AB, able-bodied; AIS, American Spinal Injury Association Impairment Scale; SD, standard deviation.

Table 2.2 – Characteristics of participants in sympathetic skin response reliability study.

Participant	SCI Level	AIS	Time since SCI (weeks)	Age (yrs)	Height (cm)	Weight (kg)
1	C2	D	9	71	180	72.2
2	C4	A	4	39	177	86.0
3	C4	B	11	29	173	59.1
4	C4	C	7	60	173	78.7
5	C5	A	11	45	179	70.0
6	C5	A	24	22	163	44.8
7	C5	B	416 (8 yrs)	50	191	79.5
8	C5	B	10	60	180	68.2
9	C5	D	1352 (26 yrs)	41	173	75.0
10	C7	A	1664 (32 yrs)	36	170	54.5
11	T3	A	7	79	170	81.8
12	T4	A	14	23	195	102.3
13	T4	C	9	44	179	77.3
14	T7	A	6	19	198	72.7
15	T10	B	4	21	163	56.0
16	T11	D	7	59	175	72.7
Mean $\pm$ SD	N/A	N/A	4 $\pm$ 2 yrs	43 $\pm$ 19	177 $\pm$ 10	71.9 $\pm$ 13.8
Minimum	N/A	N/A	4 wks	19	163	44.8
Maximum	N/A	N/A	32 yrs	79	198	102.3

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; SD, standard deviation

Table 2.3 – Characteristics of participants in tilt-table and sit-up test agreement study.

Participant	SCI Level	AIS	Time since SCI (wks/yrs)	Age (yrs)	Height (cm)	Weight (kg)
1	C4	A	10/0.19	17	173	77.3
2	C4	A	17/0.33	47	173	77.3
3	C4	B	364/7	39	158	63.9
4	C4	B	11/0.21	29	173	59.1
5	C5	A	11/0.21	45	179	70.0
6	C5	A	24/0.46	22	163	44.8
7	C5	B	10/0.19	60	180	68.2
8	T3	A	7/0.13	79	170	81.8
9	T7	A	6/0.11	19	198	72.7
Mean $\pm$ SD	N/A	N/A	51 $\pm$ 39 wks	40 $\pm$ 21	174 $\pm$ 4	68 $\pm$ 4
Minimum	N/A	N/A	6 wks	17	158	44.8
Maximum	N/A	N/A	364 wks	79	198	81.8

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; SD, standard deviation.

1) No significant differences were observed between days for any of the cardiovascular measures. Intraclass correlation coefficients showed moderate to high reliability (ICC $\geq$ 0.5) for over half of the BP measures at the various time points in the supine and seated positions for participants with SCI and AB. In both groups, measures of SBP were all

highly reliable with lower ICCs (fair to highly reliable) for DBP. These ICCs are listed in Table 2.4.

Table 2.4 – Reliability of the sit-up test.

Comparison time	ICC		Mean difference, 95% CI	
			SBP (mmHg)	
	SCI	AB	SCI	AB
Supine	0.91	0.90	0.76 ± 1.58, -0.79 to 2.31	3.14 ± 0.29, 2.86 to 3.42
Sit up 3 mins	0.89	0.78	0.29 ± 1.17, -0.87 to 1.44	1.39 ± 1.17, 0.24 to 2.53
Sit up 5 mins	0.87	0.70	1.90 ± 0.56, 1.35 to 2.45	0.94 ± 0.30, 0.64 to 1.23
Sit up largest drop	0.84	0.89	1.77 ± 2.52, -0.71 to 4.24	1.55 ± 0.40, 1.16 to 1.94
			DBP (mmHg)	
			SCI	AB
Supine	0.86	0.63	-0.42 ± 0.47, -0.88 to 0.04	3.63 ± 0.62, 3.03 to 4.24
Sit up 3 mins	0.74	0.24	1.02 ± 0.67, 0.56 to 1.48	2.39 ± 0.481.92 to 2.86
Sit up 5 mins	0.65	0.14	1.27 ± 0.25, 1.02 to 1.51	2.12 ± 1.190.96 to 3.30
Sit up largest drop	0.54	0.69	0.80 ± 0.32, 0.49 to 1.11	2.42 ± 1.43, 1.03 to 3.83

Values are mean ± SEM. SCI, spinal cord injury; AB, able-bodied, SBP, systolic blood pressure; DBP, diastolic blood pressure; mmHg, millimeters of mercury; CI, confidence interval, mins, minutes.

2) No significant differences were observed between days for any of the HRV measures. Intraclass correlation coefficients showed a moderate to high reliability (ICC ≥ 0.5) for over half of the HRV measures in the supine and seated positions for both participants with SCI and AB. These values are listed in Tables 2.5 and 2.6. Measures with ICCs from 0.7 to 0.8 (high reliability) in the supine position in participants with SCI were mean RRI, total variance, LF, HF, VLF powers. For AB this included LF power. The measures with ICCs from 0.7 to 0.8 in the sit-up position for participants with SCI included mean RRI, total variance, LF, HF, and VLF powers and LF/HF. For AB these included mean RRI, LF and HF powers.

Table 2.5 – Reliability of heart rate variability measures in the supine position.

Supine	Day 1	Day 2	ICC
SCI (n=5)			
Mean RRI, ms	1011.4 ± 47.7	971.8 ± 67.1	0.71
Total Variance, ms^2	6882.0 ± 2775.3	5314.0 ± 1633.6	0.83
LF, Hz	0.05 ± 0.01	0.04 ± 0.0	0.91
LF power			
ms^2	844.1 ± 215.5	596.1 ± 158.5	0.47
nu	81.6 ± 10.2	83.4 ± 23.7	0.69
%	40.4 ± 6.4	29.0 ± 5.3	0.52
HF, Hz	0.22 ± 0.04	0.25 ± 0.04	0.79
HF power			
ms^2	617.5 ± 140.0	472.4 ± 200.0	0.15
nu	24.8 ± 2.4	23.3 ± 5.7	0.54
%	50.8 ± 3.4	49.6 ± 7.3	0.73
LF/HF	1.7 ± 0.3	2.1 ± 1.0	0.54
VLF, Hz	0.02 ± 0.01	0.01 ± 0.004	1.00
VLF power			
ms^2	692.3 ± 183.5	757.3 ± 198.0	0.83
nu	65.9 ± 15.0	113.3 ± 41.1	0.11
%	30.0 ± 5.3	38.0 ± 6.9	0.32
AB (n=11)			
Mean RRI, ms	1018.5 ± 30.5	1001.4 ± 44.5	0.64
Total Variance, ms^2	6398.8 ± 1519.5	6873.7 ± 2559.0	0.38
LF, Hz	0.04 ± 0.0	0.05 ± 0.01	0.88
LF power			
ms^2	800.1 ± 131.1	761.2 ± 146.0	0.67
nu	70.4 ± 7.0	81.1 ± 5.3	0.28
%	33.8 ± 3.2	33.7 ± 3.0	0.65
HF, Hz	0.22 ± 0.02	0.23 ± 0.02	0.70
HF power			
ms^2	672.7 ± 92.8	617.2 ± 128.1	0.45
nu	28.5 ± 3.0	23.3 ± 2.6	0.32
%	57.2 ± 4.4	56.2 ± 4.9	0.55
LF/HF	1.4 ± 0.2	1.7 ± 0.3	0.30
VLF, Hz	0.01 ± 0.0	0.01 ± 0.0	1.00
VLF power			
ms^2	618.0 ± 55.4	879.9 ± 130.5	0.47
nu	69.2 ± 8.8	107.7 ± 13.0	0.16
%	3.7 ± 3.6	41.6 ± 2.9	0.30

Values are ± SEM. SCI, spinal cord injury; AB, able-bodied; RRI, RR interval, LF, low frequency; HF, high frequency; VLF, very low frequency; Hz, Hertz, ms^2, milliseconds squared; nu, normalized units.

Table 2.6 – Reliability of heart rate variability measures in the seated position.

Sit-up	Day 1	Day 2	ICC
SCI (n=5)			
Mean RRI, ms	879.0 ± 42.5	895.8 ± 76.4	0.81
Total Variance, ms^2	4821.1 ± 2088.1	6704.2 ± 4232.2	0.83
LF, Hz	0.04 ± 0.0	0.05 ± 0.01	0.93
LF power			
ms^2	421.2 ± 36.9	575.4 ± 178.4	0.30
nu	111.5 ± 22.4	120.6 ± 28.2	0.93
%	31.5 ± 4.2	33.0 ± 4.7	0.78
HF, Hz	0.22 ± 0.04	0.16 ± 0.01	0.14
HF power			
ms^2	231.0 ± 99.4	280.0 ± 178.5	0.82
nu	44.2 ± 9.3	46.6 ± 5.3	0.55
%	15.4 ± 5.3	17.0 ± 5.7	0.97
LF/HF	2.1 ± 0.5	2.4 ± 0.8	0.76
VLF, Hz	0.01 ± 0.0	0.01 ± 0.0	1.00
VLF power			
ms2	724.8 ± 186.6	698.1 ± 152.5	0.46
nu	201.8 ± 70.8	192.6 ± 64.6	0.94
%	48.8 ± 6.9	47.1 ± 7.1	0.82
AB (n=11)			
Mean RRI, ms	942.7 ± 24.8	906.1 ± 25.5	0.76
Total Variance, ms^2	6586.3 ± 2226.2	5655.8 ± 1916.6	0.00
LF, Hz	0.04 ± 0.0	0.05 ± 0.01	0.89
LF power			
ms^2	912.5 ± 125.3	960.1 ± 170.5	0.52
nu	104.1 ± 12.0	96.6 ± 12.0	0.33
%	38.3 ± 3.5	37.2 ± 4.1	0.83
HF, Hz	0.20 ± 0.02	0.20 ± 0.02	0.71
HF power			
ms^2	509.4 ± 59.8	595.3 ± 109.6	0.49
nu	47.1 ± 5.4	48.6 ± 4.2	0.86
%	18.0 ± 2.3	19.7 ± 2.2	0.51
LF/HF	2.9 ± 0.7	2.2 ± 0.3	0.25
VLF, Hz	0.01 ± 0.0	0.01 ± 0.02	1.00
VLF power			
ms^2	1079.8 ± 891.4	1045.9 ± 129.0	0.29
nu	174.9 ± 53.5	115.9 ± 24.2	0.13
%	42.5 ± 2.4	39.4 ± 3.8	0.47

Values are ± SEM. AB, able-bodied; RRI, RR interval, LF, low frequency; HF, high frequency; VLF, very low frequency; Hz, Hertz, ms^2, milliseconds squared; nu, normalized units.

3) No significant differences were observed between days for SSRs. Intraclass correlation coefficients for SSRs resulting from median nerve stimulation and the deep breath are presented in table 2.7. The average ICCs for all measurement sites (LH, RH, LF, RF) was 0.72 ± 0.1 and 0.81 ± 0.01 for median nerve stimulation and deep breath, respectively.

Table 2.7 – Reliability of sympathetic skin responses.

	Stimulated site			
SSR to median nerve stimulation	**LH**	**RH**	**LF**	**RF**
ICC	0.84	0.81	0.5	0.73
SSR to deep breath				
ICC	0.79	0.8	0.83	0.82

SSR, sympathetic skin response; ICC, intraclass correlation coefficient; LH, left hand; RH, right hand, LF, left foot; RF, right foot.

4) Agreement between BP measures during the sit-up and tilt-table tests was examined by comparing the two tests at multiple time points, notably during supine rest and following 5, 10, and 15 minutes (corresponding to 0^o, 30^o, 45^o, and 60^o of tilt). Group mean data are shown in Table 2.8. The limits of the agreement reveal that at any time point during the tests, the SBP of the sit-up test may be at most 7 mmHg lower or 9 mmHg higher than measurements of SBP taken during tilt-table testing. The limits of agreement also reveal that at any time point during the tests, the DBP of the sit-up test may be at most 9 mmHg lower or 2 mmHg higher than measurements of DBP taken during tilt-table testing.

Table 2.8 – Agreement for systolic blood pressure and diastolic blood pressure between the sit-up and tilt-table tests.

Time	Mean difference	Limits of agreement	95% CI	95% CI lower limit	95% CI upper limit
			SBP (mmHg)		
0 mins	0.2 ± 0.04	-0.08 to 0.5	0.1 to 0.3	-0.1 to 0.02	0.4 to 0.5
5 mins	-6.2 ± 1.5	-7.3 to 2.4	-9.0 to -3.5	-8.9 to -5.7	0.8 to 4.0
10 mins	-0.8 ± 0.2	-1.8 to 0.3	-1.1 to 4.0	-2.0 to -1.6	0.1 to 0.5
15 mins	3.7 ± 0.9	-1.4 to 8.8	2.1 to 6.0	-2.4 to -0.5	7.9 to 9.8
			DBP (mmHg)		
0 mins	0.8 ± 0.2	-0.3 to 2.0	0.5 to 1.2	-0.5 to -0.1	1.8 to 2.2
5 mins	-5.7 ± 1.3	-9.3 to 2.2	-8.2 to -3.2	-10.7 to -7.8	0.8 to 3.6
10 mins	-2.6 ± 0.6	-6.2 to 1.0	-3.7 to 0.7	-6.8 to -5.5	0.3 to 1.6
15 mins	-0.5 ± 0.1	-1.1 to 0.2	-0.7 to 4.2	-1.2 to -1.0	0.1 to 0.3

Values are mean ± SEM. Time indicates 0 mins, supine; 5, 10 and 15 mins during orthostatic challenges. SBP, systolic blood pressure; DBP, diastolic blood pressure; mmHg, millimeters of mercury; CI, confidence interval; mins, minutes.

2.4 Discussion

Main findings. The purpose of these reliability and agreement studies was to justify the use of the measures used in this thesis to assess cardiovascular autonomic function. Test-retest reliability was evaluated using ICCs[116] and agreement between two methods of clinical measurement, namely the sit-up and tilt-table tests, was assessed by calculating limits of agreement[117]. For BP responses during the sit-up test, the majority of ICCs revealed at least moderate reliability for both groups. More specifically, ICCs were moderate and strong in participants with SCI and fair to strong in AB for measures of SBP and DBP. Intraclass correlation coefficients for SSRs indicate that that across all sites (palmar and plantar) and in response to both types of stimulation, responses were all at least moderately reliable, with the majority being highly reliable. Our findings that there is at least moderate reliability for cardiovascular measures of the sit-up test and SSRs reveals that these measures may be used reliably in the population with SCI, which has not been shown previously. The majority of HRV measures were found to be moderately reliable (ICC $\geq$ 0.05). Finally, the strength of agreement between two different tests often requires judgement for how far apart measurements between them may be, and this is dependent upon the measure of interest. In this study, the agreement between the sit-up and tilt-table tests was moderate. The limits of agreement provide a fairly small range of differences in BP between the two methods. Based on this and the greater feasibility and ease with which the sit-up test is performed, it is a suitable alternative to the tilt-table test.

Test-retest reliability. Measures of SBP and DBP in the supine and seated positions were at least moderately reliable for participants with SCI. In both positions, measures of SBP had strong reliability. This has not been investigated previously in persons with SCI and these findings demonstrate that the sit-up test is reliable for use in this population. Intraclass correlation coefficients revealed that SSRs are moderately to highly reliable in response to median nerve stimulation and a deep breath. This has not been shown previously for SSRs in individuals with SCI. It is reasonable to suggest that these tests may be used to reliably to examine cardiovascular and sudomotor autonomic function in individuals with SCI. Despite our findings that some HRV

measures had poor reliability, the majority had moderate reliability. Our confidence in using HRV is supported by previous investigations showing good reliability[99, 100].

Agreement. The average mean difference across all the time points compared between the sit-up and tilt tests is small for SBP (-0.8 $\pm$ 1.9 mmHg) and DBP (-2.0 $\pm$ 0.6 mmHg) with average 95% CIs -2.0 to 1.7 mmHg for SBP, and -3.0 to 0.7 mmHg for DBP. The 95% CIs for SBP and DBP for the lower and upper limits are reasonably narrow suggesting that the variation of the differences between these methods is limited. Furthermore, the limits of agreement define the differences between the two tests for SBP as the sit-up test being no more than 7 mmHg lower or 9 mmHg higher than the tilt-table test, and for DBP the sit-up test being no more than 9 mmHg lower or 2 mmHg higher than the tilt-table test. Based on these findings, it is reasonable to believe the sit-up test may be used as a bedside test in individuals with SCI and that it may appropriately identify individuals with orthostatic intolerance. It may also be considered an appropriate alternative to the tilt-table test since it is a bedside test that is easily applied in the clinic and represents an ideal test to use in the population with SCI since it corresponds well with sitting in a wheelchair. The comparability of the sit-up to tilt-table test has not been examined previously and shows that the more clinically feasible sit-up test is an appropriate alternative to the gold standard tilt table test.

Methodological considerations. The majority of HRV measures were found to be at least moderately reliable. However, there are several factors that could have affected our analysis that should be acknowledged. First, it is known that the length of the electrocardiogram recording can impact analysis. The duration of recording has to be sufficiently long and stationary to allow for good frequency resolution. The duration of the recording should be at least twice the wavelength of the lowest frequency recording for frequency domain measurements. As such, the minimum duration for the HF (0.15 Hz) component is 13.3 seconds and for LF (0.04 Hz) component 50 seconds[118]. Since we obtained electrocardiogram recordings of 10 minutes, the length of our recording is appropriate to use with HRV analysis. Second, with the use of autoregressive modeling, it is important to ensure an appropriate model order is selected for analysis. A model order that is too low will contain too much noise and have poor resolution, and a model order that is too high will smooth the signals too much[119]. Our choice of model order was based on

what is commonly used in the literature[120, 121] and visual observation to ensure appropriate resolution was obtained. Third, the selection of autoregressive modeling over Fast Fourier Transform was appropriate since the latter is not able to identify the central frequency of a given frequency component[121]. Finally, power spectral analysis was chosen over time domain analysis because the former allows the quantification of variance or power at specific frequencies[121] whereas there is a lack of discrimination between the different branches of the ANS with the latter[118].

It is important to recognize that although our cohorts are fairly homogenous, some participants may be considered outliers with regards to their level of lesion as well as their time post-injury. Those with lesions below T5 are likely to have greater integrity of spinal autonomic pathways in comparison to those with higher lesion levels since sympathetic control of the heart remains intact in the former but not in the latter[39]. Furthermore, the longer the time post-injury, the more improvement to cardiovascular autonomic function has likely taken place. However, since the studies in this chapter examined reliability and agreement of measures, these participants who appear as outliers did not have to be treated as such since the magnitude of response wass not of interest, but rather the differences in response to the tests within each participant (e.g., over consecutive testing days for reliability and between the two orthostatic challenges). Differences between participants were not the main focus of these reliability and agreement studies.

For the agreement study, the two orthostatic challenges were performed on the same day and consecutively which may cause the first test to affect the cardiovascular responses to the second test. To help reduce the possible effects on our measures, we ensured that BP returned to baseline values prior to starting the second orthostatic challenge.

For the sit-up test, lesion level may affect postural control making it difficult to passively transition from the supine to seated position during the sit-up test. Individuals with higher lesion levels that have reduced postural stability may require manual support to remain relaxed during

the change in posture. Some participants were able to maintain proper posture independently and those who required help were provided manual assistance. Support was provided without jeopardizing measures of BP by passively adjusting position or manually holding the participant in place with as little movement and disturbance as possible. However, in comparison to the tilt-table test, the sit-up test requires minimal strapping and this may help to reduce reflexive increases in sympathetic tone, and subsequently, changes in BP unrelated to the orthostatic challenge.

Conclusion. Autonomic testing in individuals with SCI is very important to determine the presence and extent of autonomic dysfunction so that it may be properly managed. This is because autonomic dysfunction following SCI as related to impaired cardiovascular control can negatively impact daily living. Using appropriate tools to assess cardiovascular function is of paramount importance to help identify conditions like OH and life-threatening AD. The majority of HRV measures were found to be reliable. We are confident in the reliability of HRV measures based on our findings along with previous work[99, 100]. The measures of BP during the sit-up test and SSRs were found to be moderately to highly reliable in individuals with SCI. The sit-up test was also shown to be an appropriate alternative to the tilt-table test. This is beneficial since the sit-up test is a simple bedside assessment, more comfortable for individuals since it requires less strapping, and represents the orthostatic challenge of sitting in a wheelchair that is faced daily by individuals with SCI. These data support the use of these tests to assess cardiovascular autonomic function in the population with SCI.

Chapter 3: Determining the Integrity of Spinal Autonomic Pathways and the Changes in Cardiovascular Autonomic Function Over Time in the Sub-acute Phase of Spinal Cord Injury

3.1 Introduction

The initial period of time following injury is characterized by a marked decrease of sensory, motor, and reflex function below the level of SCI, known as spinal shock[122]. It is characterized by a decrease or complete loss of somatic and/or reflex functions of the spinal cord caudal to the injury and can last anywhere from several days to six weeks[122]. Neurologic function after SCI depends on the lesion level and completeness as assessed via detailed examination of the various dermatomes (sensory) and myotomes (motor) according to the evaluation of individuals with SCI as outlined by the American Spinal Injury Association and ISNCSCI[1]. Spinal shock is often accompanied by neurogenic shock which manifests as severe cardiovascular dysfunctions whereby individuals present with severe hypotension and bradycardia[67]. This is a disorder of the ANS that is commonly observed among individuals with high thoracic and cervical SCI and may last from days to weeks, often requiring careful monitoring of cardiovascular parameters[123-125]. The heart receives dual innervations from the parasympathetic (vagal nerve) and sympathetic (upper thoracic segments (T1 to T5) of the spinal cord) components of the ANS. Cardiovascular dysfunction varies dramatically with the level of SCI[9, 55] with a very strong correlation between the level and severity of autonomic dysfunction, similar to neurologic dysfunction. High-level SCI alters the ability of the medullary vasomotor centre to maintain sympathetically-mediated efferent control of vasomotor tone and HR[56]. As such, the higher the level of SCI, the greater the clinical manifestations of sympathetic dysfunction[55-57] and loss of spinal autonomic integrity. The recent addition of the International Standards to document remaining Autonomic Function after Spinal Cord Injury (ISAFSCI)[83] to the ISNCSCI[1] is used to document changes in autonomic control of various systems[2]. Though it is clear the effect of lesion level on autonomic impairment, the relationship between neurologic and autonomic severity, or completeness, of injury is not known[1, 4, 5].

Both divisions of the ANS have two neurons that transmit signals between the central nervous system and the target. The first neuron is called the preganglionic neuron and its cell body is within the gray matter of the brain or spinal cord. Its axon, called a preganglionic fibre, travels to the periphery within the ventral root of the spinal cord or within certain cranial nerves. The second neuron is called the postganglionic neuron. These lie outside the central nervous system and are located within autonomic ganglia. The postganglionic axons relay impulses to the effector organ. Sympathetic preganglionic neurons send efferent tonic signals from the central nervous system to different target organs such as blood vessels and the heart. Sympathetic postganglionic neurons from the upper thoracic segments of the spinal cord from T1 to T5 provide sympathetic innervation to the heart and the majority of the vasculature in the upper extremities[39, 40]. The sympathetic postganglionic neurons contained in the more caudal segments from T5 to L2 innervate the major vasculature in the splanchnic region and lower extremities[39, 40].

Abnormal BP control is a common consequence following SCI. Loss of sympathetic control of the splanchnic vasculature may lead to unstable BP. Previous studies have demonstrated that severe hypotension can be problematic beginning during the acute phase following injury[123] and interfere with and delay rehabilitation[19, 126]. Specifically, OH is characterized by a decrease in SBP of $\geq$ 20 mmHg or DBP of $\geq$ 10 mmHg when upright, in the presence or absence of symptoms (e.g., lightheadedness, dizziness, fatigue, etc.) within three minutes of a change in posture[85]. In the upright posture in individuals with SCI who have lost descending supraspinal control of the splanchnic vasculature, pooling occurs in these dependent vessels[127].The upright posture translocates a large fraction of the thoracic blood volume into the compliant veins of the lower body, reducing venous return. Individuals with SCI lose the ability to utilize the skeletal muscle pump whereby contractions of the leg and gluteal muscles usually propel venous blood back to the heart[128, 129]. Following injury, impaired sympathetic activity and muscle paralysis below the level of the lesion lead to an absence of sympathetic-mediated vasoconstriction and skeletal-muscle pump action, respectively[10], leading to venous pooling and orthostatic intolerance. It has been demonstrated previously that the skeletal muscle pump has an important role in ameliorating orthostatic intolerance[130], which is obviously compromised following SCI.

Fortunately, OH has been found to improve over time[127] following the acute phase of injury. Mechanisms for this improvement are unclear, but some may include vascular wall receptor hypersensitivity, increased skeletal muscle tone, recovery of postural reflexes at a spinal level, and adaptation of the renin-angiotensin system[9]. Specifically, in autonomically intact individuals, there is normally a low level of sympathetic activity in the renal nerves and thus, minimal adrenergically mediated vasoconstriction.

Another condition that results from cardiovascular dysfunction is AD. In contrast, it is characterized by extreme hypertension and a dysreflexic episode is considered to occur when there is an increase in SBP from baseline of greater than 20 to 30 mmHg[9], with SBP reaching up to 300 mmHg. Autonomic dysreflexia may be provoked by a wide range of noxious and non-noxious stimuli which include bowel and bladder distension, spasms and pressure sores[9]. Following stimulation by noxious or non-noxious stimuli below the level of injury, there is a loss of the normal ability to increase the rate of inhibitory potentials to modify outflow via the descending spinal tracts to the intermediolateral cell column so that activity to the SPNs is decreased[127]. An episode of AD may be accompanied by a pounding headache, slow HR, and upper body flushing[11]. Although AD occurs more commonly in the chronic stage of SCI in individuals with injuries at T5 or above, episodes of AD have been documented within the first few days and weeks following injury[131, 132]. There is a greater loss of descending inhibition of sympathoexcitatory signals in individuals with higher levels of SCI which may lead to acute episodes of extreme hypertension. With injuries at or above T5, loss of descending tonic inhibition is crucial to the development of AD since there is a marked reduction in supraspinal control to the splanchnic bed and vasculature of the lower extremities. With SCI below T5, there is generally sufficient sympathetically innervated vasculature under sympathetic control, especially in the important splanchnic blood vessels to limit significant cardiovascular autonomic dysfunction. Individuals with injuries below T5 are more likely to have resting BP within the normal range[11, 55] and experience episodes of AD less frequently[58]. A relationship between neurologic severity of injury and a greater predisposition to AD has been found in individuals with complete cervical SCI[58]. However, it is unclear if this relationship may be generalized across all cardiovascular impairments. In addition to complications with BP control, bradycardia

has been commonly reported in over two-thirds of individuals with cervical SCI, with the most severe episodes occurring within the first five weeks[123-125]. Efferent cardiac parasympathetic nerve pathways remain intact but sympathetic activity is more greatly disrupted in individuals with cervical SCI which makes them more predisposed to unopposed vagal overactivity[55, 56]. Unfortunately, unopposed vagal overactivity has also been documented to result in cardiac arrest[55, 56] which, along with more severe bradycardia[133], is more common in individuals with cervical SCI and more neurologically severe injuries[123]. Lehmann et al.[123] has previously found that HR in individuals with cervical SCI in the acute phase of injury returned to normal two to six weeks after injury. Individuals with lower lesions (mid-thoracic and lower) have upper thoracic cardiac sympathetic neurons and the vagus nerve intact, and this combination provides more balanced cardiac control which makes bradycardia less common in these individuals. Furthermore, the extent to which prolonged and severe hypotension requires vasopressive therapy to help manage bradycardia has been demonstrated to be well correlated with outcomes of recovery after injury[11]. Cardiovascular dysfunctions such as persistent orthostatic intolerance as well as transient episodes of extreme hypertension (AD) [22, 40] often become lifelong issues that individuals with SCI must deal with. It is important to understand the time course of changes in cardiovascular function initially following injury to ensure proper management and to maximize recovery outcomes.

The majority of investigations in individuals with SCI have focused on motor outcomes[84] and only recently have studies begun to focus on the relation between neurologic and autonomic severity of injury[5, 134]. Curt et al.[134] acknowledged that the ANS and somatic nervous system are organized differently within the spinal cord and proceeded to investigate changes in sympathetic nervous system activity following injury via SSRs. They found that neurologically complete injuries were related to preservation of SSRs, but this was only the case for 50% of individuals with neurologically incomplete SCI. Work from our lab also pioneered the examination of autonomic completeness of injury[5] showing more clearly that neurologic severity of injury as assessed by the AIS is not correlated with autonomic completeness as assessed by SSRs. The relationship between motor and sensory with autonomic dysfunction is still unclear but warrants

the inclusion of autonomic assessment into clinical practice to identify changes in ANS following SCI.

There are several mechanisms that are thought to contribute to the pathophysiology of abnormal cardiovascular autonomic control in individuals with SCI. Understanding these mechanisms provides insight into how the integrity of spinal autonomic pathways may be impacted following injury[5]. As such, three elements of autonomic circuits that potentially contribute to cardiovascular dysfunction include: 1) the disruption of the descending cardiovascular (or vasomotor) pathways; 2) morphological changes in the cardiac and vasomotor SPNs; and 3) aberrant afferent sprouting and potential formation of inappropriate synapses with spinal interneurons. First, during the acute phase of injury, disruption of the descending cardiovascular (or vasomotor) pathways has been proven with the observation of significantly fewer preserved axons caudal to the site of injury in comparison to individuals without signs and symptoms of abnormal cardiovascular control[135]. This disruption contributes to impaired cardiovascular control in individuals with mid-thoracic or higher level injuries and manifests as severe hypotension, bradycardia, and AD during the acute stage of injury. Second, morphological changes in spinal SPNs has been previously documented in rats[42] and associated with conditions resulting from abnormal cardiovascular function such as OH[64] and AD[13, 22, 32, 55]. Finally, the aberrant afferent sprouting and potential formation of inappropriate synapses with spinal interneurons have been proposed to help mediate the sympathetic hyper-responsiveness associated with existing AD[62]. In this study the assessment of the integrity of spinal autonomic pathways was performed initially during the sub-acute stage following injury when individuals were admitted to a rehabilitation centre and again one month later. We hypothesized that: 1) lesion level and neurologic completeness of injury affect the integrity of spinal autonomic pathways. This could ultimately be responsible for abnormal cardiovascular parameters at rest and during an orthostatic challenge in individuals with SCI; and 2) individuals with at least partial preservation of spinal autonomic integrity would demonstrate greater cardiovascular function.

3.2 Materials and methods

Participant characteristics. Twenty-two participants with SCI (C2 to T11; 13 with cervical and 9 with thoracic SCI; AIS A to D; 9 with AIS A and 13 with AIS B to D) (43 $\pm$ 4 years; 6 females) of normal height (177 $\pm$ 2 cm) and weight (75 $\pm$ 3 kg) in the sub-acute stage of injury (8 $\pm$ 1 wks) participated in the study (Table 3.1). Sympathetic skin responses were only collected from nine participants. Participants were recruited from an in-patient unit located at the G.F. Strong Rehabilitation Centre in Vancouver, British Columbia. Participants must have sustained a traumatic SCI. The neurological exam was performed by a qualified physician. Participants were only included if they had been injured less than six months and were free of any coincident cardiac or pulmonary diseases or active medical issue such as hypertension, decubitus ulcers or urinary tract infections. Prior to the initiation of the study, written informed consent was obtained. All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia which conforms to the *Declaration of Helsinki*.

Experimental protocol. Participants completed two identical days of testing that were one month apart. Assessments were performed in the morning. Participants withheld their medication and breakfast until testing was completed. Upon arrival to the laboratory, they were asked to empty their bladders to minimize the influence of reflex sympathetic activation on peripheral vascular tone. They were transferred (sliding board or sling) from their hospital bed to the chair used for the sit-up test[5]. There was an additional period of 5 to 10 minutes of supine rest when we instrumented research participants with electrocardiogram electrodes, and arm and finger blood pressure cuffs prior to the initiation of data collection. Following instrumentation, the experimental session began with a minimum of 10 minutes in the supine position during which time resting data were recorded. Following the 10 minutes of resting measures, participants performed a VM. Participants were then passively moved to the seated position for a minimum of 10 minutes whereby the head of the chair was raised by 90^{o} and the base of the chair dropped by 90^{o} at the knee. They were questioned for the presence of symptoms of hypotension (i.e., dizziness, fatigue, blurred vision, syncope, lightheadedness). Following the sit-up test, they were

returned to the supine position to undergo SSR testing. The following is a flow chart outlining the time course of the first and second assessment.

Day 1 and 1 month-post assessments included: sit-up test, heart rate variability, sympathetic skin responses, Valsalva manoeuvre

Figure 3.1 – Timeline of initial and second assessment.

Physiological measures. During the sit-up test, a single-lead electrocardiogram was continuously recorded to determine HR. Beat-by-beat BP was monitored at the finger with photoplethysmography (Finometer, Finapres Medical System; Arnhem, The Netherlands). Automated BP was also measured at the brachial artery (Dinamap, GE Pro 300V2; Tampa, FL). A pulse oximeter was used to measure SpO_2 (Dinamap, GE Pro 300V2; Tampa, FL).

Heart rate variability. Spectral analysis of cardiovascular parameters was used to evaluate autonomic tone from HR based on the observation that basal RRIs (the time between two successive R waves of an electrocardiogram) continually fluctuate. To investigate the responses to the sit-up test, time series of successive beats were extracted from the electrocardiogram recordings for RRIs. Power spectral analysis was performed using an autoregressive model fitted to each time series (aHRV, Nevrokard, Slovenia)[114]. Occasional ectopic beats were "corrected" by the linear interpolation of adjacent normal beats. Oscillations from 0.04 to 0.15 Hz were designated as LF, and oscillations from 0.15 to 0.4 Hz were designated as HF. Powers were normalized by dividing the power by the total variance minus VLF (<0.03 Hz) and multiplied by 100[115].

Valsalva manoeuvre. Following 10 minutes in the supine position, participants performed a VM and measures were continuously recorded. Participants were asked to exhale forcefully against a resistance (closed glottis/nose and mouth). A response to the VM is characterized by four phases depicted by the beat-by-beat BP recordings. Phase I: an increase in intrathoracic pressure leads to a transient decrease in BP. Subsequently, the baroreceptors are activated resulting in a decrease in HR (bradycardia), leading to a reduction in venous return and stroke volume. Phase II: as a result of the decrease in venous return and stroke volume, there is a drop in BP with a concomitant increase in HR (tachycardia). Phase III: upon completion of the expiration, BP continues to decrease transiently while HR increases. Phase IV: likely as a result of baroreceptor activation, there is a rise in BP above the initial values with simultaneous bradycardia. To quantify the response, the Valsalva ratio was derived from the maximum HR divided by the minimum HR and used as measure of (cardiovagal) baroreflex sensitivity[86]. A value of less than 1.21 is considered abnormal[86]. Adrenergic baroreflex sensitivity was also calculated by dividing the decline in SBP during Phase III by pressure recovery time[79] which is the time, in seconds, that it took for SBP to return to baseline from its lowest value during Phase III. There were two participants who were unable to perform the VM due to open tracheotomies or other oral conditions.

Sympathetic skin response. The integrity of sympathetic sudomotor function was assessed via SSRs[110, 111] which record a change in potential from the surface of the skin generated by sweat in response to a stimulus[113]. Sympathetic skin responses allow for examination of the common efferent pathways of the sympathetic nervous system from the spinal cord to the sweat glands of the hands (palmar) and the feet (plantar) relayed by preganglionic and postganglionic sympathetic nerve fibres[134]. Self-adhesive recording electrodes were placed on sites with maximum eccrine sweat gland density (palms of the hands and soles of the feet; left hand, LH; right hand, RH; left foot, LF; right foot, RF). Sympathetic skin responses were recorded simultaneously at all sites following a single electrical pulse (duration 0.2 ms; intensity 8-10 mA) applied to the median nerve. Five consecutive stimuli were applied to the median nerve at the wrist and for a second stimulus participants were instructed to take five consecutive deep breaths. Data were recorded using an analog-to-digital converter (Keypoint, Alpine Biomed,

California, USA). To minimize habituation, stimuli were applied in random order and with variable time delays (minimum delay of 90 s). Sympathetic skin responses were deemed present when there was a clear positive deflection from baseline. Any potential that coincided with muscle spasm, limb movement, or cough was excluded from the analysis. Responses were qualified by the number of reproducible SSRs elicited[113]. A response indicated a preserved spinal autonomic pathway.

Data and statistical analyses. All data were acquired using an analog to digital converter (Powerlab/16SP model ML795; ADInstruments, Colorado Springs, CO) interfaced with a computer and sampled at 1 kHz. Data were stored on a personal computer for subsequent offline analysis (Powerlab version 7.2, ADInstruments). The sit-up test was examined based on cardiovascular responses measured in the supine and seated positions. Differences between these positions and between participants within a given position were determined with two-way analysis of variance (ANOVA), and differences over time from the first to second assessment were determined with repeated measures ANOVA. In the case of a significant *F* ratio, differences were further investigated with Tukey's post-hoc analysis. There were differences when comparisons were made amongst participants with neurologically complete and incomplete injuries, and when comparisons were made amongst participants with cervical and thoracic injuries, therefore the findings of both of these comparisons are presented since the means of either combination were deemed not appropriate. For SSRs, the maximum response at each site was five, in which case all five stimuli elicited a response. Correlations between variables were determined using Pearson's correlation coefficients. The level of significance was set at $P < 0.05$ for all statistical calculations. Group data are presented as means $\pm$ SEM.

3.3 Results

Participant characteristics.

Table 3.1 – Characteristics of participants with sub-acute spinal cord injury undergoing rehabilitation.

Participant	SCI Level	AIS	Time Since SCI (wks)	Age (yrs)	Height (cm)	Weight (kg)
1	C2	D	9	71	180	72.7
2	C4	C	5	23	177	75.0
3	C4	A	4	39	177	86.0
4	C4	C	7	60	173	78.7
5	C4	B	11	29	173	59.1
6	C5	C	5	35	177	84.1
7	C5	C	1	53	177	80.0
8	C5	B	10	60	180	68.2
9	C5	A	11	45	179	70.0
10	C5	A	24	22	163	44.8
11	C5	A	10	37	163	50.0
12	C6	D	11	24	177	71.0
13	C6	D	5	61	175	74.0
14	T3	A	4	21	163	56.0
15	T4	C	7	59	175	72.7
16	T4	A	7	79	170	81.8
17	T6	A	9	44	179	77.3
18	T7	A	14	23	195	102.3
19	T9	A	4	54	177	86.4
20	T9	C	6	19	198	72.7
21	T10	B	14	48	180	79.5
22	T11	D	3	33	173	80.0
Mean $\pm$ SD	N/A	N/A	8 $\pm$ 1	43 $\pm$ 4	177 $\pm$ 2	75 $\pm$ 3
Minimum	N/A	N/A	1	19	163	44.8
Maximum	N/A	N/A	24	79	198	102.3

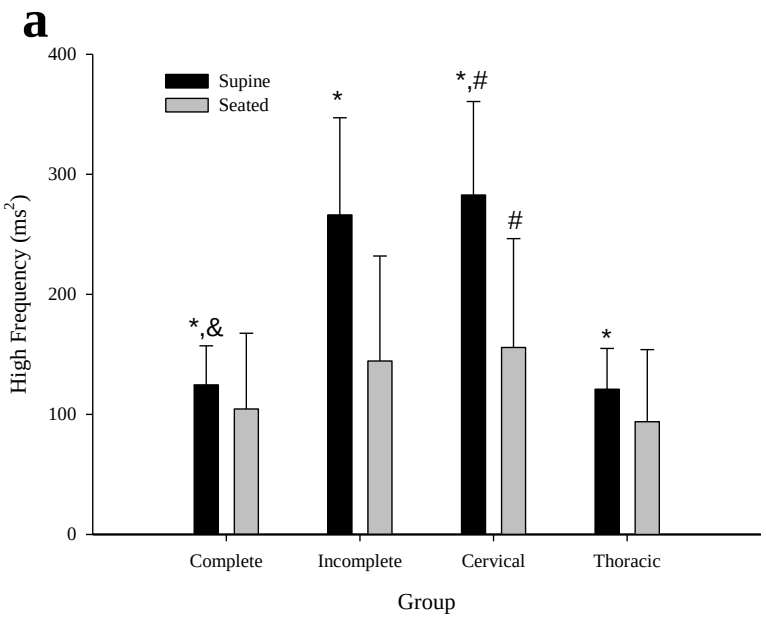
SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; SD, standard deviation.

Effect of lesion level on cardiovascular and autonomic parameters. *Heart rate variability.* There was a main effect of lesion level on HRV. Group mean data for HRV are presented in Table 3.2. Participants with cervical SCI had higher HF power than participants with thoracic SCI in the supine and seated positions (Figure 3.2 a). Participants with thoracic SCI had higher LF power than participants with cervical SCI in the supine and seated positions (Figure 3.2 b). A sample tracing of frequency spectra are shown in Figures 3.3 a and b.

Table 3.2 – Measures of heart rate variability during the first assessment in patients.

	Complete	Incomplete	Cervical	Thoracic
		Supine		
Mean RRI, ms	725.8 ± 51.8	871.0 ± 50.0	871.0 ± 53.8	725.7 ± 42.9
Total variance, ms^2	1594.0 ± 575.1	1010.8 ± 204.8	1234.2 ± 263.6	1271.2 ± 544.1
LF, Hz	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.0
LF power				
ms^2	205.9 ± 53.5^{bc}	268.1 ± 122.7^{c}	202.9 ± 115.7^{ac}	251.0 ± 60.6^{c}
nu	123.0 ± 23.3^{c}	137.8 ± 20.4^{c}	125.0 ± 17.9^{c}	141.7 ± 27.0^{c}
%	20.8 ± 3.7	27.1 ± 2.8	21.0 ± 3.2	26.9 ± 2.8
HF, Hz	0.22 ± 0.03	0.26 ± 0.02	0.29 ± 0.02	0.24 ± 0.03
HF power				
ms^2	124.6 ± 32.5^{c}	266.2 ± 80.9^{bc}	282.7 ± 77.9^{ac}	101.1 ± 34.0^{c}
nu	47.6 ± 5.0	53.0 ± 3.6	51.7 ± 3.6	49.4 ± 5.1
%	21.2 ± 5.1	26.2 ± 3.9	24.5 ± 3.8	21.4 ± 5.3
LF-to-HF ratio	3.1 ± 0.4	3.7 ± 0.8	2.9 ± 0.5^{a}	4.3 ± 1.0
VLF, Hz	0.01 ± 0.003	0.01 ± 0.0	0.01 ± 0.002	0.01 ± 0.0
VLF power				
ms^2	199.1 ± 83.2	489.1 ± 83.2	503.8 ± 149.0	177.8 ± 38.7
nu	110.2 ± 34.1	236.9 ± 73.6	149.7 ± 51.9	236.1 ± 88.8
%	38.0 ± 7.8	46.2 ± 5.8	40.5 ± 5.6	46.2 ± 8.3
		Seated		
Mean RRI, ms	708.3 ± 45.9	771.5 ± 40.9	804.9 ± 34.4	670.9 ± 45.8
Total variance, ms^2	1351.1 ± 407.0	1391.8 ± 329.5	1637.5 ± 349.9	1028.0 ± 332.6
LF, Hz	0.04 ± 0.0	0.04 ± 0.0	0.04 ± 0.0	0.04 ± 0.0
LF power				
ms^2	113.0 ± 32.7	193.7 ± 63.0	120.8 ± 33.2	194.6 ± 65.4
nu	44.5 ± 7.3	93.4 ± 13.7	71.5 ± 12.1	76.2 ± 17.9
%	21.0 ± 3.1	23.2 ± 3.4	21.5 ± 2.5	23.5 ± 4.5
HF, Hz	0.24 ± 0.04	0.24 ± 0.02	0.26 ± 0.02	0.22 ± 0.03
HF power				
ms^2	104.5 ± 63.1	144.5 ± 87.5	155.8 ± 90.6^{ac}	93.9 ± 60.0
nu	40.6 ± 4.6	45.9 ± 3.6	48.0± 2.7	38.4 ± 5.3
%	11.1 ± 4.3	11.8 ± 3.3	12.6 ± 3.3^{a}	10.1 ± 4.1
LF-to-HF ratio	3.8 ± 1.1	4.1 ± 0.5	3.5 ± 0.8	6.1 ± 0.7
VLF, Hz	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0
VLF power				
ms^2	288.1 ± 76.7	346.8 ± 75.8	326.9 ± 84	273.2 ± 57.0
nu	371.1 ± 81.6	438.3 ± 87.2	414.3 ± 78.0	410.6 ± 102.6
%	58.5 ± 7.0	59.6 ± 5.9	59.4 ± 6.1	59.0 ± 6.8

Values are mean ± SEM. RRI, RR interval; LF, low frequency; HF, high frequency; VLF, very low frequency; Hz, Hertz, ms^2, milliseconds squared; nu, normalized units.. $^{a}P < 0.05$ cervical vs. thoracic; $^{b}P < 0.05$ complete vs. incomplete; $^{c}P < 0.05$ supine vs. seated.



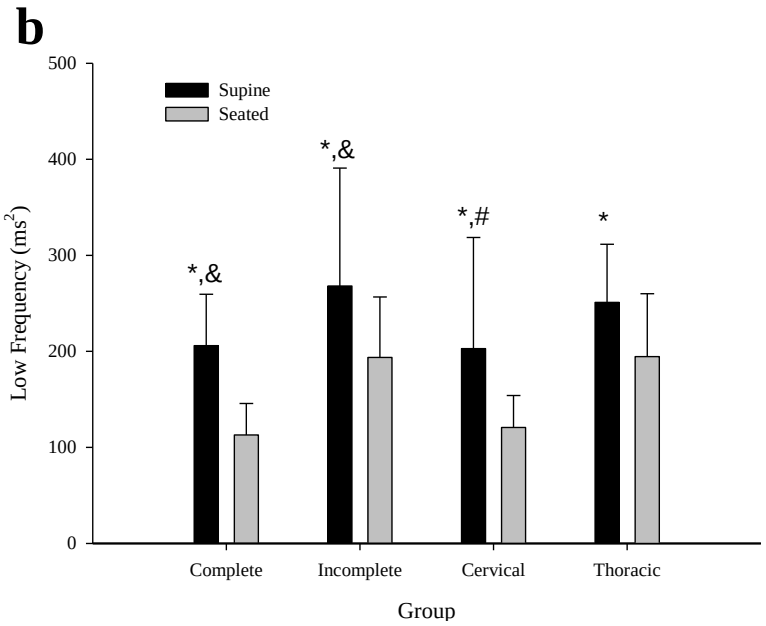


Figure 3.2 a – High frequency heart rate variability changes during the sit-up test for the first assessment in patients. *P < 0.05 supine vs. sit-up; $^{\#}$P < 0.05 vs. thoracic; $^{\&}$P < 0.05 vs. incomplete.

Figure 3.2 b – Low frequency heart rate variability changes during the sit-up test for the first assessment in patients. *P < 0.05 supine vs. sit-up; $^{\#}$P < 0.05 vs. thoracic; $^{\&}$P < 0.05 vs. incomplete.

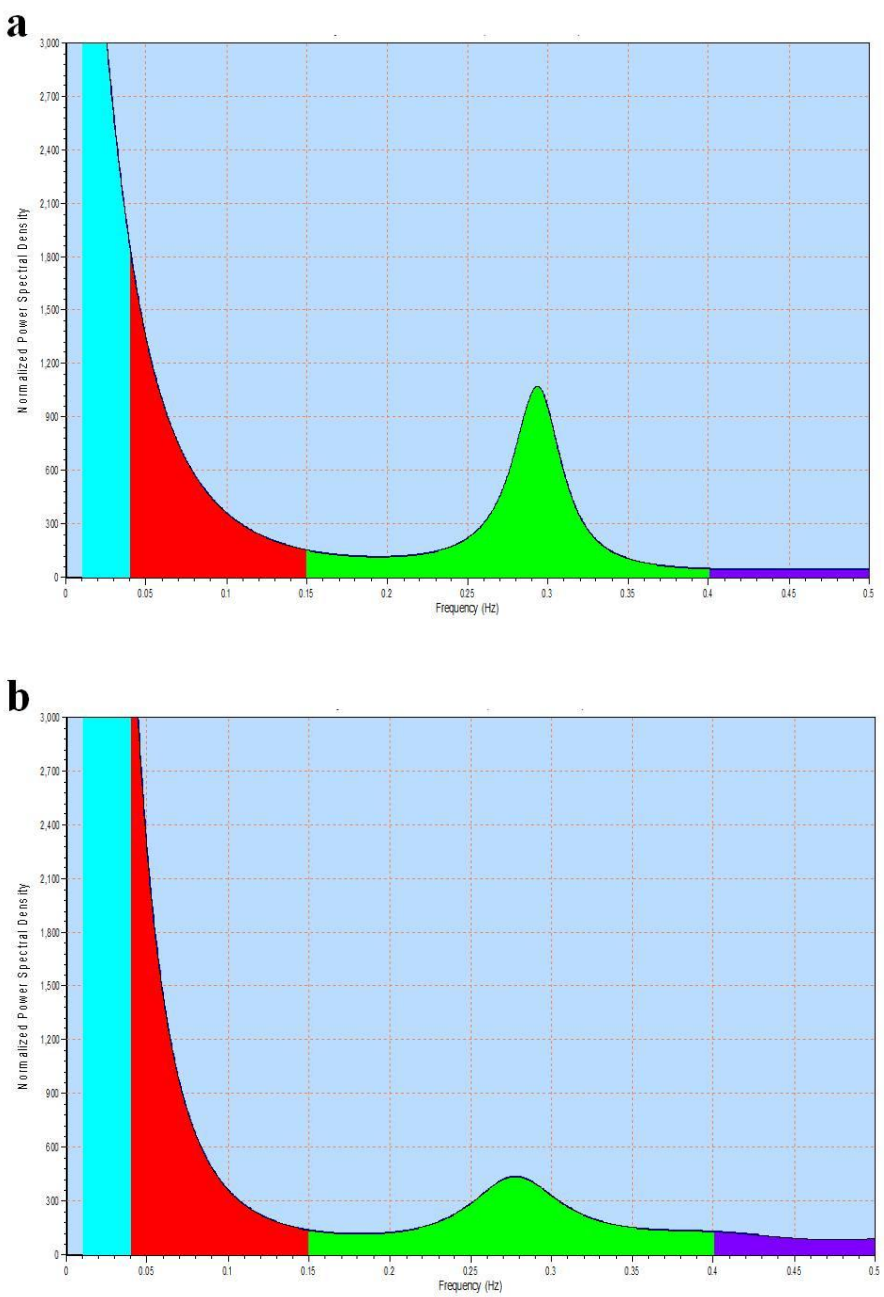


Figure 3.3 a – Representative frequency spectra in the supine position for the first assessment in a patient (C4 American Spinal Injury Association Impairment Scale B).

Figure 3.3 b – Representative frequency spectra in the seated position for the first assessment in a patient (C4 American Spinal Injury Association Impairment Scale B).

Cardiovascular responses to sit-up test. Overall, participants with thoracic SCI proved to have greater cardiovascular function than participants with cervical injury. Participants with cervical SCI experienced greater decreases in BP in the seated position than participants with thoracic injury (Table 3.6). Heart rate was also significantly higher in those with thoracic than cervical SCI.

Sympathetic skin responses. Participants with better cardiovascular responses also had better preservation of SSRs. Sympathetic skin responses revealed that with median nerve stimulation and a deep breath, 25% of participants with cervical SCI had partial preservation, and 75% had no preservation, and 60% of participants with thoracic SCI had partial preservation and 40% had no preservation. Individual participant data are presented in Table 3.3 a for SSRs, and Table 3.3 b presents the corresponding patients' experience of OH and symptoms.

Table 3.3 a – Qualification of sympathetic skin responses for the first and second assessment in patients.

Participant	SCI	AIS	First Assessment								Second Assessment (1-month post)							
			SSR to median nerve stimulation				SSR to Valsalva				SSR to median nerve stimulation				SSR to Valsalva			
			LH	*RH*	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*	*LH*	*RH*	*LF*	*RF*
1	C4	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	C4	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	C5	B	1	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0
4	C5	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	T3	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	T4	C	5	5	0	0	5	5	1	1	0	1	0	0	2	3	0	0
7	T4	A	5	5	1	0	5	5	0	0	4	4	0	0	3	4	0	0
8	T7	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	T10	B	5	5	3	3	5	5	3	2	5	5	5	5	5	5	5	5

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; SSR, sympathetic skin response; LH, left hand; RH, right hand; LF, left foot; RF, right foot.

Table 3.3 b – Presence or absence of orthostatic hypotension and associated symptoms for the first and second assessment in patients with spinal cord injury who also had sympathetic skin responses assessed.

Participant	SCI	AIS	Orthostatic hypotension 1	Orthostatic hypotension 2	Symptoms 1	Symptoms 2
1	C4	C	Y	N	N	N
2	C4	B	N	N	N	N
3	C5	B	Y	Y	Y	Y
4	C5	A	Y	N	Y	Y
5	T3	A	Y	N	Y	N
6	T4	C	N	Y	N	N
7	T4	A	N	N	N	N
8	T7	A	Y	N	Y	N
9	T10	B	N	N	N	N

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; Y, yes; N, no; 1 and 2 refer to the first and second assessments, respectively.

Baroreflex function. Group mean data for responses to the VM and calculations for indices of baroreflex function are presented in Table 3.4. There were no differences in Valsalva ratio regardless of lesion level and participants had values above the requirement to be considered normal. There were no differences between participants for changes in BP (SBP overshoot, lowest SBP, greatest SBP drop) during the VM. Adrenergic baroreflex sensitivity was significantly greater in participants with thoracic than cervical SCI during both the first and second assessment. An example tracing of a participant with a typical VM response is shown in Figure 3.4 a, and an example tracing of a participant with an altered VM response is shown in Figure 3.4 b.

Table 3.4 – Responses to Valsalva manoeuvre and indices of baroreflex function for the first assessment in patients.

	Complete	Incomplete	Cervical	Thoracic	Complete	Incomplete	Cervical	Thoracic
	Test 1				**Test 2**			
Valsalva Ratio	1.50 ± 0.07	1.38 ± 0.11	1.36 ± 0.09[c]	1.43 ± 0.10	1.48 ± 0.03[b]	1.42 ± 0.10	1.44 ± 0.05	1.53 ± 0.10
SBP overshoot	23.3 ± 11.7	12.4 ± 2.1	12.1 ± 6.0	19.8 ± 9.3	12.7 ± 3.6	13.5 ± 5.1	17.7 ± 3.3	10.1 ± 4.7
Lowest SBP	72.5 ± 9.9	67.6 ± 5.9	60.2 ± 30.1	75.8 ± 6.0	72.8 ± 4.0	70.0 ± 6.6	74.9 ± 3.2	68.6 ± 6.6
Greatest SBP drop	43.5 ± 1.8	44.2 ± 7.5	44.1 ± 8.4	43.9 ± 5.4	43.9 ± 7.1	39.4 ± 6.4	33.1 ± 5.8	46.6 ± 5.9
BRSa	3.3 ± 1.3[c]	3.3 ± 1.1[c]	1.43 ± 0.3[ac]	4.5 ± 1.0[c]	4.1 ± 1.3[b]	5.2 ± 2.6	2.3± 2.1[a]	3.3 ± 0.6

Values are mean ± SEM. SBP, systolic blood pressure; BRSa, adrenergic baroreflex sensitivity (calculated from SBP values). [a]P < 0.05 vs. thoracic; [b]P < 0.05 vs. incomplete; [c]P < 0.05 vs. Test 2.

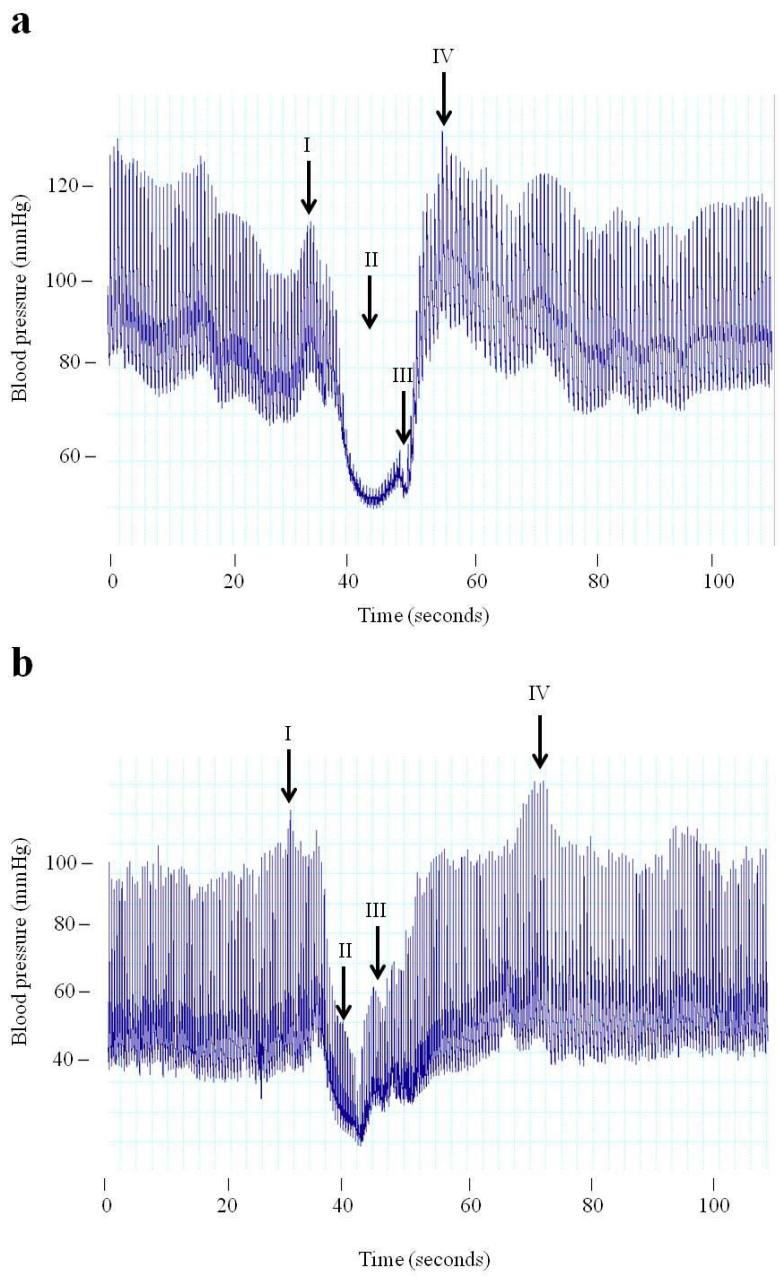


Figure 3.4 a – Example trace of normal blood pressure response to the Valsalva manoeuvre during the first assessment in a patient (T10 American Spinal Injury Association Impairment Scale B). I, II, III, and IV indicate phases of the Valsalva.

Figure 3.4 b – Example of trace of altered blood pressure response to the Valsalva manoeuvre during the first assessment in a patient (C4 American Spinal Injury Association Impairment Scale A). I, II, III, and IV indicate phases of the Valsalva.

Orthostatic hypotension. Orthostatic hypotension and its associated symptoms were experienced by participants with cervical (n=13; 8 experienced orthostatic hypotension, 6 experienced symptoms) and thoracic (n=9; 2 experienced orthostatic hypotension, 3 experienced symptoms) SCI (Figure 3.5).

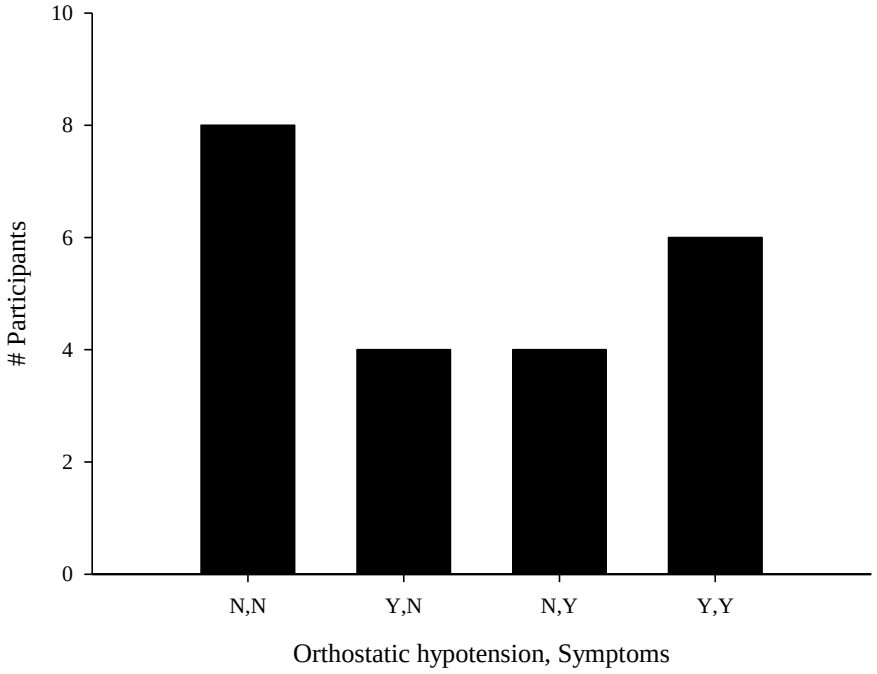


Figure 3.5 – Presence of orthostatic hypotension and its associated symptoms during the first assessment in patients. Y, yes; N, no.

Effect of neurological completeness on cardiovascular and autonomic parameters. *Heart rate variability.* Group mean data are presented in Table 3.2. Participants with complete SCI had lower supine LF power and participants with incomplete SCI had greater supine HF power across both tests than those with incomplete and complete SCI, respectively.

Cardiovascular responses to the sit-up test. Those with complete SCI had significantly greater HR response than individuals with incomplete SCI in the seated position (Table 3.6). In the

seated position, there was a main effect of completeness of SCI on BP response. Individuals with neurologically incomplete injury had smaller drops in SBP throughout the sit-up test in comparison to those with neurologically complete SCI. A positive correlation between neurological completeness of injury and decrease in SBP during the orthostatic challenge was found ($r^2 = 0.64$, $p=0.001$).

Sympathetic skin responses. Sympathetic skin responses elicited by median nerve stimulation and a deep breath revealed that 25% of participants with neurologically complete SCI had partial preservation, and 75% had no preservation, and 60% of participants with neurologically incomplete SCI had partial preservation and 40% had no preservation.

Baroreflex function. During the second assessment cardiovagal baroreflex sensitivity was significantly greater in individuals with complete than incomplete SCI, and BRSa was significantly greater in those with incomplete than complete SCI. Participants had values above the requirement to be considered normal for the Valsalva ratio.

Orthostatic hypotension. Symptoms of OH were experienced by those with complete (n=9, 5 participants experienced OH, 7 participants experienced symptoms) and incomplete (n=13, 5 participants experienced OH, 2 participants experienced symptoms) SCI (Table 3.3 b).

Effect of position. *Heart rate variability.* Participants with cervical SCI and those with incomplete SCI had significantly higher HF power in the supine than seated position. There was a main effect of position on HRV. Low frequency power was greater in the supine than sit-up position.

Cardiovascular responses to the sit-up test. Systolic blood pressure decreased (Figure 3.6) and HR (Figure 3.7) increased for all participants from the supine to seated position. Group mean data are presented in Table 3.2.

Effect of time. *Heart rate variability.* Group mean data for measures of HRV during the second assessment are presented in Table 3.5. Consistently over time, though not significant, participants with cervical SCI had greater HF and lower LF powers than individuals with thoracic SCI. Participants with complete SCI had lower LF and HF powers than those with incomplete SCI.

Cardiovascular responses to the sit-up test. Participants with complete SCI consistently experienced greater drops in SBP after assuming the seated position than participants with incomplete SCI. Similarly, those with cervical SCI consistently experienced greater drops in SBP after assuming the seated position than participants with thoracic SCI (Figure 3.6). The HR response to assuming the seated position decreased from the initial to the second assessment for participants with thoracic SCI (Figure 3.7). Group mean data for cardiovascular measures are presented in Table 3.6.

Baroreflex function. All participants except those with thoracic SCI experienced a significant improvement in BRSa from the first to the second test (Table 3.4). The Valsalva ratio (cardiovagal baroreflex) increased significantly in participants with cervical SCI.

Table 3.5 – Measures of heart rate variability during the second assessment (1-month post) in patients.

	Complete	Incomplete	Cervical	Thoracic
		Supine		
Mean RRI, ms	829.7 ± 62.1	892.6 ± 58.5	895.1 ± 63.2	826.1 ± 49.7
LF, Hz	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.0	0.05 ± 0.01
LF power				
ms^2	147.0 ± 41.2	360.9 ± 122.1	205.5 ± 64.0	276.3 ± 112.8
nu	124.7 ± 34.2	345.8 ± 108.7	168.7 ± 84.1	316.5 ± 100.5
%	23.1 ± 3.8	28.6 ± 2.5	23.7 ± 2.9	27.6 ± 4.4
HF, Hz	0.26 ± 0.03	0.22 ± 0.02	0.20 ± 0.02	0.24 ± 0.03
HF power				
ms^2	241.9 ± 164.5	472 ± 165.6[a]	443.5 ± 169.1[a]	217.8 ±167.4
nu	49.8 ± 4.5	51.3 ± 5.8	51.0 ± 5.7	50.3 ± 4.6
%	18.9 ± 6.4	24.5 ± 4.9	23.1 ± 5.2	21.7 ± 6.0
LF-to-HF ratio	3.4 ± 1.1	2.5 ± 0.8	3.4 ± 1.0	2.2 ± 0.6
Total variance, ms^2	679.6 ± 147.5	606.2 ± 90.5	560.6 ± 85.8	745.6 ± 146.3
VLF, Hz	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
VLF power				
ms^2	491.6 ± 145.5	497.5 ± 134.4	551.4 ± 145.8	413.6 ± 113.6
nu	301.6 ± 84.1	263.5 ± 119.1	321.5 ± 117.1	217.8 ± 86.1
%	49.2 ± 6.5	41.6 ± 6.5	47.3 ± 6.1	41.1 ± 8.4
		Seated		
Mean RRI, ms	675.8 ± 53.2	788.2 ± 36.6	787.3 ± 43.7	677.1 ± 39.8
LF, Hz	0.04 ± 0.0	0.04 ± 0.0	0.04 ± 0.0	0.04 ± 0.0
LF power				
ms^2	91.2 ± 23.5	141.1 ± 37.2	96.2 ± 21.9	122.6 ± 32.1
nu	119.5 ± 19.0	149.3 ± 31.0	129.2 ± 20.7	149.7 ± 28.4
%	19.6 ± 2.4	30.6 ± 5.3	22.3 ± 4.3	28.9 ± 5.0
HF, Hz	0.22 ± 0.03	0.19 ± 0.02	0.19 ± 0.02	0.21 ± 0.03
HF power				
ms^2	79.1 ± 30.1	159.5 ± 51.1	175.5 ± 48.5	121.3 ± 45.8
nu	40.1 ± 4.6	42.2 ± 3.7	44.2 ± 4.4	37.2 ± 4.2
%	12.0 ± 4.6	11.7 ± 2.5	13.2 ± 3.0	9.7 ± 3.6
LF-to-HF ratio	4.2 ± 0.8	3.9 ± 0.7	3.2 ± 0.6	5.2 ± 1.0
VLF, Hz	0.01 ± 0.01	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0
Total variance, ms^2	3454.4 ± 2008.4	1287.1 ± 583.3	2683.5 ± 803.0	2441.7 ± 1058.6
VLF power				
ms^2	328.1 ± 92.4	525.5 ± 158.1	554.0 ± 157.5	285.2 ± 80.3
nu	548.1 ± 146.6	382.6 ± 83.0	418.5 ± 102.4	494.2 ± 122.4
%	60.3 ± 8.2	56.4 ± 6.1	56.3 ± 6.3	60.5 ± 7.8

Values are mean ± SEM. RRI, RR interval; LF, low frequency; HF, high frequency; VLF, very low frequency; Hz, Hertz, ms^2, milliseconds squared; nu, normalized units.. [a]$P < 0.05$ supine vs. seated.

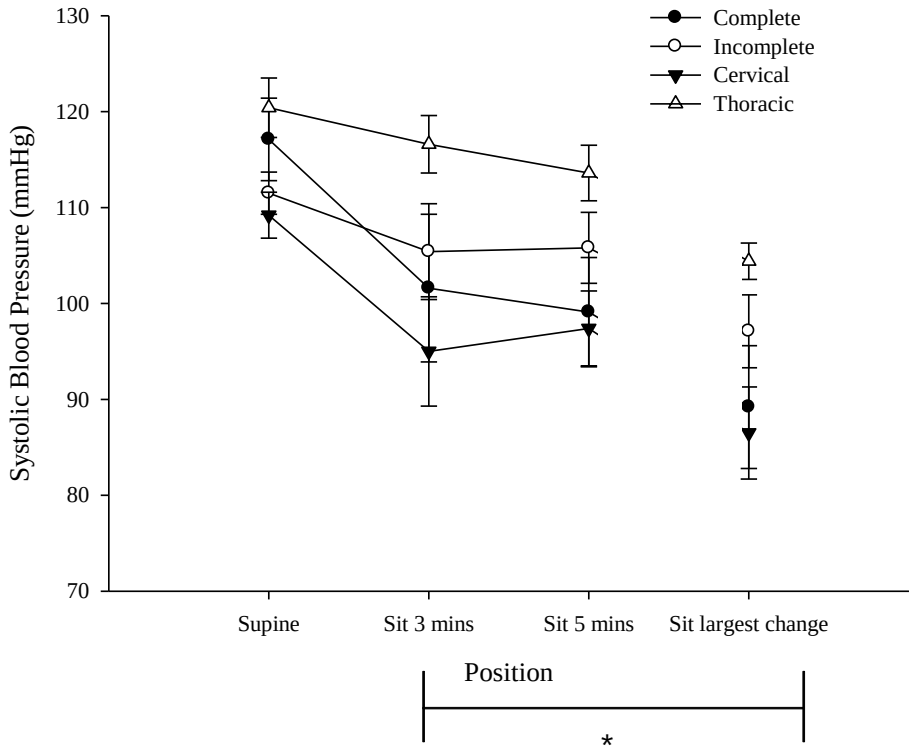


Figure 3.6 – Changes in systolic blood pressure during the sit-up test for the first assessment in patients. *P < 0.05 supine vs. sit-up.

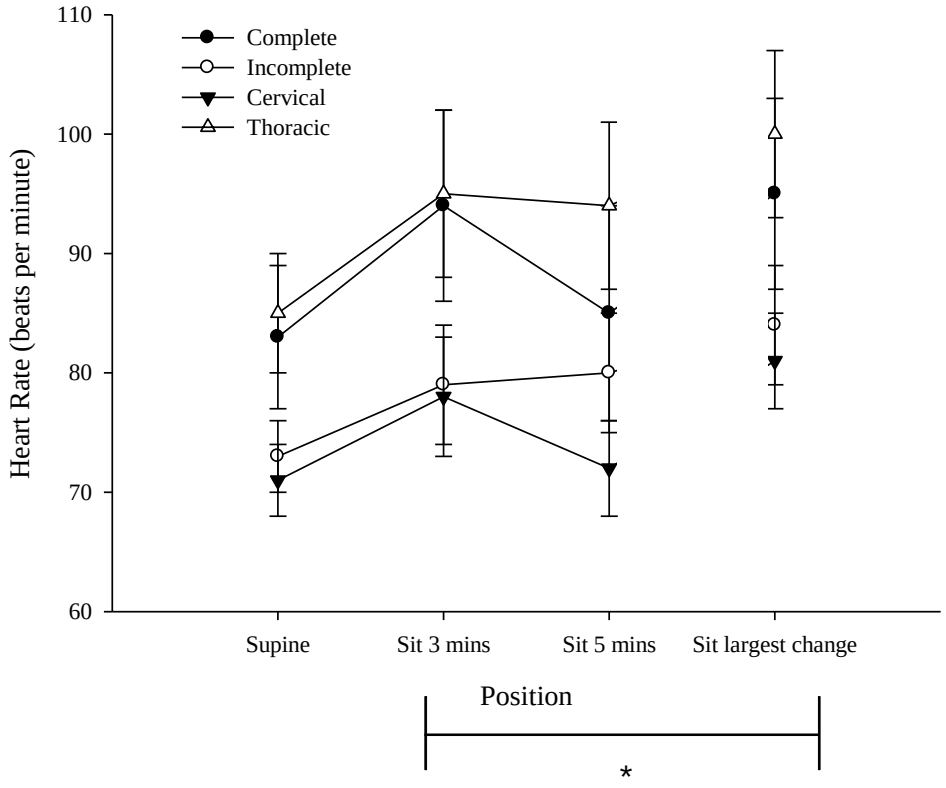


Figure 3.7 – Changes in heart rate during the sit-up test for the first assessment in patients. *P < 0.05 supine vs. sit-up.

Table 3.6 – Changes in cardiovascular measures during the sit-up test for the first and second assessments in patients.

	Complete	Incomplete	Cervical	Thoracic	Complete	Incomplete	Cervical	Thoracic
				Supine				
	Test 1				Test 2			
Mean								
SBP, mmHg	117.1 ± 4.3[c]	111.5 ± 2.2[c]	109.2 ± 2.4[c]	120.4 ± 3.1[c]	113.7 ± 5.2	111.9 ± 2.9	108.0 ± 3.5	119.1 ± 2.9
DBP, mmHg	68.5 ± 3.1[c]	65.6 ± 2.1[c]	63.3 ± 1.8[c]	71.7 ± 2.6[c]	67.4 ± 2.5	69.4 ± 2.7	69.6 ± 2.4	71.5 ± 2.8
MAP, mmHg	84.7 ± 3.1[c]	80.9 ± 1.9[c]	78.6 ± 1.8[c]	88.0 ± 2.2[c]	82.8 ± 3.0	83.6 ± 2.6	80.4 ± 2.6	87.4 ± 2.3
HR, bpm	83.2 ± 5.5[c]	72.5 ± 3.4[c]	71.4 ± 3.2[c]	84.8 ± 5.3[cd]	74.6 ± 5.6	70.6 ± 4.1	70.1 ± 5.0	75.3 ± 3.9
				Seated				
BP change								
SBP, mmHg								
3 mins	-15.5 ± 4.6[b]	-6.13 ± 4.7	-14.3 ± 5.0[a]	-3.8 ± 3.6	-18.4 ± 9.1	-8.0 ± 5.7	-19.1 ± 7.9	-2.4 ± 2.7
5 mins	-18.0 ± 3.7[b]	-5.6 ± 3.2	-12.6 ± 3.8[a]	-6.7 ± 3.7	-24.5 ± 9.0	-4.5 ± 2.7	-15.0 ± 7.0	-7.6 ± 3.1
Largest	-27.9 ± 3.9[b]	-14.4 ± 3.4	-22.7 ± 4.1[a]	-15.9 ± 3.9	-20.9 ± 5.1	-18.2 ± 9.9	-25.7 ± 9.7	-10.1 ± 3.9
DBP, mmHg								
3 mins	-7.5 ± 1.8[b]	-2.0 ± 2.5	-5.0 ± 2.7[a]	-3.2 ± 1.7	-11.9 ± 6.4	-3.1 ± 2.9	-9.6 ± 5.0	-2.6 ± 2.5
5 mins	-8.9 ± 3.6[b]	-5.6 ± 5.4	-11.1 ± 5.3[a]	-0.8 ± 3.01	-13.7 ± 7.5	-2.4 ± 1.7	-8.9 ± 5.1	-3.3 ± 2.7
Largest	-14.9 ± 3.5[b]	-12.3 ± 4.8	-16.0 ± 5.1[a]	-9.4 ± 1.9	-18.0 ± 5.9	-8.0 ± 2.4	-14.2 ± 4.5	-8.9 ± 2.8
MAP, mmHg								
3 mins	-10.2 ± 2.4[b]	-3.4 ± 3.1	-8.1 ± 3.3[a]	-3.4 ± 2.2	-14.1 ± 7.3	-4.8 ± 3.7	-12.8 ± 5.9	-2.5 ± 2.4
5 mins	-25.1 ± 3.4[b]	-20.9 ± 5.6	-26.4 ± 5.3[a]	-17.1 ± 3.6	-17.3 ± 7.8	-3.1 ± 1.9	-11.0 ± 5.7	-4.7 ± 2.3
Largest	-19.2 ± 2.9[b]	-13.0 ± 3.6	-18.3 ± 3.8[a]	-11.6 ± 2.0	-19.0 ± 4.9	-11.4 ± 4.7	-18.1 ± 5.3	-9.3 ± 3.1
HR, bpm								
3 mins	93.8 ± 8.1[b]	78.5 ± 4.7	77.8 ± 5.2[a]	94.8 ± 7.1[d]	80.9 ± 7.0	76.8 ± 3.0	80.3 ± 4.8	75.8 ± 4.1
5 mins	85.0 ± 9.3[b]	80.0 ± 5.3	72.3 ± 3.9[a]	93.9 ± 8.6[d]	76.6 ± 7.4	76.8 ± 2.9	80.0 ± 4.7	72.4 ± 4.2
Largest	95.1 ± 7.6[bd]	84.1 ± 4.7	80.8 ± 4.3[ad]	99.7 ± 7.1[d]	84.8 ± 6.9	81.9 ± 2.6	86.3 ± 4.6	78.4 ± 3.4

Values are mean ± SEM. Participants with complete SCI include lesion levels C4 to T9; participants with incomplete SCI include lesion levels C2 to T11. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate. BP change refers to changes in blood pressure (BP) from baseline. Heart rate is presented in absolute values at given time after assuming the seated position. [a]$P < 0.05$ cervical vs. thoracic; [b]$P < 0.05$ complete vs. incomplete; [c]$P < 0.05$ supine vs. seated; [d]$P < 0.05$ test 1 vs. test 2.

Sympathetic skin responses. Participants with greater preservation of SSRs proved to have better cardiovascular function in response to the sit-up test. There were some changes in SSRs from the initial to the second assessment (Table 3.3 a). There were no changes over time for participants with neurologically complete SCI (25% had partial preservation and 75% had no preservation). In the five participants with neurologically incomplete SCI, 1 (20% increase) more (2/5 to 3/5) had no preservation by the second assessment. However, of the remaining (2/5) that had at least partial preservation, 1 (20%) had improved to have complete preservation of SSRs. Individuals with cervical SCI also had 1 (25% increase) participant (3/4 to 4/4) progress from partial to no preservation by the second assessment. There was no change in the number of individuals with

thoracic SCI that had no preservation (2/5, 40%). Nevertheless, of the participants with partial preservation (3/5, 60%), 1 (20%) had improved to complete preservation of SSRs. Participants with thoracic and incomplete SCI had greater integrity of descending sympathetic pathways as indicated by SSRs and better cardiovascular control during the sit-up test.

Orthostatic hypotension. There were changes in the number of participants that experienced OH and/or symptoms over time (Table 3.7 and Figures 3.5 and 3.8). The changes in the number of individuals that experience OH and/or its associated symptoms from the first to the second assessment are summarized in Table 3.8.

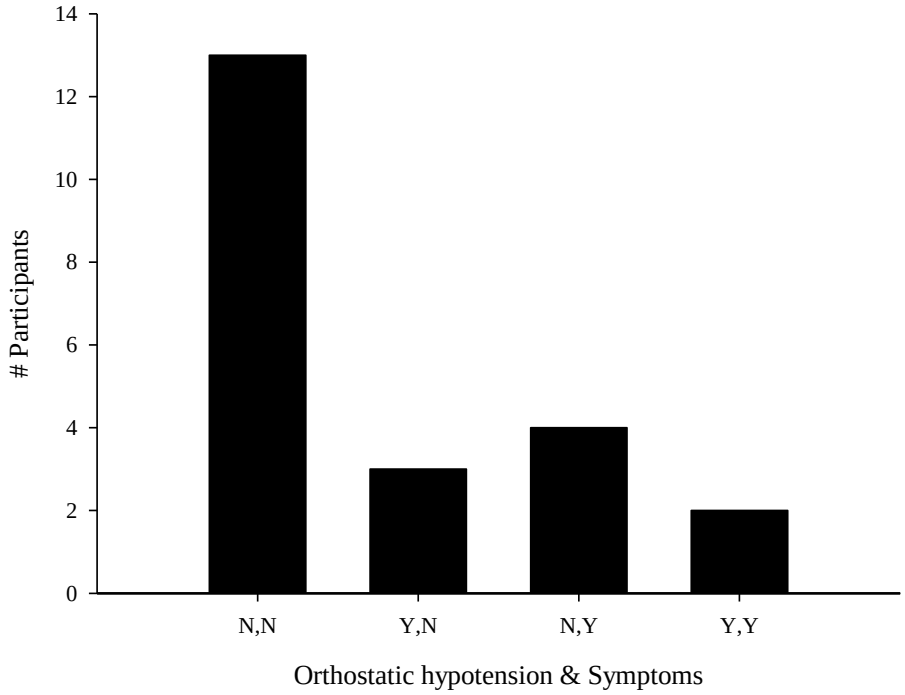


Figure 3.8 – Presence of orthostatic hypotension and its associated symptoms during the second assessment in patients. Y, yes; N, no.

Table 3.7 – Changes over time of the presence of orthostatic hypotension and its associated symptoms in patients.

Participant	SCI	AIS	Orthostatic hypotension 1	Orthostatic hypotension 2	Symptoms 1	Symptoms 2
1	C2	D	N	N	N	N
2	C4	A	N	N	Y	Y
3	C4	B	Y	N	N	N
4	C4	C	Y	Y	N	N
5	C4	C	N	Y	N	N
6	C5	A	Y	Y	Y	Y
7	C5	A	Y	N	Y	N
8	C5	A	Y	N	Y	Y
9	C5	C	N	N	N	N
10	C5	C	Y	N	N	N
11	C5	B	Y	Y	Y	Y
12	C6	D	N	N	Y	Y
13	C6	D	Y	N	N	N
14	T3	A	Y	N	Y	N
15	T4	A	N	Y	N	N
16	T4	C	N	N	N	N
17	T6	A	N	N	Y	N
18	T7	A	Y	N	Y	N
19	T9	A	N	N	N	N
20	T9	C	N	N	N	N
21	T10	B	N	N	N	N
22	T11	D	N	N	N	N

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; Y, yes; N, no; 1 and 2 refer to the first and second assessments, respectively.

Table 3.8 – Changes in number of participants that experienced orthostatic hypotension and/or symptoms from the first to the second assessment.

	Participants (n)							
	Test 1				Test 2			
	Orthostatic hypotension, Symptoms							
	No, No	No, Yes	Yes, No	Yes, Yes	No, No	No, Yes	Yes, No	Yes, Yes
Cervical	3	2	4	4	6	3	2	2
Thoracic	6	1	0	2	8	1	0	0
Complete	2	2	0	5	5	2	1	1
Incomplete	7	1	4	1	9	1	2	1

3.4 Discussion

Main findings. This study assessed the integrity of spinal autonomic pathways via HRV, BP and HR measures, SSRs, and the VM in participants with sub-acute SCI in the supine and seated positions. Autonomic integrity varied depending on lesion level and neurologic completeness of injury whereby individuals with thoracic and incomplete SCI had greater integrity than individuals with cervical and complete injuries, respectively. This was showcased by the former groups' greater integrity of SSRs and better cardiovascular responses to the sit-up test.

Cardiovascular function was always better in participants with the greatest preservation of SSRs for both the initial and second assessments. The number of individuals who experienced OH and/or its associated symptoms improved over time in all groups. There was an increase in the number of those who no longer experienced OH and/or symptoms and a decrease in the number of those who experienced OH and/or symptoms.

Lesion level. Measures of HRV in this study reveal that spinal autonomic integrity varies as a result of different levels of lesion to the spinal cord. It has been shown previously that LF and HF powers are different between individuals with cervical and thoracic SCI[71]. Heart rate variability provides insight on integrity of spinal autonomic pathways since any changes in its measures reflects changes in cardiovascular autonomic function. Individuals in this study with cervical SCI had higher HF power at rest than those with thoracic SCI. At rest, the higher HF power in participants with cervical SCI presumably indicates an increased cardiac vagal tone which is compatible with their slower HRs. The lower HF power in participants with thoracic SCI suggests reduced vagal tone, supported by their higher HRs. This has been documented previously in humans[5] and rodents[136] and suggests that autonomic integrity is greater with lower levels of injury. The reduced vagal tone and higher HRs may compensate for lower stroke volume following thoracic SCI[3, 137-139] to help minimize reductions in cardiac output. Vagal tone may have been higher in participants with cervical SCI, but their lower cardiovagal baroreflex function and associated HR response may not have been sufficient to counteract the decreases in SBP experienced when in the seated position. Heart rate increased from the supine to the seated position in all participants, but was higher in those with thoracic SCI. Low frequency power, thought to represent sympathetic[106, 140] and/or baroreflex modulation of sympathetic outflow to the heart[107, 108] decreased in all participants with SCI during the orthostatic challenge, indicating an inadequate response in the seated posture. The loss of sympathetic function contributed to the orthostatic intolerance seen in participants, especially those with higher lesion levels and neurologically complete injury[3, 71]. Clearly, lesion level and neurologic severity of injury impact autonomic dysfunction following SCI. Furthermore, it has been demonstrated in previous work that sympathetic activity normally elevates in response to an orthostatic challenge in the face of a decrease in BP[138, 139, 141-143]. Participants in our study did not elevate LF power during the sit-up

test and evidently face autonomic dysfunction that leads to OH resulting from lack of adjustment in central modulation of sympathetic system impulses in response to orthostatic stress.

The presence of LF power following SCI suggests that cardiac sympathetic control, baroreflex function, and integrity of spinal autonomic pathways remain. Mechanisms responsible for the presence of LF include: 1) the destruction of descending sympathetic pathways is incomplete[5]; and 2) the ability to modulate autonomic outflow via baroreflexes remains partially preserved in some individuals with SCI[107, 144]. Incomplete injury to autonomic pathways as indicated by preservation of palmar SSRs has been shown previously[5]. This study found a similar association between preservation of palmar SSRs and autonomic completeness of injury. That is, participants with greater preservation of palmar SSRs had better autonomic integrity and function than those with little or no preservation. The preservation or absence of palmar SSRs was related to lesion level as well. Individuals with thoracic SCI had greater preservation and, subsequently, better orthostatic tolerance than those with cervical SCI. This indicates that participants with thoracic SCI also demonstrated better integrity of spinal autonomic pathways. Participants with thoracic SCI had greater preservation of SSRs and better baroreflex function than those with cervical SCI illustrating that higher lesions produce greater deficits to autonomic function[9, 56, 57]. The greater decreases in SBP seen in those with cervical SCI is consistent with the lower BRSa and absence of SSRs in comparison to participants with thoracic SCI. Individuals with thoracic SCI had better BP responses (less decrease) to the orthostatic challenge, preservation of SSRs and adrenergic baroreflex function.

Baroreflex function is also affected by lesion level. Cardiovagal function (Valsalva ratio) was greater in participants with thoracic SCI. However, regardless of lesion level, it has been speculated that since the vagal and glossopharyngeal nerves remain intact following SCI, it may be that reductions in cardiovagal function are largely a result of increased arterial stiffening after injury. Arterial stiffening is thought to be enhanced in individuals with SCI as a result of high levels of inactivity[32]. It is known that increased arterial stiffening leads to a decrease in the activation of arterial stretch receptors for a given change in intra-arterial pressure, reflecting a

direct reduction of the sensitivity of the cardiovagal system[56, 145, 146]. Lower adrenergic baroreflex function in cervical SCI contributed to the impaired BP response to the orthostatic challenge and the increased HR through vagal withdrawal was not sufficient to prevent orthostatic intolerance and OH seen in these participants as mentioned previously. Heart rate responses were different depending on lesion level suggesting that baroreflex-mediated reductions in vagal tone were not the only cause of the increase in HR in the seated position. Adrenergic baroreflex control probably contributed to a greater extent in individuals with thoracic SCI since they had greater BRSa. Low frequency power was greater in the participants with thoracic SCI and may be indicative of sympathetic and/or baroreflex function. This is appropriate since those with thoracic SCI had greater sympathetic function as evidenced by their cardiovascular responses to the sit-up test and their greater baroreflex sensitivity. With greater supraspinal control over the sympathetic nervous system contained in the thoracolumbar spinal cord[56], it is expected that individuals with lower lesions have greater autonomic integrity.

Orthostatic hypotension and its associated symptoms were experienced by more individuals with cervical SCI, in agreement with previous findings[56, 126, 147, 148]. This may be explained by the greater impairment to the efferent sympathetic nerves with higher levels of injury[56]. However, there were three in this group who experienced OH but did not experience symptoms. In contrast, all participants with thoracic SCI who experienced OH also experienced symptoms, but one participant who did not experience OH still had symptoms. This particular individual had a decrease in SBP of 16 mmHg which helps to explain why this person may still have had symptoms as this is a fairly large reduction in SBP in the seated position. Findings from this study suggest that those with lower lesion levels have better spinal autonomic integrity following SCI. The impact on spinal autonomic integrity is more pronounced with higher levels of injury.

Neurologic completeness. Systolic BP decreased significantly during the sit-up test for both groups, but dropped more in those with complete SCI. This is in accordance with smaller LF power in these individuals (greater disruption to sympathetic function, in agreement with SSRs, and poorer baroreflex function). Nevertheless, HR response was higher in participants with

complete SCI. It may be postulated that this HR response was an attempt to help manage their greater drop in SBP when seated[3, 138, 139, 141-143]. The sit-up test leads to blood to pooling in the lower extremities and gut which decreases venous return and stroke volume. An increase in HR helps to compensate for the decrease in stroke volume and helps to prevent large decreases in cardiac output[3, 149]. All but one of the participants with complete injury had thoracic SCI and based on the HF power found for those with thoracic injury, these HR responses are reasonable. The cardiovagal component of the baroreflex response increased the HR response to help manage the drop in SBP during the sit-up test. This is consistent with HR responses to orthostatic stress resulting from baroreflex-mediated parasympathetic (vagal) withdrawal, which normally remains intact following SCI[9] and was evidenced by the normal values for the Valsalva ratio in participants. Additionally, the larger drops in SBP in those with complete SCI corresponds with their lower preservation of SSRs and lower BRSa in comparison to participants with incomplete SCI who had greater integrity of descending sympathetic pathways[5]. Similarly, the inability to elevate LF power during the sit-up test illustrates the autonomic dysfunction resulting from changes in central modulation of sympathetic system impulses. Neurologic severity impacts autonomic completeness of injury whereby individuals with greater neurologic impairment have greater cardiovascular dysfunction. This study revealed that neurologic and autonomic completeness are related which is in agreement with some studies[1] and in contrast to others[4, 5].

Position. There is clearly a change in autonomic function following SCI since response to an orthostatic challenge is diminished when compared to autonomically intact AB[5, 150]. Integrity of spinal autonomic pathways is showcased by cardiovascular responses to an orthostatic challenge when in the seated position. In this position, individuals experienced a decrease in HF and LF powers corresponding to vagal withdrawal and a decreased baroreflex and/or sympathetic response to the orthostatic stress, respectively. The reduction in baroreflex and/or sympathetic response helps to explain the decreases in SBP upon assuming the seated position seen in all participants. The decrease in HF power, reflecting baroreflex-mediated vagal withdrawal, was associated with an increase in HR in the seated position[3, 71], which likely helped to minimize drops in SBP. Those with cervical SCI experienced the greatest drops in SBP and the smallest increase in HR in the seated position, corresponding to a greater compromise in the integrity of

spinal autonomic pathways after high-level SCI. Furthermore, participants with cervical and complete SCI had lower preservation of SSRs than those with thoracic and incomplete SCI which corresponds well to their respective cardiovascular response to the orthostatic challenge and diminished autonomic integrity.

Time. Low frequency power, the Valsalva ratio, and BRSa improved over time in participants with cervical SCI, and the associated increase in HR may have served to limit the larger decrease in SBP they may otherwise have experienced. In contrast, the decrease in HR seen in other participants likely resulted from the resolution of tachycardia experienced in the sub-acute phase of injury to help maintain BP. Heart rate decreased in all participants except for those with cervical SCI and they also did not improve their SBP response to the orthostatic challenge by the second assessment. These findings correspond to preservation of SSRs as those with cervical SCI still experienced the greatest drop in SBP in the seated position and all participants with cervical SCI had no preservation of SSRs by the second assessment. The greater preservation of SSRs in those with thoracic and incomplete SCI did not improve SBP response indicating that perhaps there is a threshold for the minimal amount of preservation needed to minimize decrease in SBP. Based on the findings of this study, the minimum required preservation appears to be complete palmar preservation. Participants with thoracic SCI and those with incomplete SCI experienced a significant decrease in HR response during the sit-up test from the first to second assessment. The decrease in HR can be presumed to indicate a decrease in the amount of work required by the heart to help maintain BP by the second assessment. Regardless of level of injury or neurological completeness, participants improved their orthostatic tolerance from the initial to the second assessment. One individual with thoracic and complete SCI progressed to experiencing OH but was fairly close to a qualifying drop in SBP (14 mmHg) during the first assessment. Interestingly, this individual's (palmar) SSRs also deteriorated from the first to second assessment (participant 15, T4 AIS A, Table 3.7).

The improvement in orthostatic tolerance over time seen in our study is in agreement with previous findings[127]. Cardiovascular responses and SSRs underwent corresponding changes over

time in our study and there are several possible mechanisms that may help explain the improvement in orthostatic tolerance. First, since it is the interruption of sympathoexcitatory efferent pathways from the brainstem to the spinal SPNs involved in vasoconstriction that causes poor short-term blood pressure regulation, the recovery of spinal sympathetic reflexes may help to decrease the severity of OH[9]. Second, in individuals with higher lesion levels, the paralysis of lower extremity muscles in addition to the decrease in sympathetic efferent activity leads to excessive pooling of blood in the abdominal viscera and lower limbs, reducing systemic filling pressure at the heart[151-155]. As such, an increase in skeletal muscle tone and the development of spasticity over time may help to improve orthostatic tolerance[9] and mitigate the decrease in end-diastolic volume and, thus, stroke volume that results from venous blood pooling[153, 156]. Third, resting catecholamine levels are lower in individuals with higher lesions in comparison to those with lower lesions and AB[157]. It is possible that vascular wall receptor hypersensitivity may improve cardiovascular response to an orthostatic challenge[9]. There is evidence for hyperresponsiveness of peripheral alpha-adrenoceptors below the level of SCI from both human[55, 158] and animal studies[159]. This hyperresponsiveness may not only help to improve orthostatic tolerance but may also account for the enhanced pressor response that may be seen in some individuals with SCI. Finally, there may be adaptation of the renin-angiotensin system, which is thought to be the predominant mechanism used for BP control in individuals with cervical SCI[21]. This system increases blood volume by enhancing sodium and water retention[160]. In individuals with cervical SCI, there may be an increase in renal vascular resistance in response to an orthostatic challenge. A potential mechanism is that there may be an increase in the production of pressor substances including renin[161, 162] and norepinephrine[162]. This recovery over time in individuals with SCI may help to partially explain why individuals with high-level injuries are able to tolerate an upright posture. In contrast, in autonomically intact AB, a reduction in systemic BP is detected by baroreceptors such that the rate of afferent action potentials to the medullary vasomotor centre decreases. This reflects a reduction of the inhibitory potentials which modifies outflow via the descending spinal tracts to the intermediolateral cell column so that activity to SPNs and postganglionic sympathetic neurons is increased. This leads to an increase in vasomotor tone which augments systemic vascular resistance and increases mean arterial pressure at the level of the heart. As the first study to investigate changes in

autonomic function over time in the sub-acute phase of SCI, we revealed that level of injury and neurologic severity impact the ANS.

Correlations. The finding that neurologic completeness of injury is related to decreases in SBP during the sit-up test is in agreement with previous findings[1], suggesting that perhaps integrity of spinal autonomic pathways is related to neurologic severity of injury. Examining these participants in the chronic stage of their injuries may improve our understanding of this relationship and how it may change over time.

Methodological considerations. Lesion level may affect postural control making it difficult to passively transition from the supine to the seated position during the sit-up test. Individuals with higher lesion levels that have reduced postural stability may require manual support to remain relaxed during the change in posture. Some individuals were able to maintain proper posture independently and some required manual support to remain relaxed during the change in position. Help was provided without altering measures of BP by passively adjusting position or manually holding the individual in place. The VM can be difficult to perform since pulmonary muscles can be affected following SCI. Spinal cord injury above L1 to L2 impairs neural control to the abdominal (expiratory) muscles, and individuals with cervical lesions (C3 to C5) lose partial neural control of the diaphragm which impairs inspiration. Lower cervical injuries also compromise breathing because there is a reduction in the coordination between chest expansion and diaphragm descent as a result of impaired neural control of the external intercostals muscles[163]. Impairment to muscles required for breathing can make performing the VM challenging. It is important that it is performed correctly, and in addition to impaired breathing, individuals may perform the VM incorrectly by increasing mouth pressure without increasing intrathoracic pressure, producing abnormal changes in RRI[164]. Verbal instruction and a visual demonstration were provided to participants to help them perform the VM properly.

The Valsalva ratio and BRSa were used to assess the cardiovagal and sympathetic components of the baroreflex, respectively. Based on frequency analysis of HR, the LF/HF ratio may also be used to indicate the parasympathetic and sympathetic components of cardiac function as well. Indirect methods derived from frequency analysis of HR have suggested reduced sympathetic response to an orthostatic challenge, but these estimates cannot be interpreted to be directly related to sympathetic activity. Previous studies have found LF power from the LF/HF ratio to be related to muscle sympathetic nerve activity, although the statistical analyses was not suitable in one study[165], and less than half (40%) of participants recorded a significant relationship in another study[166]. Furthermore, in AB the changes that occur in sympathetic activation more likely result from decreases in the HF denominator (respiratory-mediated increases in vagal efferent activity) and not the LF numerator[167]. Nonetheless, since vagal withdrawal is the same[168] or increased[71] during an orthostatic challenge, the reduction in the LF/HF may indicate a reduction of the sympathetic cardiac response. Thus, the controversial findings about sympathetic cardiac function derived from the LF/HF ratio prompted the use of other indicators of baroreflex function.

It must also be acknowledged that during the recording of electrocardiogram data for the analysis of HRV, participants were allowed to breathe spontaneously since breathing was not paced. However, it has been shown that paced breathing has been found to artificially inflate HF power and deflate LF power during the upright position of an orthostatic challenge[140, 169]. Furthermore, Ditor et al.[99] tested participants using spontaneous breathing over two consecutive days and no significant difference for HF power was found between the two days. Marks and Lightfoot[100] used paced breathing on two consecutive days as well and found no improvement in HF power reproducibility with paced breathing. Not using paced breathing in this study likely did not affect results since no significant difference between consecutive testing days for the reliability study were found for HF power.

Patients in our study were likely euvolemic as most of the fluctuation in blood volume typically occurs during the acute period of injury and participants in this study were at least two to three

months post-SCI. However, it is important to recognize that changes in blood volume after injury may affect HR. Hypovolemia is common during the early period following injury and is associated with severe bleeding[170] which may be due to multiple injuries sustained during the accident or due to subsequent surgery post-injury. In the acute phase, plasma volumes may also be reduced as a consequence of hyponatremia[171]. A reduction in blood volume may explain the tachycardia seen in the early stage of injury since increased HR is associated with hypovolemia[172]. In contrast, patients in the early stages post-injury may also experience bradycardia. Heart rate immediately following injury is also affected by neurogenic shock whereby persistent bradycardia (HR less than 60 beats per minute for at least one day) is commonly seen in patients, especially individuals with severe cervical lesions[13, 123-125]. In patients with bradycardia, HR has been found to return to normal within two to six weeks following injury[123]. Hypotension that may result from hypovolemia in the acute stage is treated with fluid resuscitation to maintain tissue perfusion and to resolve neurogenic shock[173]. It is likely that our participants in the sub-acute phase of injury were euvolemic since hypovolemia is normally treated with fluid resuscitation and vasopressive drugs to maintain a minimum SBP of at least 85 mmHg during the first week following SCI[174, 175]. We presumed all of our participants were euvolemic since they all had resting SBP greater than 85 mmHg and were no longer in the acute phase of injury.

Cardiovascular deconditioning may also affect HR during the acute phase of SCI and is characterized by many physiological disorders that develop following prolonged bed rest or exposure to microgravity, such as space flight. This condition is expected after SCI as a result of long periods of bed rest during the acute phase of injury. This condition may manifest as profound orthostatic intolerance which is thought to be the result of diminished blood volume, decreased muscle or tissue pressure in the extremities, or changes to sympathetic nervous system function[176]. The orthostatic intolerance that occurs along with cardiovascular deconditioning is characterized by postural hypotension and postural tachycardia[176]. These signs are more prominent and problematic during the acute phase following injury and in the chronic phase are less likely to be responsible for OH since deconditioning effects are resolved over time. It should also be acknowledged that altered HR may persist during the chronic phase of injury, especially

in individuals with thoracic SCI who have been observed to have reduced vagal tone and higher HRs to help compensate for lower stroke volume[3, 137-139].

Conclusions. The integrity of spinal autonomic pathways following SCI varies with different lesion levels and neurologic severities of injury. This study supports the notion that there is a strong correlation between the level of injury and extent of remaining integrity of spinal autonomic pathways. Individuals with cervical SCI tend to have poorer cardiovascular response to an orthostatic challenge[71] and less preservation of SSRs[5] than their counterparts with thoracic injuries. This is expected for individuals with cervical than thoracic SCI since there is greater disruption to descending sympathetic control and thus, less control of vasculature below the level of injury[55]. The assessment of the integrity of sympathetic spinal pathways via SSRs clearly illustrated that the extent of autonomic dysfunction was related to the preservation of SSRs. All individuals with complete palmar preservation did not experience OH, indicating that preservation of SSRs indicates greater integrity of spinal autonomic pathways. In contrast, those with very little (1/5 for preservation at the palmar sites) to no preservation experienced OH, suggesting that they had poor spinal autonomic integrity. Baroreflex function also reflected integrity of spinal autonomic pathways in accordance with preservation of SSRs and changes in SSRs over time. Participants with greater integrity always had better cardiovascular function. The association between neurologic and autonomic completeness of injury found in our study is in agreement with previous findings[1].

Inconsistencies between motor and sensory completeness of injury and autonomic completeness of injury have been documented[5] but the extent of autonomic impairment is not expected to be related to the neurological completeness of injury since the AIS does not evaluate autonomic integrity. However, this study demonstrated that participants with neurologically complete injuries had greater autonomic impairment than their counterparts with incomplete SCI. As such, autonomic integrity was related to level of injury and neurologic completeness of SCI. Regardless, the importance of integrity has been highlighted previously by Furlan et al.[13]. They demonstrated that following SCI, individuals with less white matter degeneration and greater

preserved axons in the dorsolateral funiculus (location of descending vasomotor pathways) had less cardiac dysfunction than those that had more extensive white matter degeneration and fewer preserved axons, respectively. The destruction of descending vasomotor pathways leads to the loss of excitatory supraspinal input to the SPNs[55, 173]. Clearly, spinal cord components involved in cardiovascular control following SCI, including SPNs and descending vasomotor pathways, are an integral part of proper cardiovascular function.

Though OH may have been related to level and neurologic completeness of injury, it did not appear as though experiencing symptoms of OH was similarly related. It has been demonstrated previously that many individuals with SCI are asymptomatic despite marked OH[126, 177]. This is similar to other able-bodied populations who have autonomic impairments and likely represents protective alterations in cerebral autoregulation despite cerebral hypoperfusion[150, 178, 179]. Participants experienced significant changes in cardiovascular responses to the sit-up test over time demonstrating in agreement with previous research[127] that cardiovascular control can change following injury. Unique to this study, the combination of autonomic tests used allowed for the determination of integrity of spinal autonomic pathways in individuals with SCI by building upon existing knowledge of changes in cardiovascular autonomic function and sympathetic sudomotor pathways after injury. We found that lesion level, neurologic severity of injury and time post-injury impact autonomic dysfunction, and individuals with greater integrity of spinal autonomic pathways have better cardiovascular function.

Chapter 4: Integrity of Spinal Autonomic Pathways in Elite Athletes with Spinal Cord Injury: An Important Consideration in Addition to Motor and Sensory function

4.1 Introduction

The ISNCSCI traditionally evaluated only motor and spinal cord sensory pathways[2], and the final outcome of this assessment, known as AIS[1], characterizes motor and sensory completeness of SCI. Only recently has a standard assessment of autonomic function been proposed as an additional component to evaluate individuals with this devastating type of injury[2]. The sympathetic and parasympathetic components of the ANS are involved in cardiovascular control. The vagus nerve originates from the medulla and is normally spared after SCI[39, 180, 181] so vagal control of the heart remains intact after injury. Sympathetic innervation of the heart and peripheral vasculature for the upper extremity originates from the upper thoracic segments (T1 to T5). Spinal sympathetic neurons in segments T6 to L2 control the vasculature in the splanchnic region and the lower extremity[39]. The impact of changes in the integrity of spinal autonomic pathways on cardiovascular control is important to acknowledge during all stages of injury. A sub-population of those with chronic SCI is athletes and the identification of athletes with intact vasomotor pathways is of critical importance to competition in sport. In addition to the greater neurologic impairment of individuals with higher SCI, injuries at or above T5 also result in loss of sympathetic control of the visceral vasculature[5] and this disruption of spinal sympathetic pathways affects the vascular resistance responses, especially in dependent regions like the splanchnic bed which are known to play a pivotal role in BP control in the upright position[182-184]. Thus, impairment in vascular resistance responses following injury may lead to orthostatic intolerance[182, 183], illustrating the presence of abnormal cardiovascular responses to physiological stressors such as orthostatic challenges. These abnormalities in cardiovascular control may affect exercise performance as well since the importance of appropriate BP and HR responses to appropriate exercise performance are well, known[9, 185]. Appropriate spinal autonomic integrity is required to ensure proper cardiovascular control such that there is sufficient blood redistribution to the muscles during exercise[186]. There is a need for proper sympathetic control of the cardiac and regional blood vessels in addition to skeletal muscle pump activity[12, 155, 185]. It is clear that a

combination of motor and autonomic changes following SCI may impact exercise performance. In Paralympic wheelchair rugby players with cervical SCI, it has been reported that those with partially or fully intact descending vasomotor control performed significantly better during tests of aerobic function and endurance performance than athletes who did not have descending vasomotor control[187]. High-level SCI results in low resting BP[5], reduces maximum HR as a result of altered sympathetic tone and lower catecholamine release[153, 188-190], and lowers maximal oxygen uptake[153, 189] and peak power output[153, 188] in comparison to athletes with lower levels of injury. Injury below T5 leaves sympathetic control of the heart and vasculature of the splanchnic region intact, which is important for appropriate cardiovascular response to exercise (e.g., increase HR and BP)[9, 185]. In addition to the cardiovascular system, others under autonomic control are involved during activity and these include the respiratory and sudomotor systems[155, 185]. It is clear that autonomic dysfunction following SCI can largely impact sport performance and movement to acknowledge impairments to the ANS in International Paralympic Committee (IPC) classification is a current and significantly pressing issue[6]. The IPC is working alongside athletes in an effort to improve the classification system as a part of the IPC Athletics Classification Project for Physical Impairments, upon which the improved official system will be based on[191].

During the last decade more evidence has become available illustrating that the severity of motor and sensory impairment (neurologic completeness) is not necessarily related to the autonomic completeness of SCI[5, 192-194]. Our present understanding of motor, sensory and autonomic completeness of injury is disconnected with the current functional IPC classification that is primarily focused on abilities of athletes for specific sports. For example, wheelchair rugby athletes are classified based on functional movement tests and on-court observations. They are assigned a score from 0.5 to 3.5, and during competition, four players with a total score of 8 are allowed on the court. This creates a unique and often disadvantageous situation for athletes with SCI since they are grouped with individuals who have similar motor control but varying degrees of spinal autonomic integrity, and thus, significant variability in their cardiovascular control. Moreover, athletes with SCI that experience cardiovascular dysfunction are predisposed to the ability to "boost"[195, 196], or intentionally induce AD[195, 197]. Individuals with higher lesion levels

experience greater compromises to the integrity of spinal autonomic pathways. This increases cardiovascular dysfunction and subsequent physiological limitations which may serve as incentive for athletes to "boost". Boosting has been shown to improve athletic performance[197-199], and unfortunately, has also been associated with catastrophic events including intracerebral hemorrhaging, seizures, myocardial ischemia, and even sudden death[28-30, 200]. Therefore, it has been banned in the Paralympics. This highlights the importance of acknowledging that varying degrees of autonomic integrity affect the amount of cardiovascular autonomic dysfunction that athletes face. Understanding how spinal autonomic integrity impacts sport performance may help to reduce the use of boosting if a fair playing field ensues from the incorporation of an autonomic evaluation into classification. Tests that identify autonomic dysfunction in addition to neurological impairment would promote a broader understanding of all the limitations athletes may encounter.

Changes to the integrity of spinal autonomic pathways may affect the sympathetic nervous system and, subsequently, the severity of resulting cardiovascular dysfunction. Several factors may alter autonomic sympathetic tone: 1) loss of supraspinal control, which is greater with increasing levels of SCI. Spinal cord injury at or above T5 causes large reductions in sympathetic outflow and supraspinal control to the splanchnic bed and other vasculature of the lower extremities[9]. Spinal cord injury below T5 conserves more supraspinal control of sympathetically innervated vasculature which limits cardiovascular dysfunctions[9]; 2) sympathetic activity is reduced below the SCI as seen by a reduction in activity of muscle postganglionic axons[201]; and 3) SPNs below the SCI lose their descending supraspinal connection and undergo changes in morphology[202, 203]. Sympathetic preganglionic neurons send efferent tonic signals from the central nervous system to the heart and blood vessels and are crucial for central cardiovascular control[68]. Evidently, the degree of cardiovascular dysfunction depends on the severity of damage to the spinal autonomic pathways. It is currently well understood that individuals with higher lesion levels experience greater cardiovascular dysfunction. The main goal of this study was to examine the integrity of spinal autonomic pathways in elite wheelchair athletes and correlate this with the impairment to cardiovascular control. It was hypothesized that neurological completeness of injury would be related to autonomic completeness of injury.

4.2 Materials and methods

Participant characteristics. Eighteen wheelchair athletes with cervical SCI (C5 to C8; AIS A to C; 7 with AIS A and 11 with AIS B to C) (32 $\pm$ 1 yrs; all males) of normal height (183 $\pm$ 2 cm) and weight (76 $\pm$ 3 kg) participated in this study (Table 4.1). Participants were divided into two groups for comparison: 1) athletes with complete SCI (AIS A); and 2) athletes with incomplete SCI (AIS B and C). They were in the chronic stage of injury (12 $\pm$ 1 yrs) and free from cardiovascular and pulmonary diseases. Of these 18 athletes, SSRs were only collected from 13. Participants were recruited from training and competition venues in Burnaby, Victoria, and Vancouver, British Columbia. They competed at national or international levels of sport and must have sustained a traumatic SCI. The neurological exam was performed by a qualified physician. Participants were only included if they were free of any coincident cardiac or pulmonary diseases or active medical issue such as hypertension, decubitus ulcers or urinary tract infections. Prior to the initiation of the study, written informed consent was obtained. All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia which conforms to the *Declaration of Helsinki* and the IPC.

Experimental protocol. Testing was performed in the morning if possible. Upon arrival to the room where testing was conducted, participants were asked to empty their bladders to minimize the influence of reflex sympathetic activation on peripheral vascular tone and to minimize the risk of AD. None of the participants smoked, had a history of cardiopulmonary disease, or were taking medications known to influence cardiovascular responses. They were required to avoid caffeine for four hours and food for two hours prior to assessment. They transferred independently, unless help was needed, from their wheelchairs to the bed used for the sit-up test[5] and first underwent motor and sensory examination to determine neurologic severity of injury (AIS). There was an additional period of 5 to 10 minutes of supine rest when we instrumented research participants with electrocardiogram electrodes, and arm and finger blood pressure cuffs prior to the initiation of data collection. Following instrumentation, the experimental session began with a minimum of 10 minutes in the supine position during which time resting data were recorded. After 10 minutes at rest, participants were passively moved to the seated position.

Since the sit-up test was performed on a bed, they were positioned between two researchers for support and their knees dangled freely over the side of the bed. They were asked to report the presence of symptoms of hypotension (i.e., dizziness, fatigue, blurred vision, syncope, lightheadedness). Sympathetic skin response testing was done immediately prior to or following the sit-up test.

Physiological measures. During the sit-up test, a single-lead electrocardiogram was continuously recorded to determine HR. Automated BP was measured at the brachial artery (Dinamap, GE Pro 300V2; Tampa, FL). A pulse oximeter was used to measure SpO_2 (Dinamap, GE Pro 300V2; Tampa, FL).

Heart rate variability. Heart rate variability is spectral analysis of cardiovascular parameters used to evaluate autonomic tone from HR. The analysis of HRV is based on the observation that basal RRI (the time between two successive R waves of an electrocardiogram) continually fluctuate. To investigate HRV during the sit-up test, offline beat-to-beat analysis of the digitized electrocardiogram was performed and time series of successive beats were extracted from the electrocardiogram recordings for RRIs. Power spectral analysis was performed using an autoregressive model fitted to each time series (aHRV, Nevrokard, Slovenia)[114]. Occasional ectopic beats were "corrected" by the linear interpolation of adjacent normal beats. Oscillations from 0.04 to 0.15 Hz were designated as LF and oscillations from 0.15 to 0.4 Hz were designated as HF. Powers were normalized by dividing the power by the total variance minus VLF (<0.03 Hz) and multiplied by 100[115].

Sympathetic skin response. The integrity of sympathetic sudomotor function was assessed via SSRs[110, 111]. This electrophysiological test records a change in potential from the surface of the skin generated by sweat in response to a stimulus and assesses sympathetic fibres[113]. Sympathetic skin responses reveal overall autonomic sympathetic function by examining the common efferent pathways of the sympathetic nervous system from the spinal cord to the sweat glands of the

hands (palmar) and the feet (plantar) relayed by preganglionic and postganglionic sympathetic nerve fibres[134]. Self-adhesive recording electrodes were placed on sites with maximum eccrine sweat gland density (palms of the hands and soles of the feet; left hand, LH; right hand, RH; left foot, LF; right foot, RF). Sympathetic skin responses were recorded simultaneously from both hands and feet following a single electrical pulse (duration 0.2 ms; intensity 8-10 mA) applied to the median nerve. Five consecutive stimuli were applied to the median nerve at the wrist and for a second stimulus participants were instructed to take five consecutive deep breaths. Data were recorded using an analog-to-digital converter (Keypoint, Alpine Biomed, California, USA). To minimize habituation, stimuli were applied in random order and with variable time delays (minimum delay of 90 s). Sympathetic skin responses were deemed present when there was a clear positive deflection from baseline. Any potential that coincided with muscle spasm, limb movement or cough was excluded from the analysis. Responses were qualified by the number of reproducible SSRs elicited[113]. A response indicated a preserved spinal autonomic pathway.

Data and statistical analyses. All data were acquired using an analog to digital converter (Powerlab/16SP model ML795; ADInstruments, Colorado Springs, CO) interfaced with a computer and sampled at 1 kHz. Data were stored on a personal computer for subsequent offline analysis (Powerlab version 7.2, ADInstruments). The sit-up test was examined based on cardiovascular responses measured in the supine and seated positions. Differences between these positions and between participants were determined with two-way ANOVA. In the case of a significant *F* ratio, differences were further investigated with Tukey's post-hoc analysis. For SSRs, the maximum response at each site was five, in which case all five stimuli elicited a response. Correlations between variables were performed using Pearson's correlation coefficients. Group data are presented as means $\pm$ SEM.

4.3 Results

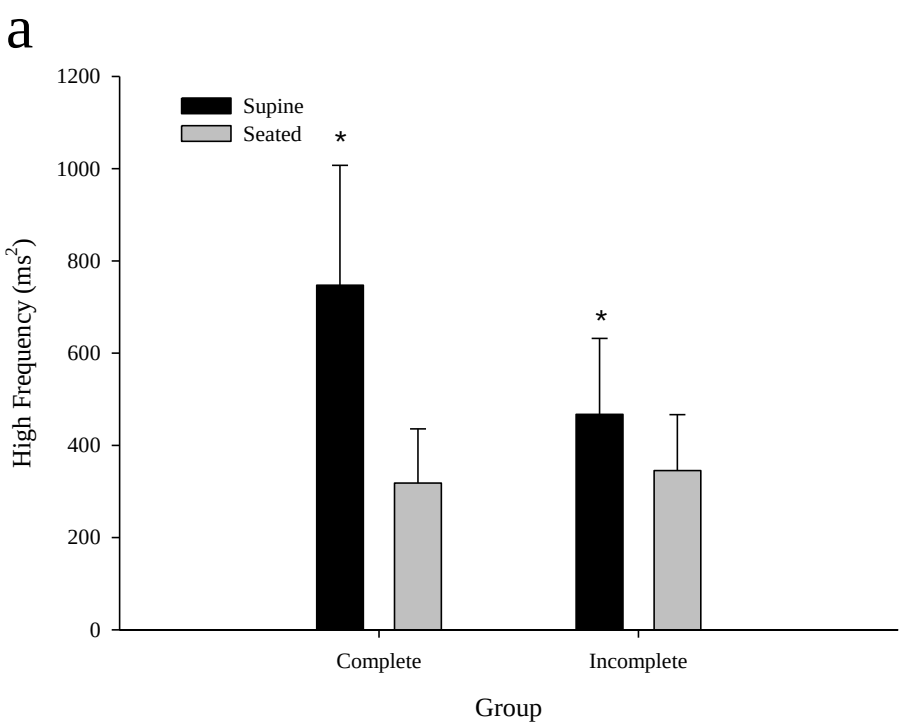
Participant characteristics.

Table 4.1 – Characteristics of wheelchair rugby athletes with chronic spinal cord injury.

Participant	SCI Level	AIS	Time Since SCI (yrs)	Age (yrs)	Height (cm)	Weight (kg)
1	C5	A	6	27	171	75
2	C5	B	9	24	173	80
3	C6	A	14	36	168	70
4	C6	A	9	26	185	78
5	C6	A	17	37	190	76
6	C6	C	10	33	196	75
7	C6	B	17	32	178	86
8	C6	B	8	28	183	78
9	C6	C	21	37	186	74
10	C6	B	6	34	191	66
11	C6	C	8	25	184	65
12	C7	A	17	35	178	64
13	C7	A	10	28	180	63
14	C7	A	9	26	183	75
15	C7	B	11	30	185	82
16	C7	B	17	36	180	77
17	C7	C	21	38	193	90
18	C8	B	13	37	186	87
Mean ± SD	N/A	N/A	12 ± 1	32 ± 1	183 ± 2	76 ± 3
Minimum	N/A	N/A	6	24	168	63
Maximum	N/A	N/A	21	38	193	90

SCI, spinal cord injury; AIS, American Spinal Injury Association Impairment Scale; SD, standard deviation.

Cardiovascular measures. Group mean data for measures of HRV during the orthostatic test are presented in Table 4.2. There were no significant differences between groups for changes in BP or HR during the sit-up test (Figure 4.1 a and b). However, SBP significantly decreased and HR significantly increased upon assuming the seated position (Figures 4.2 and 4.3). Group mean data for HR and BP are presented in Table 4.3. Analysis of HRV revealed that for both participants with complete and incomplete SCI, LF and HF powers significantly decreased upon assumption of the seated position.



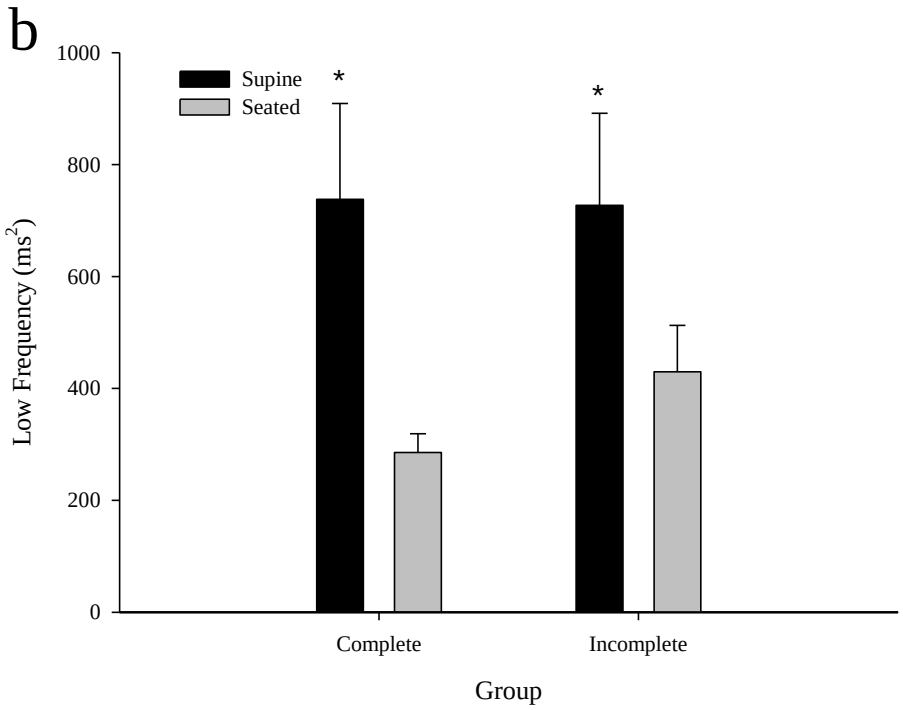


Figure 4.1a – High frequency heart rate variability changes during the sit-up test in wheelchair rugby athletes. *P < 0.05 supine vs. sit-up.

Figure 4.1 b – Low frequency heart rate variability changes during the sit-up test in wheelchair rugby athletes. *P < 0.05 supine vs. sit-up.

Table 4.2 –Measures of heart rate variability during the sit-up test in wheelchair rugby athletes.

	Neurologically complete SCI	Neurologically incomplete SCI
	Supine	
Mean RRI, ms	1064.9 ± 49.7	1088.6 ± 79.5
LF, Hz	0.04 ± 0.0	0.05 ± 0.01
LF power		
ms^2	738.0 ± 171.3[a]	727.1 ± 164.6[a]
nu	119.3 ± 19.2	109.4 ± 18.0
%	30.8 ± 4.1	26.5 ± 1.7
HF, Hz	0.2 ± 0.02	0.18 ± 0.01
HF power		
ms^2	747.2 ± 259.8[a]	467.3 ± 101.1[a]
nu	47.8 ± 5.1	51.0 ± 5.4
%	65.9 ± 3.7	52.1 ± 4.7
LF-to-HF ratio	1.0 ± 0.1	1.9 ± 0.4
Total variance, ms^2	4150.1 ± 479.1	3215.0 ± 490.8
VLF, Hz	0.01 ± 0.00	0.01 ± 0.00
VLF power		
ms^2	488.4 ± 65.6	581.2 ± 24.5
nu	102.7 ± 15.0	138.3 ± 38.9
%	41.6 ± 2.9	38.8 ± 6.2
	Seated	
Mean RRI, ms	850.1 ± 34.3	969.7 ± 68.6
LF, Hz	0.04 ± 0.0	0.05 ± 0.01
LF power		
ms^2	285.6 ± 33.6	429.8 ± 83.0
nu	62.9 ± 5.7	105.0 ± 14.8
%	25.9 ± 4.2	29.0 ± 4.7
HF, Hz	0.27 ± 0.03	0.18 ± 0.01
HF power		
ms^2	318.2 ± 117.6	345.2 ± 121.2
nu	28.8 ± 2.8	22.9 ± 3.6
%	12.9 ± 3.3	16.6 ± 3.8
LF-to-HF ratio	3.0 ± 0.7	2.6 ± 0.6
Total variance, ms^2	3499.5 ± 617.8	3643.8 ± 606.6
VLF, Hz	0.01 ± 0.00	0.01 ± 0.00
VLF power		
ms^2	658.2 ± 84.2	681.4 ± 74.1
nu	358.3 ± 115.0	282.2 ± 93.2
%	57.8 ± 5.9	52.5 ± 6.1

Values are mean ± SEM. Participants with complete SCI are AIS A; participants with incomplete SCI are AIS B to C. RRI, RR interval; LF, low frequency; HF, high frequency; VLF, very low frequency; Hz, Hertz, ms^2, milliseconds squared; nu, normalized units; SCI, spinal cord injury.
[a]$P < 0.05$ supine vs. seated.

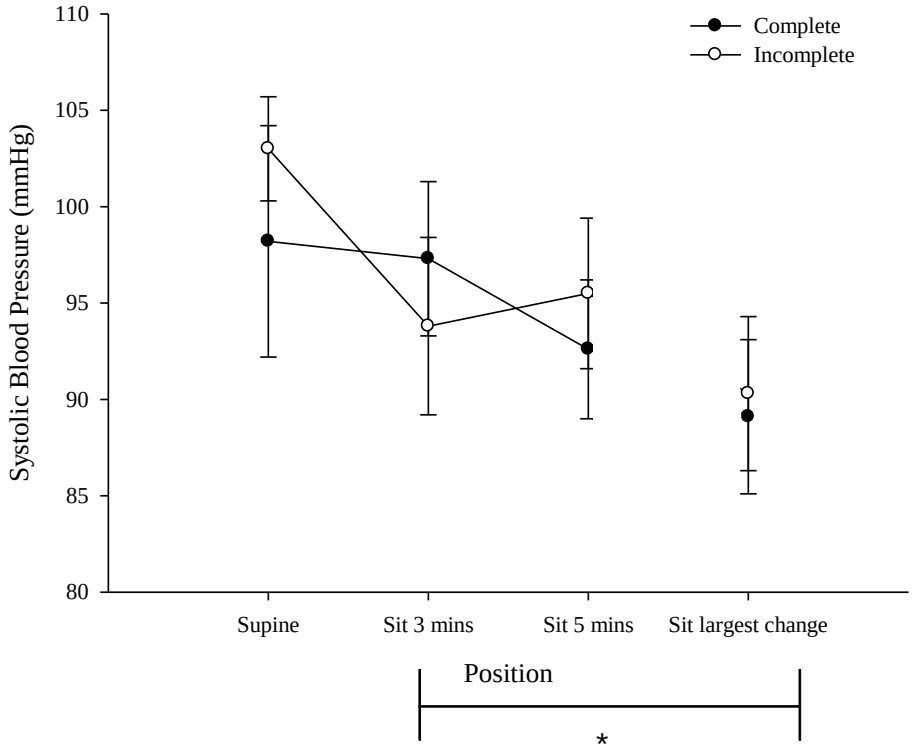


Figure 4.2 – Changes in systolic blood pressure during the sit-up test in wheelchair rugby athletes. *P < 0.05 vs. supine.

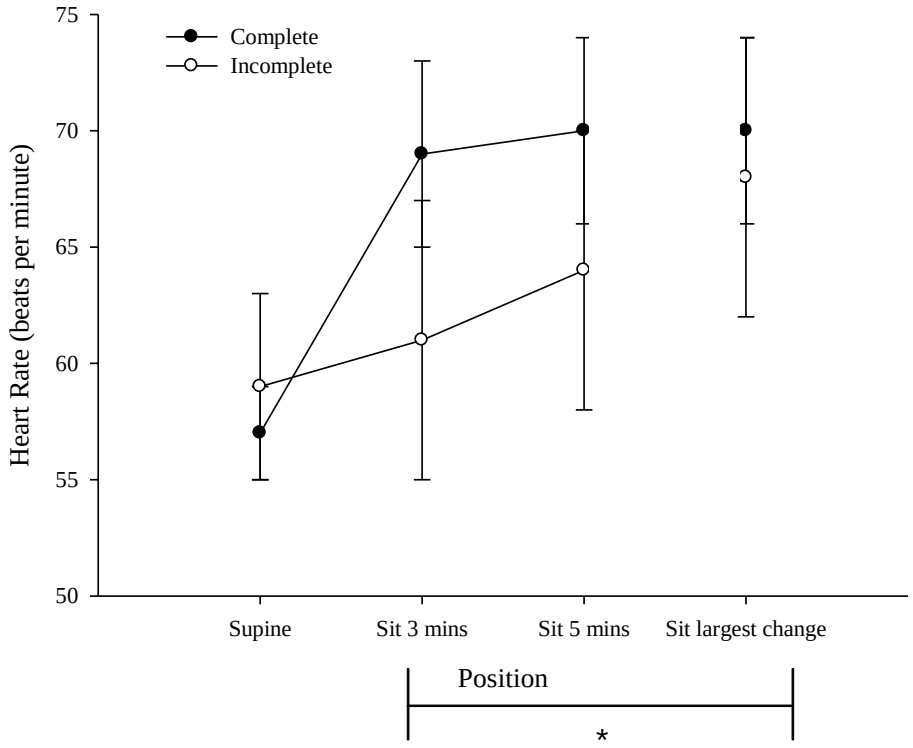


Figure 4.3 – Changes in heart rate during the sit-up test in wheelchair rugby athletes. *P < 0.05 vs. supine.

Table 4.3 - Changes in cardiovascular measures during the sit-up test in wheelchair rugby athletes.

	Complete	Incomplete
	Supine	
SBP, mmHg	98.2 ± 6.0[a]	103.0 ± 2.7[a]
DBP, mmHg	63.0 ± 3.4[a]	62.8 ± 3.1[a]
MAP, mmHg	74.7 ± 4.2[a]	76.2 ± 2.8[a]
HR, bpm	56.8 ± 2.4[a]	59.1 ± 3.7[a]
	Seated	
SBP, mmHg		
3 mins	97.3 ± 4.0	93.8 ± 4.6
5 mins	92.6 ± 3.6	95.5 ± 3.9
Largest	89.1 ± 4.0	90.3 ± 4.0
DBP, mmHg		
3 mins	68.3 ± 5.2	56.7 ± 3.8
5 mins	60.8 ± 4.3	60.8 ± 3.5
Largest	53.8 ± 1.4	53.8 ± 3.5
MAP, mmHg		
3 mins	77.9 ± 4.6	69.1 ± 3.9
5 mins	71.4 ± 3.6	72.4 ± 3.5
Largest	65.6 ± 1.9	66.0 ± 3.6
HR, bpm		
3 mins	68.5 ± 4.0	61.4 ± 5.6
5 mins	69.8 ± 3.9	64.2 ± 5.6
Largest	70.3 ± 4.2	67.7 ± 6.2

Values are mean ± SEM. [a]$P < 0.05$ supine vs. seated. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

Sympathetic skin response. Individual participant data for SSRs, along with the presence of symptoms during the sit-up test and the largest drop in SBP, are presented in Table 4.4. There were differences in the preservation of SSRs between participants. In those with complete SCI (n=5), 3 (60%) had partial preservation, 1 (20%) had complete preservation (therefore, 80% of participants with neurologically complete SCI had at least partial preservation of SSRs) and 1 (20%) participant had no preservation of SSRs. In participants with incomplete SCI that underwent SSR testing (n=8), 7 (88%) had partial preservation and 1 (12%) had no preservation of SSRs. Preservation of SSRs was positively correlated (r^2=0.60) with decreases in SBP during the sit-up test, but not correlated with AIS classification.

Table 4.4 – Qualification of sympathetic skin responses, the presence of symptoms of orthostatic hypotension, and the largest drop in systolic blood pressure during the orthostatic challenge in wheelchair rugby athletes.

		SSR to median nerve stimulation					
Participant	**CC/CI**	**LH**	**RH**	**LF**	**RF**	**Largest SBP change**	**Symptoms**
1	CC	3	2	4	2	-9	N
2	CC	5	5	5	5	12	N
3	CC	4	5	0	0	-4	Y
4	CC	3	3	2	2	-18	Y
5	CC	0	0	0	0	-24	N
6	CI	3	5	3	3	-12	Y
7	CI	5	5	5	4	-8	N
8	CI	0	0	0	0	-41	Y
9	CI	5	5	4	5	-6	N
10	CI	2	5	2	2	3	N
11	CI	3	3	3	1	-14	Y
12	CI	3	5	5	4	-3	N
13	CI	2	4	1	1	-9	N

SSR; sympathetic skin response; CC, cervical complete; CI, cervical incomplete; LH, left hand; RH, right hand; LF, left foot; RF, right foot; SBP, systolic blood pressure.

Symptoms of OH. The presence of symptoms in individual participants is presented in Table 4.4. Decreases in SBP indicative of OH were observed in 2/13 (15%) participants, one each with a complete and incomplete SCI. This was symptomatic only in the individual with incomplete SCI. The remaining participants (4/13) (31%) who experienced symptoms also experienced decreases in SBP, but none that qualified for OH, though one individual was close with a decrease in SBP of 18 mmHg.

Correlations. There were several correlations among measures in athletes in addition to the one between SSRs and drops in SBP during the orthostatic challenge. High frequency power was positively correlated (r^2=0.58, p=0.01), and LF power positively correlated (r^2=0.40, p=0.1) with decreases in SBP during the sit-up test.

4.4 Discussion

Main findings. There were no significant differences in BP or HR between participants, but BP and HR changed significantly during the sit-up test. The decrease in HF power indicates that perhaps vagal withdrawal compensated for and minimized decreases in SBP in the seated position since decreased HF power and increased HR probably reflect baroreflex-mediated vagal withdrawal[71]. Low frequency power also decreased in the seated position reflecting reduced cardiac sympathetic control[71] and impaired baroreflex function[107]. Preservation of SSRs was not related to neurologic completeness of SCI. Both groups with complete and incomplete injuries included participants with no preservation and partial preservation. This suggests that spinal autonomic integrity may not be associated with neurologic severity of injury.

Cardiovascular responses. Cardiovascular responses to the orthostatic challenge were reflected by changes in HRV power. At rest there were no differences in LF and HF powers between participants with complete and incomplete injuries. This is in accordance with similar resting HR and BP between groups. Resting HR and BP were normal for this level of injury[5]. Heart rate was found to increase significantly during the transition from the supine to the seated position and this corresponded with decreases in HF power in participants with complete and incomplete SCI. Heart rate responses to orthostatic stress were due to baroreflex-mediated parasympathetic (vagal) withdrawal, which is usually intact following SCI[9]. The decrease in SBP in the seated position is also in agreement with the decreased LF power that reflects reduced cardiac sympathetic control[71] and baroreflex control following cervical SCI[204]. Exercise training has been shown to improve cardiovascular and autonomic function[205-209] and so in addition to time following injury, it is likely that training status also impacts the ANS. Based on the involvement of the sympathetic and parasympathetic components of the ANS during exercise, it may be expected that HRV indices may change following training[210]. Long-term training is known to induce sinus bradycardia in resting conditions, and a slower increase in HR at any degree of submaximal oxygen uptake as a result of a shift in sympathovagal balance[211] towards parasympathetic dominance[212]. Larger HF power has been demonstrated via spectral frequency analysis in aerobically-trained athletes[213-220]. Beneficial effects of training on HRV parameters

has been demonstrated previously in clinical populations as well, including post-myocardial infarction patients[221], heart transplant recipients[222], and individuals with SCI[205-207]. Some of the differences in HRV measures between our participants with sub-acute and chronic SCI may be attributed not only to time, but to fitness level as well.

Sympathetic skin response. Since the AIS is the standard assessment of motor and sensory function, it is not surprising that it was not related to severity of damage to descending autonomic spinal pathways as assessed by SSRs. There were two individuals, one of each with a complete and incomplete injury, who had no preservation of SSRs. There was also one individual with a complete SCI who had complete preservation of SSRs. However, no participants with neurologically incomplete SCI had complete preservation of SSRs. The extent of cardiovascular dysfunction and remaining spinal autonomic integrity are related to the degree of impairment of descending sympathetic pathways as indicated by SSRs. The AIS provides an excellent standard of assessment of motor and sensory pathways, but it does not evaluate spinal autonomic pathways, and thus, is not expected to correlate well with other indices of autonomic integrity[177, 223, 224]. Heart rate variability measures were not significantly different between groups which explains the heterogeneity of SSRs between them. Examination of SSRs and changes in SBP upon assumption of the seated position revealed that those with no preservation also experienced the larger drops in SBP regardless of neurological completeness of injury. Participants with intact palmar SSRs to median nerve stimulation had smaller changes in SBP than those that did not have any palmar SSRs. This is not surprising, since those with intact palmar SSRs may retain descending cardiac sympathetic control and descending autonomic sympathetic control of the vasculature of the upper body, and have better baroreflex function. The link between palmar SSRs and the ability to increase sympathetic activity and sympathetically-mediated vasoconstriction has been supported by previous findings of significant correlations between palmar SSRs, noradrenaline, HR and BP responses to an orthostatic challenge[5]. As an extension of work by Curt et al.[134] we also found that the presence of SSRs is dependent on the level of injury. Additionally, the two participants with no preservation had the greatest drops in SBP during the sit-up test. The only participant with complete preservation of palmar SSRs also experienced an increase in SBP in the seated

position. It appears as though our data, in agreement with previous studies[5, 134], suggest the possibility of identifying individuals with impaired BP control from SSRs, and that they, in combination with cardiovascular autonomic measures, provide insight on integrity of spinal autonomic pathways following SCI. Sympathetic skin responses are a valuable tool to assess autonomic function as evidenced by this study which illustrates that autonomic completeness of injury may be not associated with neurologic severity of SCI.

Symptoms of OH. Of the two participants that experienced OH, only one experienced symptoms. In agreement with other studies we showed that individuals with SCI may experience OH without any symptoms[5, 126, 177]. This is also observed in AB who have autonomic disturbances and probably results from protective alterations in cerebral autoregulation despite cerebral hypoprofusion[150, 178, 179]. Similar to a previous study[5], OH was only observed in participants with absent palmar SSRs following median nerve stimulation. All those who did not experience OH had at least partial preservation of palmar SSRs indicating that better integrity of spinal autonomic pathways facilitates better cardiovascular response to an orthostatic challenge.

Methodological considerations. Assessing elite athletes is unique and to accommodate training and competition schedules meant that it was not possible to examine them all at the same time of day. Testing was conducted at competition venues and not under ideal laboratory conditions but the same room was used consistently. It is not ideal to assess athletes following competition due to the stress associated with performance so they were given at least one hour between the end of competition and the start of testing, or they were assessed at least one hour prior to competition. The benefit of testing at competition venues is the concentration of athletes in one place, allowing for direct recruitment. This should not be overlooked as recruitment of persons with SCI, particularly elite athletes, is extremely difficult. Though athletes were not taking any medications that could affect their cardiovascular response to exercise, they may have been taking medications to combat spasticity, such as baclofen and ditropan. However, the incidence of cardiovascular side effects with these medications are very low, usually transient, and only associated with the start of treatment[225].

Lesion level may affect postural control making it difficult to passively transition from the supine to seated position during the sit-up test. Individuals with higher lesion levels that have reduced postural stability may require manual support to remain relaxed during the change in posture. Some were able to maintain proper posture independently and those who required assistance were provided manual assistance and support. When help was required it was provided without altering measures of BP by passively adjusting position or manually holding the participant in place.

Conclusions. The ANS has an important role in sporting performance because it controls HR and BP, both of which must increase to meet cardiovascular demands at the onset of activity[39]. Impaired cardiovascular response in athletes with SCI may negatively affect performance since cardiovascular function and athletic performance are tightly coupled[226]. As expected since all the participants in this study had cervical SCI, they all exhibited altered cardiovascular function and control in response to the sit-up test. Evidently, exercise capacity and sport performance may be affected by changes in the integrity of spinal autonomic pathways following SCI as a result of subsequent cardiovascular impairments. This study also shed light further on the potential association between neurologic and autonomic completeness of SCI. The integrity of spinal autonomic pathways may not be evident from AIS classification as demonstrated in this study and others, and thus, may not be related to neurologic completeness of injury. Damage to spinal autonomic pathways may be independent of injury to motor and sensory pathways of the spinal cord, so individuals with similar neurologic function may have entirely different autonomic integrity. Present functional classification of athletes based solely on motor function overlooks potential autonomic and cardiovascular dysfunction creating a mismatch of abilities. We propose that the assessment of spinal cord autonomic integrity of athletes with SCI could be an important addition to the evaluation of motor function so that all of the potential limitations to performance are captured.

Chapter 5: Conclusion

5.1 Summary of major research findings

Studies investigating SCI have increased dramatically, however, the number of studies with motor outcomes is overwhelmingly higher than those with sensory or autonomic outcomes[84]. This is despite the increasing awareness that following SCI, neurologic and autonomic dysfunctions may result. Fortunately, changes to cardiovascular autonomic function following SCI are well understood and have been explored extensively in the literature. Nevertheless, studies more specifically focused on the integrity of spinal autonomic pathways are less abundant[7]. Understanding changes in spinal autonomic integrity are important. The uncertainty about the association between neurologic and autonomic completeness of injury warrants consideration since the examination of one may not necessarily lend any insight about the other. Identifying factors that affect integrity of spinal autonomic pathways is important so that the relative contribution of these factors may help provide insight on changes to integrity following injury. To properly examine the changes to autonomic function after SCI, it is imperative that appropriate tests are used. Tests that are relevant to assessing the integrity of spinal autonomic pathways include non-invasive methods such as orthostatic challenges, HRV, SSRs, and the VM. Accordingly, the series of studies in this thesis used a clinical model of SCI to investigate the reliability of several tests of autonomic function and the agreement between two tests that evaluate orthostatic tolerance. Spinal autonomic integrity was examined in patients with sub-acute SCI and elite wheelchair rugby athletes with chronic SCI. The role of several factors that include lesion level, neurologic severity of SCI and time post-injury were considered for their impact on the integrity of spinal autonomic function.

The first study (Chapter 2) focused on the reliability of various tests of autonomic function including HRV, SSRs and the sit-up test, and the agreement between two types of orthostatic challenges. Sympathetic skin responses, cardiovascular responses to the sit-up test and several measures of HRV were found to be reliable. The sit-up test is a suitable alternative to the gold standard tilt-table test for assessing orthostatic tolerance in individuals with SCI. This is

beneficial since the sit-up test is a bedside assessment that may be easily used in any clinical setting. The sit-up test requires less strapping than the tilt-table test which is preferable to minimize and prevent BP changes unrelated to the orthostatic challenge. The methods used to assess autonomic function in this study proved to have moderate to high reliability in individuals with SCI and appropriate for use in the population with SCI. Though some HRV measures were found to have only fair reliability, our findings are supported by previous work that has found HRV to be reliable for use the population with SCI. Thus, the second study (Chapter 3) used these tests to examine spinal autonomic integrity in patients in the sub-acute stage of injury. Level and neurological severity of injury were related to autonomic completeness of SCI whereby individuals with cervical SCI and individuals with complete SCI had poorer cardiovascular responses to the sit-up test than their counterparts with thoracic SCI and incomplete SCI, respectively. This was also reflected in indices of cardiovagal and adrenergic baroreflex function derived from HR and BP measures during the VM. Patients with poorer cardiovascular function also had more greatly diminished integrity of spinal sympathetic pathways (preservation of SSRs), showcasing larger impairment to overall spinal autonomic integrity in comparison to those with better cardiovascular control and preservation of SSRs. Spinal autonomic integrity improved over time as patients also experienced changes to cardiovascular function during the sub-acute stage of injury. Overall, patients that had greater preservation of SSRs indicating better spinal sympathetic integrity had enhanced cardiovascular responses to an orthostatic challenge in comparison to their counterparts with poorer preservation. Based on experiences of OH and associated symptoms in the seated position, cardiovascular parameters were shown to improve over time in patients during the sub-acute phase. Lastly, the third and final study (Chapter 4) concentrated on spinal autonomic integrity in wheelchair rugby athletes with chronic SCI by examining cardiovascular responses to the sit-up test. Contrary to findings in the second study (Chapter 3), this investigation demonstrated that neurologic completeness is not related to autonomic completeness of injury. Unlike in Chapter 3, neurologic severity of injury, as indicated by the AIS, was not related to SSRs in the athletes. The individuals with chronic SCI in our study (Chapter 4) were also elite wheelchair rugby athletes. In comparison to the patients with sub-acute SCI (Chapter 3), the trained athletes had lower resting HR. Some of this may be attributed to the tachycardia seen in patients, but patients with incomplete SCI did not experience tachycardia and still had higher resting HR than the

athletes. These findings are in agreement with research showing that endurance training reduces resting HR[227]. In agreement with changes in HRV, the lower resting HR is explained by the larger HF power seen in the athletes with SCI. Whether these beneficial improvements in autonomic function are the result of changes of time or training should be investigated, although both factors likely contribute. This cannot be said definitively from our findings since we did not include individuals with chronic SCI who were not highly trained. Based on the literature of HRV in individuals with longstanding injuries[71], it appears as though HRV parameters do change over time since they have been found to be greater than that found in the patients tested in our study.

Sympathetic skin responses were still related to autonomic completeness in athletes with chronic SCI. Participants with the greatest preservation experienced smaller drops in SBP during the sit-up test than those with little or no preservation of SSRs, regardless of motor and sensory completeness of SCI. Current classification of athletes based on motor function and on-court observation of abilities without consideration of autonomic dysfunction that may impact sport performance is problematic. With the current lack of clarity about the association between neurologic completeness and autonomic completeness of SCI, knowledge about neurologic impairment provides little to no insight on autonomic dysfunction. Unfortunately, neglecting to acknowledge the role of autonomic dysfunction in sporting competition may negatively impact performance and/or encourage the use of illegal practices to enhance performance (e.g., boosting). As a result, this study highlights the importance of incorporating an assessment of autonomic function into the classification of athletes so that all factors that influence performance are captured and considered. Together, the studies of this dissertation provide insights into the reliability and validity of different tests of autonomic function and into the factors that affect the integrity of spinal autonomic pathways in different sub-populations of SCI.

5.2 Perspectives and limitations

An overarching goal of the current thesis was to investigate the changes to integrity of spinal autonomic pathways following SCI. Participants included individuals with different lesion levels and varying neurologic severities of injury at different stages of time post-SCI. Individuals with higher levels of injury showcased greater compromise to integrity of spinal autonomic pathways. Findings that the severity of autonomic dysfunction both varies along with and in contrast to neurologic completeness of injury have been demonstrated previously[1, 4, 5]. Time proved to be a major contributing factor to alterations in spinal autonomic integrity as it was different within the sub-acute stage of injury and between individuals with sub-acute and chronic SCIs. Our laboratory has stressed the importance of acknowledging and identifying autonomic dysfunctions following SCI[5, 12, 17, 40, 71, 228-230]. Given the results of these studies that illustrate lesion level, neurologic severity of injury and time impact integrity, it is important to acknowledge the possible changes to spinal autonomic pathways as this manifests in cardiovascular dysfunction following damage to the spinal cord. Alterations over time need to be understood such that, for example, changes in preservation of SSRs may provide insight on the potential for improvement of abnormal cardiovascular parameters and overall integrity of spinal autonomic pathways. The discrepancy found between studies in this thesis regarding neurologic and autonomic completeness warrant further investigation among individuals with SCI. Accordingly, more focus on changes to autonomic integrity are required, and surrogate measures of cardiovascular autonomic function and spinal sympathetic pathways have proven to be appropriate measures to investigate changes in the ANS. Applying stimuli above and below the level of the lesion with a battery of tests that evaluate both cardiovascular and sudomotor pathways provides information on the cholinergic and adrenergic sympathetic pathways of the ANS[7]. This approach provides a means to develop better documentation of the integrity of spinal autonomic pathways. Understanding changes in spinal autonomic function following SCI is important since lives are impacted immensely by its diminished integrity. Daily living is affected by autonomic dysfunction since compromised integrity to spinal autonomic pathways manifests as cardiovascular instability that leads to unstable BP (e.g., OH and AD). Performance outcomes are of concern in athletes with SCI and the illegal practice of boosting is a major concern. Autonomic dysreflexia has been documented to lead to catastrophic consequences, which is why

its practice has been banned by the IPC. Boosting may enhance performance, but it is unsafe and its use may represent the attempt of athletes with greater cardiovascular dysfunction and autonomic impairment to level the playing field.

There was a discrepancy between two studies about the association between neurologic and autonomic completeness of injury. In patients with sub-acute SCI, neurologic completeness was found to be associated with autonomic completeness. However, in athletes with chronic SCI, the opposite was found whereby motor and sensory completeness of injury was not associated with the degree of spinal autonomic integrity. The difference in findings between these studies may result from multitude of factors, but for these studies, likely the time post-injury is the largest contributing factor. Most of the recovery following SCI occurs within the first six months following injury[231], and since individuals in the second study (Chapter 3) were injured for less than six months at the time of both assessments, they could still be undergoing recovery of autonomic function. Another contributing factor could be the training state of the athletes. They are highly trained and it is known that exercise may beneficially influence autonomic balance in individuals with SCI[205, 206] and able-bodied individuals[232].

The studies of this thesis mostly involved individuals with SCI who are difficult to recruit. However, in accordance with previous investigations also examining cardiovascular measures and SSRs in individuals with SCI[5, 99, 150, 205, 207, 233, 234], our attained sample sizes are similar (n = 9 to 22), showcasing the strong recruitment of this population for our studies. Our sample sizes are still small in comparison to other similar studies in individuals with SCI and AB[3, 144, 235-238]. This caveat is acknowledged and larger sample sizes would have been ideal but were appropriate for our studies. For the majority of the data, the magnitude of the physiological response was large, and was consistent in all participants. This provides greater confidence that the measured responses are ‘real’ and not simply due to chance. Nonetheless, interpretation of the results should be accompanied with consideration to the sample sizes since type I error may be present.

Heart rate variability is a common method used to examine the function of components of the ANS because it is simple and non-invasive[120] and unlike other techniques, such as SSRs, it is able to tease out relative contributions of cardiovascular control mechanisms (sympathetic/baroreflex and parasympathetic). On the other hand, it is important to acknowledge the discrepancy in the literature regarding LF power[104, 107-109, 239]. Despite the lack of clarity and disputes about what LF power represents, it should be acknowledged that, overall, changes in measures of HRV, such as LF, indicate that there are alterations in autonomic function. More information on integrity of spinal autonomic pathways may be obtained when several methods are used to assess the ANS as demonstrated in the studies in this thesis (e.g., HRV, SSRs, VM, cardiovascular responses to orthostatic challenges). A battery of tests to assess autonomic integrity may provide a better overview of specific changes to the components of the ANS[7].

In addition to SSRs that only provide information on the frequency of the autonomic response, we could have also examined the latency and amplitude to determine the magnitude of the responses[113]. For the purposes of identifying the integrity of spinal autonomic pathways, the qualification of responses as present or absent were suitable for our analysis as seen previously in our laboratory[5] and in line with our attempt to use simple bedside assessments that are easily transferred to the clinic. The qualification of responses was suitable for revealing pertinent information on the preservation of sympathetic function, and subsequently, spinal autonomic integrity. Furthermore, other methods that could have supplemented the SSRs are those that quantify the sweat response. These include the quantitative sudomotor axon-reflex test (QSART) and the thermoregulatory sweat test (TST). The QSART assesses postganglionic sympathetic cholinergic sudomotor function by measuring the axon-reflex mediated sweat response. Sweat production is measured as an increase in humidity[240]. The latency of the initial sweat response and the amount of sweat produced are recorded. Though this test is non-invasive, it may cause some discomfort such as transient burning or stinging[241]. Thermoregulatory sweat testing evaluates the integrity of central and peripheral sympathetic sudomotor function from the central nervous system to the cutaneous sweat glands and is a test for malfunctioning of the sweat glands. Individuals are enclosed in a heated and humidified environment with infrared heating of the skin. The resulting cutaneous precipitation is monitored with an indicator due that is a

moisture-sensitive powder that changes colour[242]. The percentage and pattern of perspiration of the body is recorded. The pattern obtained is compared to a set of "normal" patterns (normally symmetrical), and deviance from these patterns may indicate abnormal perspiration distributions that are associated with specific disorders[240]. We chose to only assess SSRs since they are simple to examine at the bedside and are easily interpreted and analyzed to obtain important information on the integrity of sympathetic pathways.

An underlying goal of the current experimental design was to investigate spinal autonomic integrity following SCI. Using several methods to assess the ANS is helpful given the complexity of different bodily systems under its control. The strength of this experimental design unfortunately suffers when attempting to understand the mechanistic rationale for the physiological observations. Often, interpretation for the responses in humans is limited by assumptions and inference. Incorporating findings of the current studies into reductionist experimental designs could provide further insight into the mechanisms responsible for the cardiovascular autonomic dysfunction experienced by individuals following SCI. Unfortunately, reductionist approaches can suffer in their ability to fully consider the integrated nature of cardiovascular autonomic control. Ultimately both the reductionist and integrated models are imperfect, but a greater understanding of cardiovascular autonomic dysfunction following SCI is dependent on our ability to integrate findings from both approaches.

5.3 Future directions and overall conclusion

This thesis provides insight into the changes in integrity of spinal autonomic pathways in individuals with SCI, nonetheless, there are still unanswered questions that warrant further investigation. For instance, what are the changes in spinal autonomic integrity during the first year of injury? Although it is understood that most motor recovery occurs within the first six months post-injury, with the greatest rate of change occurring within the first three months[243], a similar map for the time course of changes in autonomic function is not known. An understanding of the alterations that take place from the acute to chronic stages of injury would

be useful with the consideration of how these changes vary according to lesion level and more interestingly, to neurologic severity of injury. It is acknowledged that this would be a logistically difficult study to conduct with a sufficient number of participants in total and with enough participants of varying lesion levels and severities of injury that they could be reasonably compared. Additional studies focusing on the time course of changes during the first year post-injury with assessments once a month would be a useful future direction.

The selection of tests to assess autonomic function is important and the first study (Chapter 2) helped demonstrate that the assessments used throughout this thesis were appropriate. It appears that when used in combination they are more beneficial to provide information that only one cannot. Together, HRV, SSRs, cardiovascular measures from the orthostatic challenges and the VM helped to identify changes in the parasympathetic and sympathetic components of the ANS and provided an overview of integrity of spinal autonomic pathways in individuals with SCI. This proved to be helpful since HRV, though a simple and non-invasive method of assessing autonomic function is a controversial measure with respect to its ability to identify the function of the sympathetic component of the ANS. Moreover, since both orthostatic challenges used in this thesis provided agreeable and similarly comparable cardiovascular responses, this ensured that both tests provided an appropriate orthostatic stimulus. Overall, results from the various tests used corresponded to one another to reflect the cardiovascular responses observed. For example, changes in LF power over time corresponded with indices of baroreflex function. Consequently, the changes in the Valsalva ratio and BRSa reflected the expected changes in HR and SBP during the initial and second sit-up tests. Changes in LF and HF power also reflected the HR and BP responses to the sit-up test. Additionally, preservation of SSRs indicated individuals with the greatest spinal autonomic integrity and cardiovascular function. Those with greater preservation initially had better cardiovascular control in response to an orthostatic challenge than those with little or no preservation. It would be of value to further investigate what other tests of autonomic function would be valuable to include in a battery of tests designed to identify individuals with autonomic impairment beyond those used in the studies in this thesis. An ideal combination would include tests that are simple bedside assessments which are valid and reliable.

The purpose of this thesis was to understand the integrity of spinal autonomic pathways following SCI. Lesion level, neurologic severity of injury and time post-SCI were demonstrated to affect integrity which ultimately leads to cardiovascular dysfunction. To achieve our purpose, we concentrated on changes in the sub-acute stage of injury and examined elite athletes with chronic SCI. Individuals had various levels and neurologic severities of injury so that the association between neurologic and autonomic completeness could be examined along with how it may be different at different stages of SCI. Tests used to assess autonomic function were determined to be appropriate for use in individuals with SCI in the first study. As such, with the use of these assessments in the final two studies of this thesis, it was demonstrated that during the sub-acute stage of injury, neurologic and autonomic completeness are associated with one another, and how this relationship changes over time is left to be investigated. Furthermore, the opposite is true for athletes with chronic SCI whereby autonomic completeness cannot be identified from neurologic completeness. The latter has implications for the classification of athletes since current classification does not include an evaluation of autonomic function. Together, these studies extend our understanding of spinal autonomic integrity following injury to the spinal cord. Future work is needed to expand our understanding of the changes in the ANS over time in individuals with varying severities of injury, and to promote fair play and safety for athletes with SCI given that neurological and cardiovascular changes may not be related.

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