

**Cardiovascular Disease Risk after
Spinal Cord Injury:
The Role of Autonomic Dysfunction**

by

Henrike Joanna Cornelie (Rianne) Ravensbergen

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Approval

Name: **Henrike Joanna Cornelia (Rianne) Ravensbergen**

Degree: **Doctor of Philosophy**

Title of Thesis: **Cardiovascular Disease Risk after Spinal Cord Injury: The Role of Autonomic Dysfunction**

Examining Committee: **Chair:** Angela Brooks-Wilson
Associate Professor

Victoria Claydon
Senior Supervisor
Assistant Professor
Department of Biomedical Physiology
and Kinesiology

Scott Lear
Supervisor
Professor
Department of Biomedical Physiology
and Kinesiology

Shubhayan Sanatani
Supervisor
Associate Professor
Department of Pediatrics
Faculty of Medicine, University of
British Columbia

Will Cupples
Internal Examiner
Professor
Department of Biomedical Physiology
and Kinesiology

Vaughan Macefield
External Examiner
Professor
School of Medicine
University of Western Sydney

Date Defended/Approved: August 7, 2013

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Abstract

Cardiovascular disease (CVD) is the leading cause for mortality and morbidity in those with spinal cord injury (SCI), with an earlier onset and more rapid progression compared to the general population. Lifestyle changes after injury have been suggested to be the main contributor to CVD risk, but I proposed that the issue is more complicated. Although less well-known, autonomic function is affected by SCI, in addition to motor and sensory dysfunction. Cardiovascular autonomic dysfunction is a particular concern in individuals with high lesions (above T5) due to the possible disruption of descending spinal sympathetic pathways to the heart and main vascular resistance bed. In this thesis, I propose that cardiovascular autonomic impairment plays a role in the elevated CVD risk. The thesis starts with an evaluation of the prevalence and progression of cardiovascular dysfunction after SCI. Then, the contribution of autonomic dysfunction on CVD risk is investigated. In addition, markers for obesity-related CVD risk specific to individuals with SCI and ECG markers for cardiac arrhythmias in relation to autonomic impairments are explored. Prevalence of cardiovascular dysfunction was found not to improve over time after injury and it was highest in those with lesions above T5. The second study showed that autonomic dysfunction contributes to overall CVD risk and specifically to glucose intolerance, either directly or through an interaction with physical activity levels. The data showed that waist circumference is the best marker for obesity considering ability to detect adiposity and CVD risk, and practicality of use. A specific cut-off for waist circumference was found to be lower compared to the general recommendations. The final study showed increased values for the ECG markers Tpeak-Tend variability, P-wave variability and QT variability index, only in those with impairments to descending cardiac sympathetic pathways. The ECG characteristics may be indicative of susceptibility to cardiac arrhythmia related to autonomic dysfunction. Implications of these findings are that management of cardiovascular autonomic dysfunction should remain a priority into the chronic phase of injury, not merely due the direct impact on quality of life, but also due to its contribution to the elevated cardiovascular disease risk after SCI.

Keywords: Spinal cord injury; autonomic nervous system; cardiovascular disease risk; obesity; cardiac arrhythmia; autonomic dysfunction

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List of Acronyms

Term	Definition
AD	Autonomic dysreflexia
AIS	American Spinal Injury Association (ASIA) Impairment Scale
ASIA	American Spinal Injury Association
AUC	Area under the curve
BMI	Body mass index
BPM	Beats per minute
BPV	Blood pressure variability
C1-7	1 st to 7 th cervical spinal levels
CVD	Cardiovascular disease
DAP	Diastolic arterial pressure
DEXA	Dual energy X-ray absorptiometry
ECG	Electrocardiogram
FES	Functional electric stimulation
HDL-C	High density lipoprotein cholesterol
HF	High frequency
HR	Heart rate
HR _{rest}	Resting heart rate
HR _{peak}	Peak heart rate
HRV	Heart rate variability
ISCoS	International Spinal Cord Society
L1-5	1 st to 5 th lumbar spinal levels
LDL-C	Low density lipoprotein cholesterol
LF	Low frequency
LF _{SAP}	Low frequency power of systolic arterial pressure
MRI	Magnetic resonance imaging
NC	Neck circumference
NTS	Nucleus tractus solitarius
OH	Orthostatic hypotension
PWD	P-wave dispersion
PASIPD	Physical activity scale for individuals with a physical disability
QTVI	QT variability index

RMR	Resting metabolic rate
ROC	Receiver operator characteristic
S1-5	1 st to 5 th sacral spinal levels
SAP	Systolic arterial pressure
SCI	Spinal cord injury
SFU	Simon Fraser University
SSR	Sympathetic skin response
T1-12	1 st to 12 th thoracic spinal level
TC	Total cholesterol
TDR	Transmural dispersion of repolarization
TG	Triglyceride
$T_{\text{peak}}-T_{\text{end}}$	Period from peak of the T-wave to the end of the T-wave
UTI	Urinary tract infection
VLF	Very low frequency
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio

Chapter 1.

Background

Spinal cord injury

Spinal cord injury (SCI) is defined as damage to the spinal cord that may be either traumatic or non-traumatic in nature. Spinal cord injuries are devastating not only to the individual's health, but also to their quality of life, resulting in loss of sensation, paralysis, and many other complications and co-morbidities. Other complications can include chronic pain, spasticity, mood disorders, fatigue, pressure sores, bowel and bladder dysfunction, and many other autonomic dysfunctions. Some of these complications or co-morbidities can be life threatening.

In people with SCI the spinal cord is not usually completely transected, since most injuries are commonly caused by compression, contusion or a combination of both, leaving the cord damaged, but not severed. The consequences of SCI are, therefore, diverse and dependent on the level and severity of the injury. To explain differences in dysfunction following injury, SCI is commonly classified as either a complete or an incomplete lesion. A complete SCI refers to injuries that cause the individual to have no sensory or motor function remaining below the lesion level, while after an incomplete SCI some sensory or motor function is preserved. In general, the higher in the cord that the injury is located, the greater the severity of dysfunction. Differences in level of dysfunction after SCI are also coarsely classified by the terms tetraplegia (previously termed quadriplegia) and paraplegia. Tetraplegia is an injury that leads to a loss of function that extends to all four extremities, and paraplegia is an injury with preserved motor and sensory function in the upper extremities.

Incidence in Canada

The incidence of SCI in Canada was estimated at 4,071 new cases per year, in 2010¹. Of those cases 42% were sustained following trauma, and 58% were non-traumatic in nature (due to other disease). The prevalence of SCI in Canada (how many individuals currently live with SCI in Canada) was estimated at 85,556, of which 51% was traumatic and 49% non-traumatic in nature. The incidence of SCI, both traumatic and non-traumatic, is related to age. For traumatic injuries the incidence is bimodal, showing the first peak in adolescents and young adults (15-29 years) when injuries are mostly a result of motor vehicle accidents, sporting accidents or violence². The second peak lies at the older adult stage (over 70 years), when injuries are mostly a result of falls² (Figure 1.1A). For non-traumatic SCI the incidence rate steadily increases with age³ (Figure 1.1B). The incidence of SCI is also related to sex. In both traumatic and non-traumatic SCI, and at all ages, the incidence is higher for males than for females¹ (Figure 1.1). Whether and how the incidence of SCI in Canada has changed over time is not entirely clear. A study in the province of British Columbia showed a fairly constant incidence of traumatic SCI from 1995 to 2004⁴, while a study in Ontario showed that the incidence almost doubled from 1997 to 2000⁵. Neither of these studies report changes over time in the incidence of non-traumatic SCI, but taking into account the increasing incidence with age and the aging population, an increase in incidence over time seems likely¹.

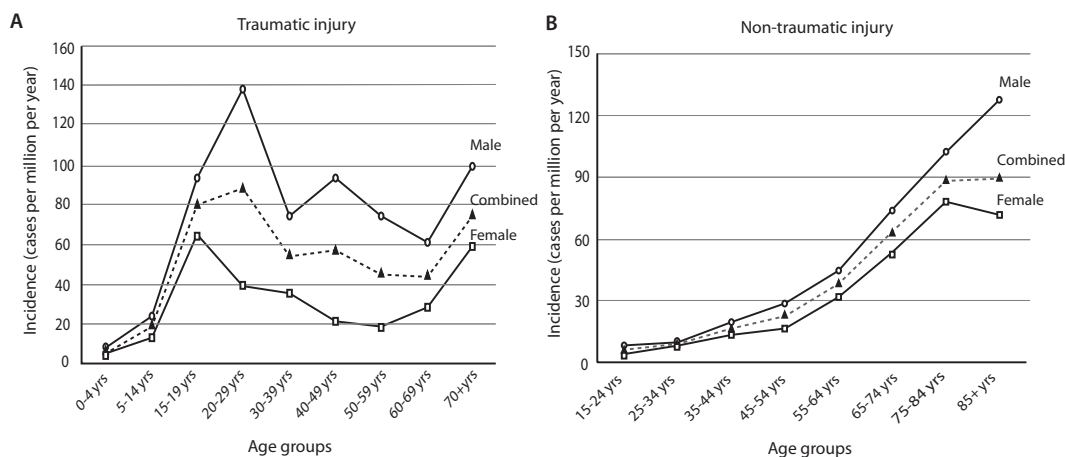


Figure 1.1. Incidence rate of spinal cord injury in Canada according to age and sex

A. Incidence of traumatic injury. Adapted with permission from Dryden et al. 2003².

B. Incidence of non-traumatic injury. Adapted with permission from New et al. 2008³.

The economic burden associated with SCI is quite substantial, as it places a financial burden on the individual, the health care system, and society as a whole. There are both direct costs, associated with acute health care, necessary equipment, and long-term care, and indirect costs due to loss of income, caregiving by family members, and premature mortality. The total lifetime cost per individual with SCI in Canada was estimated at \$1.6 million for paraplegia and \$3.0 million for tetraplegia, totalling \$3.6 billion for all current traumatic SCI cases in Canada⁶.

Care and cure

The main obstacle in finding the 'cure' for SCI is the limited ability of the central nervous system to regenerate damaged tissue⁷. Interestingly, regeneration of the nerve axon is very common in peripheral nerves, leading us to believe that there are features of the environment of the central nervous system, especially after injury, that limit the ability of axon regeneration. Amazing effort from the basic science field has been and still continues to be put towards identifying these factors in the CNS that inhibit regeneration and towards finding the cure for SCI by overcoming these factors^{7,8}. At the moment a full cure for SCI is lacking, but much insight into regeneration mechanisms has been gained in recent decades. In the absence of a cure for SCI, other pathways are followed to improve functional and quality of life outcomes after SCI.

Care after SCI starts even before coming to the hospital and the emergency room, where spinal immobilization helps prevent secondary injury to the cord after the initial spinal trauma⁹. The next step is the acute care in the hospital, where the two main goals are stabilizing the spinal column and decompression of the cord. In recent years much research has been done into surgical techniques with respect to spinal fixation, decompression, and the optimal timing of surgery¹⁰.

The primary injury to the cord due to direct impact is not the only concern after SCI. A cascade of events such as ischaemia, inflammation, and immune responses that expand the injury site occurs, leading to secondary injury. Neuroprotection strategies aim to reduce further damage to the cord due to these events. Pharmacological treatment in the acute phase of SCI is one of these strategies, and is mostly aimed at limiting secondary injury by controlling the inflammatory response. Steroids like

methylprednisolone have been widely used, but more recent studies have raised concerns about potential toxicity and complications, and it is therefore no longer standard treatment in Canada^{8,11,12}. In recent decades extensive research has focused on new pharmacotherapies after SCI, including investigations into the use of antibiotics and the calcium-channel blocker riluzole as neuroprotective agents¹². Unfortunately, a standard, optimized treatment strategy is not currently in place. Another aim for pharmacotherapy in the acute stages of SCI is to address the severe hypotension due to spinal shock and related extreme hypovolemia in the acute phase of injury.

After the acute care, most individuals will stay in an in-patient rehabilitation centre with the goal of restoring or improving function, and preparing the individual by teaching them the skills necessary to return to independent living. The beneficial effect of rehabilitation is probably mediated through neuroplasticity of the spared pathways in the cord. Rehabilitation and other strategies can help in the development of new beneficial connections of the intact pathways in the cord in order to take over functions of the damaged pathways. Due to extensive research in this field, many advances have been made in rehabilitation therapy for individuals with SCI in the past 25 years. Most prominent are technologies such as functional electric stimulation (FES) and robotic bodyweight-supported treadmill training. FES is a technique that uses electrical stimulation of the peripheral nerves via surface electrodes (although implanted options are available) to generate muscle contractions. It can be used on the extremities for exercise training, but research has also been conducted to investigate its use for respiratory muscles, as well as to improve bladder, bowel and sexual function¹³. Robotic body-weight supported treadmill training is slowly replacing locomotor training manually performed by physiotherapists. The length of stay at in-patient rehabilitation centres has decreased in the US and Canada potentially due to the advances in technology described above¹⁴.

A decrease in mortality following SCI is consistently reported in many countries over recent decades; most likely due to advances made in acute care¹⁵. However, despite this improvement, compared to the general population the mortality rates are still approximately three times higher in individuals with SCI¹⁴. The life expectancy for

individuals living with SCI has increased over time, similar to the general population, but it remains lower compared to an age-matched able-bodied population¹⁵.

Secondary complications of spinal cord injury

The first documented report of SCI was written approximately 2,500 years ago, when two cases of SCI were reported in the Egyptian Edwin Smith papyrus. The condition was determined as “an ailment not to be treated”, and thus most people would die shortly after sustaining the injury. Surprisingly, this advice was followed for millennia, until the early part of the 20th century. It was only after the Second World War, when many advances in surgical techniques and acute care were made, that the outcome of the condition changed dramatically. We have now reached a stage where people live fairly long lives with SCI, but as a result the challenges now lie more in the secondary complications after SCI.

Rehospitalization after SCI due to secondary complications occurs frequently, especially in the first year after discharge from rehabilitation¹⁶. The most common reasons for rehospitalization are urinary tract infections (UTI), chronic pain, pressure ulcers, or respiratory complications (e.g. pneumonia)¹⁶. Bladder dysfunction is very common in individuals with SCI as the autonomic parasympathetic pathways supplying the urinary bladder, exit at the sacral level of the spinal cord¹⁷. Both urine storage and voiding can be impaired due to dysfunction of autonomic control mechanisms (referred to as neurogenic bladder), so most people with SCI use some form of catheterization in order to void. Inserting the catheter evidently increases the infection risk in the urinary tract.

Chronic pain is a complicated issue after SCI; it can have many different underlying causes, ranging from nociceptive pain that originates in the periphery, to neuropathic pain that is generated by changes in the central nervous system. Most nociceptive pain can be treated as long the necessary time is taken to find the right approach (e.g. physiotherapy or medication etc.). Neuropathic pain is generally extremely difficult to manage. Pharmacological treatment designed to target the hyper-excitability of neurons (e.g. opioids, antidepressants or anti-epileptics) is commonly used in both acute and chronic phases¹⁸. Research is ongoing into the effects of electrical nerve stimulation,

spinal cord or even brain stimulation, but the use of these treatments remain controversial¹⁹.

Pressure ulcers or pressure sores are lesions of the skin and underlying tissues due to prolonged, uninterrupted pressure for example due to being 'bedridden' in hospital. It can lead to large open wounds that have long healing times due to poor circulation as a consequence of SCI. During rehabilitation, it is important to educate individuals with SCI about pressure sores and how to prevent them after discharge from rehabilitation. Much can be done to prevent pressure sores with self-management, trained caregivers, pressure-release routines, and the use of specialized equipment that reduces or redistributes pressure.

Respiratory complications are a major reason for rehospitalization due to the seriousness of the problem. Diminished expiratory function due to SCI impairs the effectiveness of coughing immensely and therefore increases the susceptibility to respiratory tract infections²⁰.

Many secondary complications after SCI are related to autonomic dysfunction, which impacts most organs. This can lead to bladder and bowel dysfunction, sexual dysfunction, and altered thermoregulation and metabolism. Up until the past decade, autonomic dysfunction has been a 'forgotten' topic in SCI research. Most research focuses either on finding a "cure" for paralysis, or improving motor and/or sensory function after SCI. In 2004, Anderson conducted a survey of 681 individuals living with SCI, asking them to rank a list of functions in order of importance to their quality of life²¹. She showed that regaining arm and/or hand function was the highest priority for individuals with tetraplegia. For both individuals with paraplegia and tetraplegia, regaining sexual function, bowel and bladder function, and eliminating autonomic dysreflexia (AD, episodes of dramatic increases in blood pressure unique to SCI) had the highest priority when both first and second ranked functions were combined (Figure 1.2). This large survey highlights that autonomic function (including sexual, bowel, bladder and cardiovascular function) has a large impact on quality of life for those with living with SCI, and should be a priority for research.

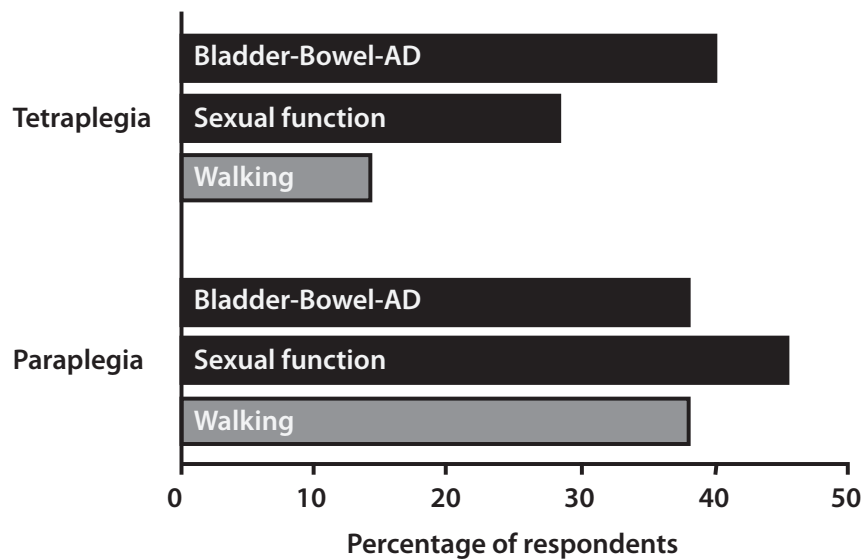


Figure 1.2. Functional priorities for the improvement of quality of life for individuals with spinal cord injury.

Percentage of individuals who ranked these functions as their first or second most important function to improve quality of life. AD, autonomic dysreflexia, one form of cardiovascular dysfunction encountered by individuals with SCI. Adapted with permission from Anderson 2004²¹.

Cardiovascular disease (CVD) is one of the major complications after SCI. It is the leading cause of morbidity and mortality in chronic SCI, and it has an earlier onset and more rapid progression than in the general population²². One obvious factor contributing to the high cardiovascular morbidity and mortality after SCI is the more sedentary lifestyle and reduced physical function that is associated with the loss of motor function after injury^{23,24}. This lifestyle change most likely contributes to the elevated cardiovascular risk factors (e.g. unfavourable lipid profile, obesity, and insulin resistance) that have been found in this population^{25,26}. However, SCI can also cause disruption of cardiovascular autonomic pathways, disturbing the normal cardiovascular control mechanisms. Although evidence is growing to support the concept that autonomic disturbances might influence CVD risk, it remains unclear how the different factors contribute to the development of increased risk of CVD²². My thesis will focus on the role of autonomic dysfunction in the increased risk of CVD after SCI, as well as examining the possible interactions between autonomic impairment and lifestyle changes.

Autonomic function after spinal cord injury

Before we examine the pathophysiology of the autonomic nervous system after SCI, it is prudent to consider the normal anatomy and physiology of this system.

Autonomic pathways

The autonomic nervous system is comprised of two generally opposing components: the sympathetic and the parasympathetic nervous systems. The parasympathetic nervous system is primarily involved in maintaining the resting state of the body, for example by slowing down the heart rate. The sympathetic system on the other hand is mostly excitatory in nature and is involved in fight or flight responses, for example by increasing blood pressure and heart rate.

The cell bodies of the preganglionic sympathetic neurons are located in the intermediolateral column of the thoracic and upper lumbar spinal cord (T1-L22)^{17,27}. The sympathetic preganglionic axons exit the spinal cord via the ventral root at different spinal levels to synapse onto the postganglionic neurons in the paravertebral and prevertebral ganglia^{17,27}. The paravertebral ganglia are interconnected by the sympathetic trunk and lie on either side of the vertebral column. The prevertebral ganglia are not connected and lie ventral to the vertebral column. All sympathetic ganglia lie remotely from their target organs, in contrast to the parasympathetic ganglia^{17,27}. Most parasympathetic fibres exit the central nervous system above the level of the spinal cord, via the cranial nerves, although there are parasympathetic pathways that travel through the spinal cord to the sacral levels and innervate the pelvic viscera and genitals^{17,27}.

The autonomic ganglia are complex structures where pre- and postganglionic neurons are connected via synapses. Pre- and postganglionic neurons do not appear in a one-to-one ratio in the ganglia, and so there is not a direct connection from one specific preganglionic to one specific postganglionic neuron. Mechanisms of divergence and convergence play a role in these connections. Divergence is a mechanism by which one preganglionic neuron connects to multiple postganglionic neurons. The role for divergence is believed to be to distribute a central signal widely across peripheral neurons with the use of minimal central neurons¹⁷. Convergence is the opposite process by which one postganglionic neuron receives input from multiple preganglionic neurons. It remains unclear what the functional explanation is for this mechanism¹⁷.

An overview of both sympathetic and parasympathetic pathways is shown in Figure 1.3.

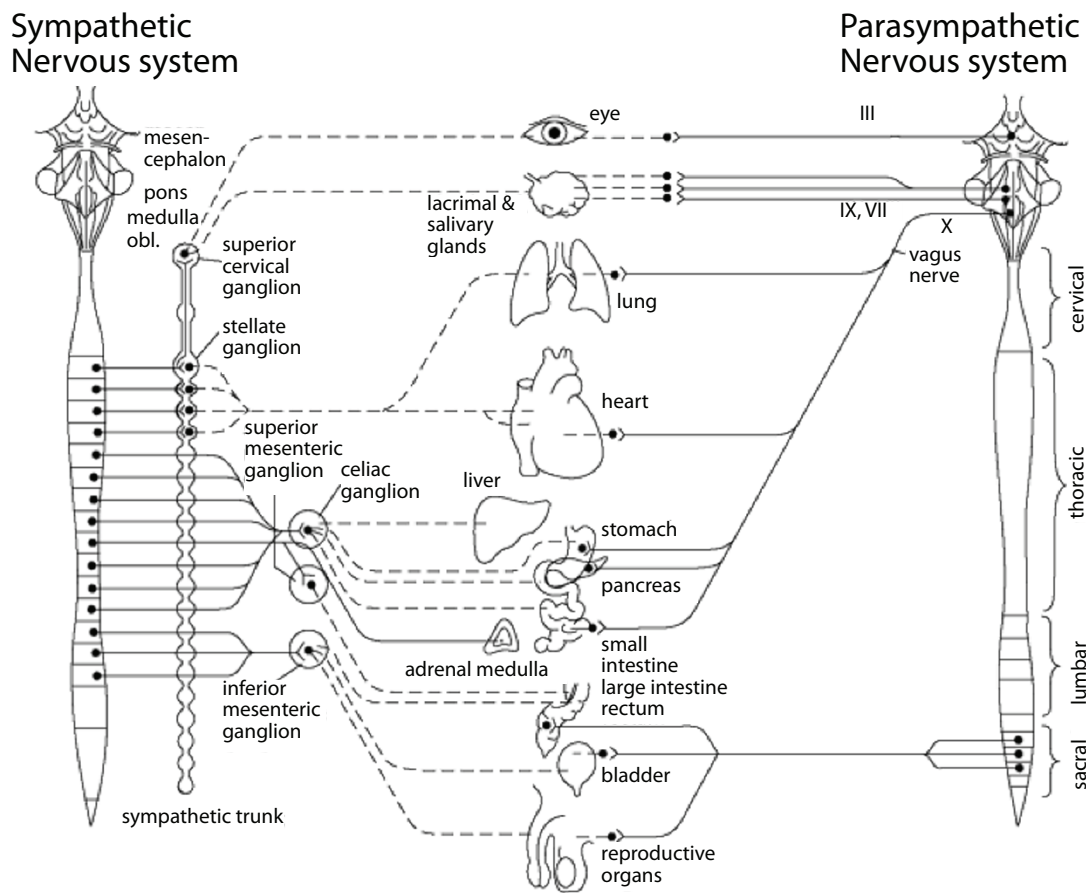


Figure 1.3. Overview of the anatomy of the autonomic nervous system

The continuous lines represent preganglionic neurons; the dotted lines represent postganglionic neurons. Abbreviations: III: oculomotor nerve; VII, facial nerve; IX, glossopharyngeal nerve; X vagus nerve. The sympathetic outflow to the skin, vasculature and deep somatic structures are not shown. Adapted with permission from Jänig 2006¹⁷.

Given the high priority of autonomic dysfunction for individuals with SCI, coupled with the high cardiovascular morbidity and mortality, this thesis will focus on cardiovascular autonomic control. The next paragraph will discuss the autonomic control of the cardiovascular system.

Cardiovascular autonomic pathways

The two major components of the cardiovascular system are the heart and the blood vessels. The heart is innervated by both the sympathetic and the parasympathetic nervous system, while the vasculature almost exclusively receives sympathetic innervation. The parasympathetic preganglionic neurons that innervate the heart travel through the vagus nerve (cranial nerve X) and exit the central nervous system above the spinal cord at the level of the brainstem. The postganglionic neurons lie in the cardiac ganglion, close to the heart, to then act on the sinus and atrial-ventricular nodes, the Purkinje fibres, and the atria. The heart receives sympathetic input from preganglionic neurons that lie in the upper thoracic spinal cord (T1-T5), via the postganglionic neurons in the paravertebral ganglia. The blood vessels are innervated by sympathetic fibres exiting the spinal cord from the levels T1-L2, depending on the region in which the blood vessels lie. The splanchnic vascular bed is particularly important in cardiovascular control due to its capacitance and resistance function, and potentially even more so after SCI when skeletal muscle mass in the lower extremities is reduced and this region therefore becomes less important in terms of control of total peripheral resistance. The splanchnic bed consists of highly compliant vessels and contains around 20-25% of the total circulating blood volume at rest²⁷. It is, therefore, the most important capacitance bed in the body. Sympathetically induced vasoconstriction of the arteries of the splanchnic bed causes a large reduction in blood flow, a vast increase in total peripheral resistance, and thus an increase in blood pressure. At the same time vasoconstriction of the veins reduces the blood volume in the region, leading to increased venous return, and thus increases blood pressure via the increased cardiac preload²⁷. Sympathetic preganglionic nerves originating in the spinal cord at the levels T6-T10 come together in the celiac ganglion where the postganglionic nerves exit to innervate this vascular bed¹⁷. See Figure 1.4 for an overview of the cardiovascular autonomic pathways.

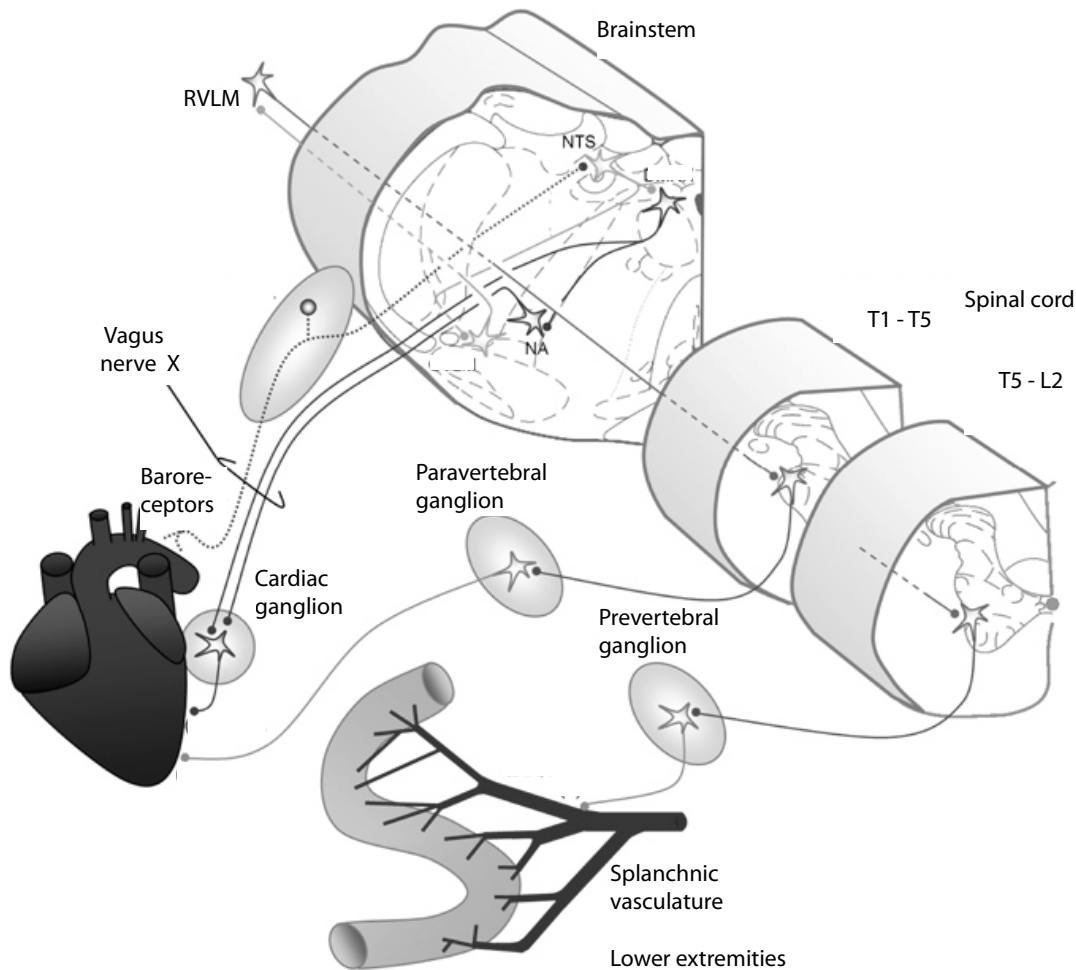


Figure 1.4. Overview of cardiovascular autonomic pathways

Parasympathetic input to the heart runs via the vagus nerve (cranial nerve X) that exits at the level of the medulla, and the cardiac ganglion close to the heart. Baroreceptor afferents travel through the vagus nerve to reach the nucleus tractus solitarius. Sympathetic input to the heart originates from preganglionic neurons in the spinal cord at levels T1-T5 and reaches the heart via a paravertebral ganglion. The vasculature in the splanchnic region and the lower extremities receive sympathetic input from spinal levels T5-L2 via prevertebral ganglia. Abbreviations: RVLM; rostral ventrolateral medulla; NTS, nucleus tractus solitarius; NA, nucleus ambiguus. Adapted with permission from Inskip 2009²⁸.

Effects of spinal cord injury on cardiovascular autonomic pathways

Considering this introduction to the anatomy of the cardiovascular autonomic pathways, the following section will discuss the effects, including both acute effects of injury and adaptations over time, that SCI can have on these pathways and how it disrupts normal function. Differences between lesion levels and completeness will also be addressed.

Acute effects on cardiovascular autonomic control

SCI has an acute effect on cardiovascular autonomic control by separating the autonomic pathways below the injury from supraspinal control. The normal functioning of these pathways and the reflexes that use these pathways will, therefore, be affected. The most important reflex mechanism of cardiovascular autonomic control is the arterial baroreflex, which plays an important role in the regulation of blood pressure.

Arterial baroreflex and blood pressure control

As noted previously, normal baroreceptor reflex function (Figure 1.5) can be interrupted by damage to cardiovascular autonomic pathways in the spinal cord after SCI; the extent of impairment is dependent on the lesion level. Baroreceptors are a type of mechanoreceptors located in the carotid sinus, the aortic arch, and the coronary arteries^{29,30}. Depolarization of the baroreceptors results from stretch of the arterial wall due to an increase in blood pressure. This signal originating from the baroreceptors travels to neurons in the nucleus tractus solitarius (NTS), and modulates two efferent components: a pathway limiting sympathetic excitation and a cardio-inhibitory pathway³¹. The first pathway involves increased activity from inhibitory neurons in the NTS, directed, via interneurons, towards neurons in the rostral ventrolateral medulla that without this inhibition would cause excitation of the preganglionic sympathetic neurons in the spinal cord. Inhibition of this pathway ultimately leads to limited excitation of the postganglionic sympathetic neurons innervating both the vasculature and the heart, causing vasodilation and a decrease in heart rate and force of contraction³². The cardio-inhibitory pathway includes the input from the NTS to vagal preganglionic neurons located in the nucleus ambiguus. These neurons project onto cardiac postganglionic vagal neurons that inhibit cyclic AMP production in the sinus node, and thus inhibit the open probability of the hyperpolarized-activated cyclic nucleotide-gated (HCN) channels, which are in turn responsible for the diastolic depolarization of the pacemaker cells of the sinus node³²⁻³⁴. This slowing down of the diastolic depolarization of the sinus node results in a slower heart rate³²⁻³⁴. This pathway remains intact after SCI as it lies completely outside the spinal cord. The sympathetic pathway on the other hand can be affected by the injury; the input from the NTS cannot reach the spinal sympathetic neurons below the lesion. Depending on the level of the lesion some preganglionic sympathetic neurons can still be reached. As the cardiac

preganglionic neurons are located in the spinal levels T1-T5, lesions below T5 will leave these pathways intact, but lesions in this region can partially damage them, and cervical lesion can completely disrupt these pathways. As noted previously, sympathetic control over the splanchnic vasculature is extremely important for blood pressure control and SCI above T6 can therefore lead to pronounced impairments to blood pressure control. Completeness of injury is an also important factor, as these impairments to autonomic control only occur when the lesion affects spinal sympathetic descending pathways.

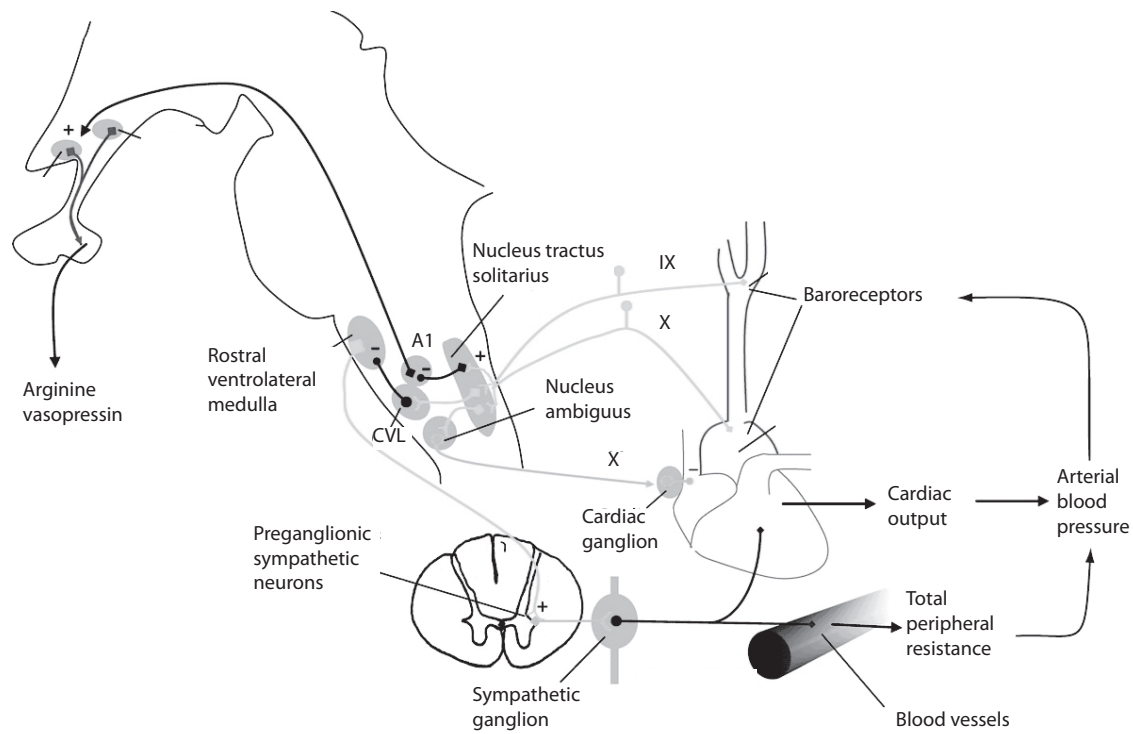


Figure 1.5. Overview of the baroreceptor reflex

Baroreceptors send signals to the nucleus tractus solitarius resulting in adaptations in sympathetic activity (via the rostral ventrolateral medulla and the spinal cord) as well as the parasympathetic activity (via the nucleus ambiguus and the vagus nerve). Changes in autonomic activity result in changes in cardiac output and total peripheral resistance, and thus blood pressure. Baroreceptors respond to these changes in blood pressure, completing the feedback loop. Abbreviations: A1, group of noradrenergic cells; CVL, caudal ventrolateral medulla; IX, glossopharyngeal nerve; X vagus nerve. Adapted with permission from Benarroch 2008³¹.

Adaptations to cardiovascular autonomic pathways

In addition to the acute effects of SCI on cardiovascular autonomic pathways, interruption of these pathways leads to adaptations in many structures involved in autonomic control. Damage to sympathetic pathways reduces overall sympathetic

activity and therefore catecholamine levels (specifically the neurotransmitter noradrenaline). However, the reduced input to the α -adrenoreceptors due to reduced activity from sympathetic postganglionic axons^{35,36}, makes those receptors more sensitive to noradrenaline. This hypersensitivity has been shown in both animal and human experiments. In humans it is known that individuals with cervical SCI have an enhanced pressor response to noradrenaline^{37,38}. These studies showed that the blood pressure response to a known concentration of noradrenaline was larger, and the concentration needed to increase blood pressure by a given amount was less in individuals with cervical SCI, indicating hypersensitivity of the α -adrenoreceptors^{37,38}. Another study showed α -adrenoreceptor hyperresponsiveness by determining the concentration of noradrenaline required to vasoconstrict the dorsal foot vein to half of the baseline diameter³⁹. They found that the concentration needed was six to seven times lower in individuals with cervical SCI compared to able-bodied controls³⁹.

In addition, sympathetic preganglionic neurons undergo morphological changes in response to reduced input from supraspinal centres. In a small study in humans, atrophy of the soma of sympathetic preganglionic neurons early after injury has been shown, which recovers with time after injury⁴⁰. The recovery can possibly be explained by sprouting of primary afferents in the dorsal root ganglion, which increases the input to the sympathetic preganglionic neurons⁴⁰. Investigations in rats have shown that the primary afferents in the dorsal root ganglion indeed show sprouting after complete spinal transection^{41,42} as well as after a severe compression induced SCI⁴³.

Other adaptations that contribute to counteract the limited ability to vasoconstrict due to damage to cardiovascular sympathetic autonomic pathways are seen in endothelin-1 and angiotensin II function^{44,45}. Endothelin-1 is an endothelium-derived factor that causes vasoconstriction and thus affects peripheral resistance. Thijssen et al. showed that blocking the receptors for endothelin-1 induced an increase in blood flow (e.g. vasodilation) in individuals with SCI, but not in able-bodied controls, suggesting an increased contribution of endothelin-1 to vascular tone⁴⁴. The same study showed that an exercise program reduced the effects of endothelin-1 on vascular tone, suggesting that the adaptations are in response to inactivity after SCI⁴⁴. Similarly angiotensin II is a hormone that causes vasoconstriction, but in able-bodied controls it does not regularly

contribute extensively to vascular resistance during orthostatic stress. In individuals with SCI, it has been shown that the effects of angiotensin II on vascular resistance are enhanced as the same blood pressure increase is reached with lower concentrations of angiotensin II^{38,45}.

Consequences for cardiovascular function

The main concerns of the altered cardiovascular autonomic control after SCI are the unopposed vagal activity to the heart, and the lack of sympathetic control over the vasculature. The consequences are most detrimental after lesions above T5, when sympathetic control over the splanchnic vascular bed is lost. Also, lesions above this level can lead to damage to the sympathetic control over the heart. This section describes how the functionality of the cardiovascular system is altered as a consequence of the changes in autonomic control.

Altered cardiac function

The unopposed vagal activity to the heart brings about a low resting heart rate and a limited heart rate reserve. These heart rate changes are accompanied by a decrease in contractility of the cardiac tissue, negatively affecting stroke volume and cardiac output^{46,47}. After a complete lesion of the autonomic pathways above T1, the predominant mechanism intact to increase heart rate (e.g. in response to exercise) is the withdrawal of the vagal tone. It has been shown that with maximum vagal withdrawal in the absence of sympathetic activity, the maximum heart rate is around 100 beats per minute⁴⁸. This is similar to peak heart rates found in individuals with cervical SCI⁴⁹.

Consequences of altered blood pressure control

Three main consequences of impaired blood pressure control after SCI are: a low supine blood pressure; orthostatic hypotension; and autonomic dysreflexia.

Low supine blood pressure

At rest in the supine position, the unopposed vagal activity to the heart and the sympathetic hypoactivity to the vasculature innervated by spinal sympathetic nerves below the lesion level, lead to a lower than normal blood pressure. Sympathetic activity at rest is generally low (but dependent on the severity of the injury), as quantified by

low resting plasma noradrenaline and adrenaline levels in individuals with a cervical SCI⁵⁰. These low blood pressures are seen in individuals with either acute or chronic SCI. In the acute phase, when the individual goes into spinal or neurogenic shock, the muscles undergo flaccid paralysis and neurogenic shock induces profound vasodilation and associated severe hypotension^{51,52}. However, neurogenic shock is not the only cause for the lowered blood pressure, because this phenomenon is seen in individuals with chronic SCI when the neurogenic shock has been relieved. Supine resting systolic arterial pressures between 95 and 114 mmHg in individuals with cervical lesions are reported, significantly lower than able-bodied controls⁵³⁻⁵⁶. In reality, values for resting blood pressure can actually be much lower taking a few considerations into account. These values are group averages including individuals with complete and incomplete lesions, who can have a normal supine blood pressure. Also, the clinical standard for blood pressure measurement is to record blood pressure in a seated position. The research studies in SCI populations normally record blood pressure in a supine position, because there is the risk for orthostatic hypotension and fainting in the seated position.

Orthostatic hypotension

In addition to a low supine blood pressure, individuals with SCI can also experience orthostatic hypotension (OH, see the example trace in Figure 1.6A). This refers to a further drop in blood pressure that occurs with a change in posture, often leading to symptoms such as light-headedness, dizziness and nausea, and can ultimately result in syncope (fainting). OH is traditionally defined as a decrease in systolic arterial pressure of 20 mmHg or more, or in diastolic arterial pressure of 10 mmHg or more⁵⁷ upon the transition from a supine to an upright posture. The mechanism underlying the development of OH in individuals with SCI is not well understood, however, there are likely multiple contributing factors. One of those factors is the disturbance of the normal baroreceptor reflex (Figure 1.5). A blood pressure drop due to venous pooling in the legs during a postural change is sensed normally by the baroreceptors, but when the reflex loop is interrupted in the spinal cord, heart rate and vascular resistance will fail to increase. Another contributing factor could be the enhanced venous pooling due to diminished skeletal muscle pump activity as a result of paralysis⁵⁰. During activation, skeletal muscles compress the veins and thus promote venous return to the heart when upright. This pumping action of the muscle is an important means of maintaining cardiac

output during orthostatic stress in able-bodied individuals⁵⁰. The blood flow to the extremities is highly affected by muscle mass, and active muscle needs a greater blood supply. Blood flow into the paralyzed legs would therefore be decreased⁵⁸, but the effect of losing the muscle pump activity seems to override this decrease in blood flow. Spasticity in the muscles below the lesion is common after SCI and can be seen as a type of muscle pump activity (although not under voluntary control), but the influence of spasticity on venous return and OH is unknown.

Given the changes in blood pressure control described above, the problem of OH after SCI could be expected to be even more severe than reported. The most likely explanation for this less than expected severity of OH, is an increase in peripheral resistance during orthostatic stress, which appears despite the loss of sympathetic control over those vessels⁵⁹. Different mechanisms can potentially underlie this increase in peripheral resistance. Recent studies have ruled out the following mechanisms: a release of catecholamines from the adrenal medulla, because plasma catecholamine levels are low at rest and do not improve during orthostatic stress⁵⁰; reduced responses to endothelium-derived nitric oxide⁶⁰. Although an increase in catecholamine levels in response to orthostatic stress has been refuted, the α -adrenoreceptor hypersensitivity that occurs after SCI induces a stronger vasoconstriction response to a given plasma catecholamine concentration³⁹. The increased action of endothelin-1 and angiotensin II also contribute to the increased peripheral resistance during orthostatic stress^{44 45}.

The repeated episodes of hypotension that occur throughout the day have severe implications for quality of life, leading to feelings of general fatigue^{61,62}, difficulty in participating in activities of daily living⁶³ and even impaired cognitive function^{64,65}. OH is a very prevalent condition in individuals with lesions above T5, reported to occur in around 50% of the population⁵³. Another major implication of this condition is the fact that it interferes with rehabilitation. Illman et al. showed that the prevalence of OH was as high as 73.7% during rehabilitation sessions⁶³. In 33% of these sessions the signs and symptoms of OH were so severe that they were perceived as limiting the treatment⁶³.

Autonomic dysreflexia

In contrast to the two conditions described above, AD encompasses episodes of extremely high blood pressure evoked by sensory stimuli from below the lesion (see an example in Figure 1.6B). Systolic arterial pressures as high as 250-300 mmHg and diastolic arterial pressures of 200-220 mmHg have been reported⁶⁶. These episodes can be evoked by many different sensory stimuli, both noxious and non-noxious, such as a full urinary bladder, bowel stimulus, ingrown toenails, sexual stimuli, pressure sores or a bone fracture among many more⁶⁷. Common symptoms are pounding headache, sweating and flushing above the lesion and the opposite, goose bumps and pallor below the lesion. However, in some cases individuals with SCI do not experience any symptoms during an episode of AD. This is called 'silent' AD, and it is actually a very dangerous presentation of AD, because an asymptomatic individual will not seek treatment, and the hypertension can be left unchecked⁶⁸. AD is a condition that is specific to those with lesions above T6, because these lesions lead to impairment of sympathetic splanchnic vascular control and thus have the largest effect on blood pressure control⁶⁹.

A clear definition of AD has not yet been established. The first description of the condition involved symptoms such as sweating, and a rash of the head and the neck⁷⁰. Only much later did researchers start to include a measure of blood pressure into their criteria⁷¹, but the magnitude of the blood pressure increase considered diagnostic for AD still varies widely, ranging between a 20 to 40 mmHg rise in systolic pressure⁷². Including a measure of the magnitude of the blood pressure rise seems necessary, particularly given that symptoms are not always reported, as discussed above. The reported prevalence of AD in individuals with lesions above T6 is dependent on many factors, such as the definition used, research technique (laboratory examination or a survey), and completeness of the lesions⁶⁶. The variation in the reported prevalences is therefore large, but all reported values are high, ranging from 48% to 70%^{66,73}. Perhaps the most distressing aspect of this condition is that it is unavoidable, as it occurs in response to stimuli common in activities of daily living, and individuals thus experience episodes (almost) on a daily basis. The symptoms can be so profound and disturbing that it interferes with activities of daily living and normal sexual experiences.

Reflex loop underlying autonomic dysreflexia

The episodes of AD are caused by a spinal reflex (Figure 1.7), which in the able-bodied is normally inhibited by supraspinal centres. The sensory stimulus triggers a spinal reflex arc that induces widespread sympathetic activity, leading to elevated blood pressure in response to massive vasoconstriction. This increased blood pressure would, in an intact system, trigger baroreflex-mediated reduction of sympathetic excitation. Due to the disruption in the spinal sympathetic pathway, sympathetic activity below the lesion remains high. Therefore vasodilation arises above the lesion, as these regions are still under normal supraspinal control (resulting in facial flushing and a headache)⁶⁶.

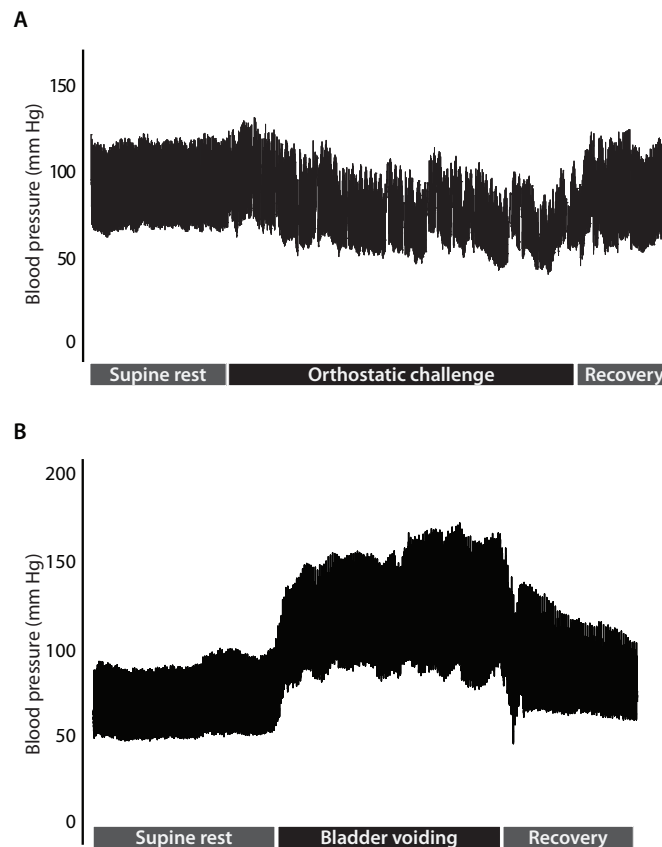


Figure 1.6. Example traces of abnormal reflex control of blood pressure after spinal cord injury.

- A. Blood pressure recording from a 26-year-old male with a C5 AIS B SCI during an orthostatic challenge. During orthostasis the blood pressure progressively declines, meeting criteria for OH, with a nadir of 78 mmHg systolic pressure. Blood pressure recovers upon return to supine.
- B. Blood pressure recording from a 34-year-old male with C6 AIS A SCI during bladder voiding that triggered AD. During bladder voiding the blood pressure rapidly increases, with a peak of 173 mmHg systolic pressure. Blood pressure recovers after bladder voiding is completed.

Severe vasoconstriction and pilomotor activity as part of extreme general sympathetic activity emerges below the lesion, often resulting in pallor and goose bumps respectively⁶⁶. Extremely high sympathetic activity below the lesion level is accompanied by high vagal activity to the heart, as the vagal component of the efferent baroreflex arc continues to function normally. Therefore, it has classically been thought that AD is accompanied by bradycardia. However, when the connection between the brain and the sympathetic pathways to the heart is impaired (cervical lesions), a pathophysiological state arises where both sympathetic and parasympathetic activity to the heart are simultaneously elevated. In individuals with lesion levels between T1-T5 this connection between the brain and the cardiac spinal sympathetic pathways remains (partly) intact. The baroreflex-mediated inhibition of cardiac sympathetic neurons and excitation of the vagal nerve to the heart in response the blood pressure increase can thus still occur, leading to a decrease in heart rate. Figure 1.6 illustrates the reflex loop and how the disruption of sympathetic supraspinal control leads to AD.

AD has generally been thought to develop in a later phase of SCI, while it is believed that the damage to descending sympathetic pathways is not the only mechanism underlying this phenomenon. The adaptations in response to the damaged spinal sympathetic pathways, such as α -adrenergic hyper-responsiveness and sprouting of primary afferents, are proposed to play a role in the development of AD. In the same line of thought, reduced reuptake of noradrenaline is thought to contribute to increased response to sympathetic activity and development of AD⁷⁴. However, more recently, AD has also been identified during the acute phase of injury SCI, but the underlying mechanism remains unknown⁷⁵.

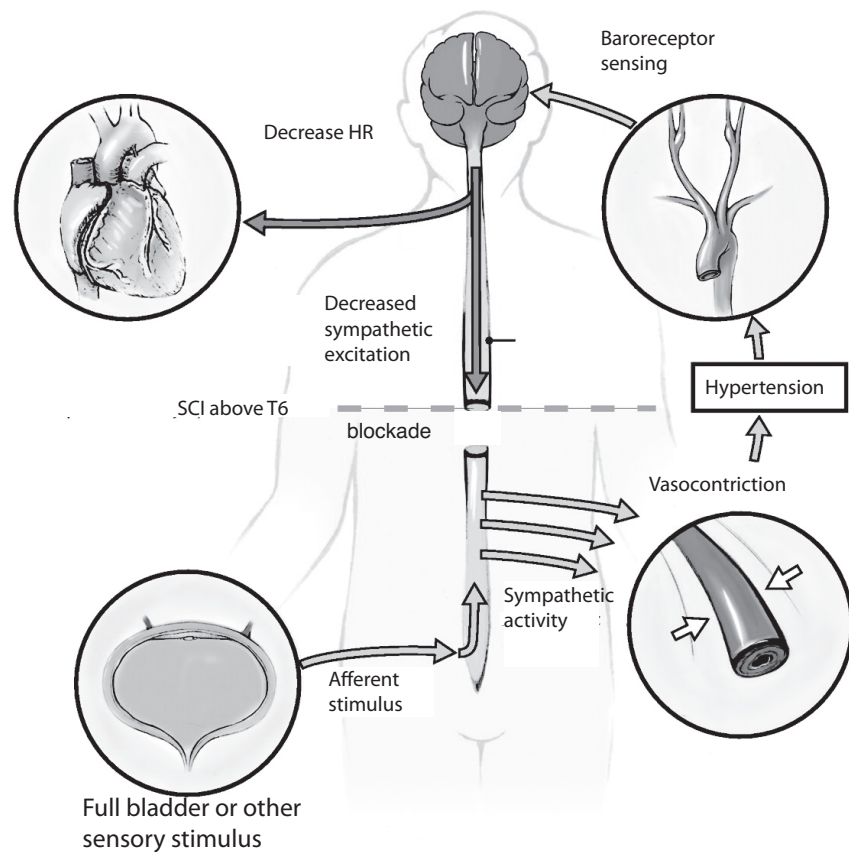


Figure 1.7. Schematic of the spinal reflex underlying autonomic dysreflexia

A sensory stimulus below the lesion triggers reflex sympathetic activity resulting in vasoconstriction and an increase in blood pressure. This increase in blood pressure triggers the baroreceptor reflex, but the spinal cord lesion blocks the inhibition of sympathetic activity. Adapted with permission from Blackmer 2003⁷⁶.

Impaired blood pressure responses to exercise

Similar to the heart rate response to exercise, the blood pressure response to exercise is also blunted. The normal increase in blood pressure with exercise is not seen in individuals with complete lesions to autonomic pathways above T6^{77,78}. This limits the performance of athletes with SCI (above T6). Some athletes use intentional induction of AD, commonly called 'boosting', to enhance their performance. This tactic has been shown to be effective in a research setting. Tetraplegic athletes were tested on a 7.5 kilometer simulated road race (wheeling) and performed significantly better in the boosted condition (over-distended bladder) improving their time by 9.7%⁷⁹. In this setting blood pressure was recorded and a safety protocol was in place, but of course this would not be the case in regular training or competition settings. The concept of

boosting is therefore still a topic of debate, where clinicians are concerned about the devastating medical consequences of the induced AD, and athletes are cognizant of the improvements in performance associated with boosting. The international Paralympic Committee prohibits athletes from competing in a dysreflexic state (intentionally induced or not), because of its classification as a health risk.

Assessment of autonomic function after injury

The standard assessment of severity of neurologic injury is primarily focused on motor and sensory function based on the American Spinal Injury Association (ASIA) Impairment Scale (AIS). However, Claydon et al. showed that individuals with a complete injury to motor and sensory pathways (AIS A) did not necessarily have a complete injury with respect to autonomic pathways⁸⁰. Conversely, individuals with incomplete lesions determined by AIS score can show signs of damage to descending autonomic pathways. This neglect of assessing autonomic consequences of injury in the clinical setting is very worrisome. A separate assessment of autonomic function in addition to the AIS assessment is pertinent. In order to address this issue a group of experts from ASIA and the International Spinal Cord Society (ISCoS) created a document that provides diagnostic criteria for the symptoms of SCI with respect to cardiovascular, sudomotor, bladder, bowel, broncho-pulmonary and sexual function⁸¹. However, although this tool is a great benefit to implement evaluation of symptomatic clinical components of autonomic dysfunction after SCI, a quantitative tool for assessment of cardiovascular autonomic function is not (yet) incorporated in the standard clinical examination of SCI.

At present, there is no gold standard for measuring cardiovascular autonomic function after SCI. Several techniques for autonomic function testing such as the Valsalva manoeuvre, heart rate responses to deep breathing, and cardiovascular responses to sustained hand grip are not suitable for individuals with SCI in whom hand function as well as breathing can be impaired. The cold pressor test is another autonomic function test that is not suitable for those with SCI, because the cold-water stimulus when applied below the level of the lesion can trigger an episode of AD, masking the normal cardiovascular response.

Plasma noradrenaline

One technique that provides measurement of general sympathetic activity is measurement of the resting level of plasma noradrenaline, or noradrenaline spillover. Noradrenaline in the plasma represents (mostly) the spillover from noradrenaline released from sympathetic postganglionic fibres. It is the most used marker for sympathetic activity in humans, especially with the use of high-pressure liquid chromatography to determine plasma levels with strong accuracy⁸². Plasma noradrenaline can be tested at rest as well as during an orthostatic stress test, to determine the ability to increase sympathetic activity in response to an induced stress. Individuals with high-level SCI have been shown to have low resting levels of noradrenaline, and fail to increase plasma noradrenaline with orthostatic stress⁵³, compatible with severe injury to descending spinal sympathetic pathways. However, there are limitations to this technique: firstly, it is an invasive test and it provides a global index of sympathetic activity with no information on regional sympathetic function. Other limitations are at a more physiological level. The noradrenaline level in the plasma is not purely dependent on the secretion of the neurotransmitter from the nerve terminals, but also on clearance and reuptake processes^{83,84}. Thus, increased plasma NA may reflect not only increased release, but also decreased clearance or reuptake, and vice versa. However, in practice this caveat applies more to findings of unexpectedly high noradrenaline level, when clearance from the plasma is impaired⁸².

Muscle sympathetic nerve activity

Microneurography is a method which allows direct recording of spontaneous muscle sympathetic nerve activity from efferent postganglionic sympathetic nerve fibres (commonly from the peroneal or the brachial nerves)⁸⁵. This technique involves inserting a microelectrode into a single nerve fascicle with a reference electrode placed subcutaneously⁸⁶. The amplitude and the number of bursts of sympathetic activity are determined and multiplied to get the total burst amplitude. Sometimes the number of bursts is corrected for heart rate and expressed as burst incidence (the number of bursts per 100 heart beats)⁸². The main advantage of this technique is the ability to directly measure sympathetic activity and to record changes in activity with different manoeuvres stressing the system. The outcomes have also been shown to be highly reproducible in healthy volunteers⁸⁷. The major downside to this technique is that it is

an invasive and time-consuming procedure. It might therefore be a more useful technique in the laboratory setting than in a clinical setting.

Sympathetic skin responses

Another test of sympathetic activity is the measurement of sympathetic skin responses. This technique measures changes in electrical conduction of the skin (from the hands and feet) resulting from sympathetic sudomotor activity. Electrical stimuli imposed on the median nerve or tibial nerve trigger a reflex via afferents to the dorsal root ganglion and into the spinal cord, where input from intra- and supraspinal pathways is received, and finally to the sympathetic postganglionic neurons eliciting sudomotor activity. Cariga et al. found the involvement of supraspinal pathways to be necessary to elicit the sympathetic skin response, as their results showed that the isolated spinal cord could not generate the response, making this a technique suitable to identify the integrity of spinal sympathetic pathways⁸⁸. There remains some controversy in the literature regarding the involvement of supraspinal pathways as these responses have occasionally been found after stimulation below the lesion⁸⁹⁻⁹¹. The main limitation of this technique is that it investigates a cholinergic pathway and thus cannot be used to directly determine adrenergic function. Also, the response has been shown to be variable and dependent on the strength of the electrical stimulus (and habituation to the stimulus), and therefore the test usually needs to be repeated a number of times⁹².

Cutaneous vasomotor responses

In the same line as measuring sympathetic skin responses (sudomotor), the cutaneous vasomotor responses (e.g. decreased skin blood flow) to arousal stimuli have been proposed as a measurement to assess autonomic function after SCI^{89,91,93}. The vasomotor responses rely on sympathetic adrenergic pathways in contrast to the cholinergic pathways involved in the sudomotor response. Another difference between the two responses is that the vasomotor response involves a spinal reflex without necessary involvement of the supraspinal centres⁸⁹. A stimulus below the lesion would thus cause a response below the lesion, but when spinal sympathetic pathways are disrupted, a stimulus above the lesion would not cause a response below the lesion. Either laser Doppler flowmetry or pulse plethysmography can be used for detection of cutaneous vasomotor responses non-invasively⁹⁴.

Heart rate and blood pressure variability

Heart rate and blood pressure are not static signals; they undergo spontaneous fluctuations that are caused by different control systems, one of which is the autonomic nervous system. Analyzing these fluctuations in heart rate and blood pressure can therefore be used to determine autonomic function.

Spectral analysis is a mathematical operation that can be used to break down the measured heart rate variability (HRV) and blood pressure variability (BPV) into their frequency components. Three main frequencies have typically been identified; a high frequency (HF, ~ 0.25 Hz), a low frequency (LF, ~ 0.1 Hz), and a very low frequency (VLF, ~ 0.01 Hz)^{95,96}. The spectral powers at these frequencies, as quantified by spectral analysis, are used as parameters of autonomic function. The HF component of BPV has been shown to result from changes in intrathoracic pressure due to breathing, while the LF component results from sympathetic activity to resistance vessels^{95,96}. The VLF power is influenced by many different factors (e.g. heat stress, catecholamine levels, and hypovolemia), that seem to be linked to one common mechanism: the myogenic vascular response via L-type Ca^{2+} channel function⁹⁷. The mechanisms underlying the frequency components of HRV are different from those underlying BPV fluctuations. The HF component of HRV represents cardiovagal control, mediated largely through respiratory sinus arrhythmia^{95,96}. The VLF is (similar to the VLF in BPV) influenced by many different control systems such as thermoregulation, the renin-angiotensin system and endothelial factors. The origin of the LF power of HRV is still controversial, but is thought to be due to both vagal and sympathetic components of the baroreflex and to be linked to LF oscillations in BPV^{98,99}.

Given that systemic blood vessels that are important in blood pressure control are supplied exclusively by sympathetic axons, quantification of sympathetic function can be determined from the spectral power of the LF component of BPV. This measure has been shown to be well correlated with supine plasma noradrenaline¹⁰⁰. The advantage of this technique is that it is non-invasive and fairly easy to record in a clinical setting. It can also be used to quantify changes in autonomic function from the resting state to different stress situations. A downside to this technique is that different algorithms can be used, as well as different lengths of data recordings, that could influence the

results⁸⁶. The technique also requires data recordings in a 'steady' state⁹⁵; inherently there will be fluctuations in the signal, but data should be recorded in a fairly steady state instead of during a sudden change in blood pressure or heart rate (e.g. during a stress manoeuvre).

We chose to use plasma noradrenaline and spectral analyses of HRV and BPV to determine autonomic function in individuals with SCI. Both have been well documented as measurements of sympathetic function, and both are easy to measure in a clinical setting. Spectral values of HRV and BPV are shown to be highly reproducible in individuals with SCI¹⁰¹ and can also detect alterations in cardiovascular autonomic function in rodents with SCI²⁸. As there is not yet a gold standard for the evaluation of autonomic function after SCI, using these two techniques in combination can be beneficial in providing a more comprehensive assessment of autonomic function.

Relationship between cardiovascular autonomic dysfunction and cardiovascular disease risk

As discussed previously, the evidence is slowly growing that the increased risk of CVD after SCI is related to cardiovascular autonomic dysfunction. There are many different ways in which cardiovascular autonomic dysfunction can have an impact on CVD risk. Firstly, episodes of AD can have devastating consequences like stroke, cardiac arrest, myocardial ischaemia, or even death¹⁰²⁻¹⁰⁶. High level SCI and the related abnormal autonomic control of the cardiovascular system has also been associated with modulations in cardiac electrophysiology and increased risk of cardiac arrhythmias^{107,108}. In addition to these direct relationships between autonomic dysfunction and CVD risk, the blood pressure control issues and inability to increase heart rate normally may lead to both decreased participation in physical activity and poor exercise tolerance. These changes in lifestyle might lead to increases in adiposity and abnormalities in metabolism. The next paragraphs describe these links in more detail, starting with the consequences of AD.

Consequences of autonomic dysreflexia

Episodes of AD can be severe and even life threatening. It is important to recognize the symptoms and subsequently discover the stimulus that triggers the reflex. When this

stimulus can be found and relieved, the blood pressure will return to the resting level quickly. Particular problems arise when the individual does not have any symptoms (silent AD⁶⁸) or when the stimulus cannot be quickly removed (e.g. a bone fracture or when the individual has limited motor function and is unable to address the triggering stimulus without assistance). In these cases an episode of AD can become life threatening. Serious events like seizures and cerebral hemorrhage have been described, in some cases even leading to death^{109,110}. Another serious complication reported is myocardial ischemia, and angina pectoris, that may go untreated due to loss of sensory function as a consequence of SCI¹¹¹. Also, neurogenic pulmonary edema has been reported as a complication of AD; this was thought to be due to the prolonged vasoconstriction, specifically in the pulmonary veins, which increases the capillary hydrostatic pressure producing pulmonary edema¹¹².

Even in the cases that the episodes are recognized and the stimuli found and relieved fairly quickly, the frequent episodes of very high blood pressure have a negative impact on the vasculature. The high pressures are in great contrast to the low resting blood pressure and could cause increased shear stress and related damage to the endothelial layer of the vascular wall.

Elevated risk of cardiac arrhythmias

The occurrence of arrhythmias in individuals with SCI is more apparent during episodes of AD, when both parasympathetic and sympathetic tone are high⁸⁰. Widespread and uncontrolled sympathetic activity leads to extreme hypertension, but also increases the dispersion of ventricular repolarization between the different layers in the ventricular wall, increasing the likelihood of re-entry arrhythmias¹¹³. An increase in transmural dispersion of ventricular repolarization (TDR) has been shown to be a substrate for ventricular arrhythmias, specifically Torsade des Pointes^{114,115}.

TDR corresponds to the difference in timing and duration of repolarization in the different layers of the ventricular wall. The myocardium is heterogeneous in composition of cells and these different cell layers repolarize at different rates. The epicardium is the first to repolarize, followed by the endocardium, leaving the mid-myocardial (M) cells to repolarize last. Antzelevitch et al. have shown in canine wedge models that the differences in repolarization between the layers of the ventricular wall determine the

morphology of the T wave on the electrocardiograph (ECG)¹¹⁴. The peak of the T wave corresponds to when the epicardial cells are fully repolarized and the end of the T wave to when the M cells finally return to the resting membrane potential. The $T_{\text{peak}}-T_{\text{end}}$ interval on the ECG is consequently thought to correspond to TDR, the duration between the first layer to be repolarized and the last cell layer to return to resting potential (Figure 1.8). The differences in repolarization properties of the different layers of the ventricular wall can be accentuated by extreme autonomic nervous system activity (both sympathetic and parasympathetic) to the heart (such as during AD)¹¹³. When TDR increases, a substrate for re-entry between the different layers of the ventricular wall is created, because current can flow from the depolarized ventricular cells to the repolarized ventricular cells, which could lead to a new action potential (afterdepolarisation). Hence, $T_{\text{peak}}-T_{\text{end}}$ can be used as a risk assessment parameter for ventricular arrhythmias¹¹⁶.

In addition to a higher risk of ventricular arrhythmias, atrial arrhythmias such as atrial fibrillation are more common in individuals with SCI, and this also particularly occurs during AD¹¹⁷. The predisposition to atrial fibrillation during AD is thought to be related to an altered pattern of repolarization in the atria¹¹⁷. The pattern of repolarization in the atria can be studied in two different domains, the spatial domain and the time domain. In the spatial domain, an inhomogeneous prolongation of propagation of sinus impulses in the atria is associated with atrial fibrillation¹¹⁸. It is proposed that this inhomogeneous atrial conduction could be identified by variation in P wave duration in differently oriented surface ECG leads, a measure called P wave dispersion (PWD). P wave dispersion is defined as the difference between the maximum P wave duration and the minimum P wave duration on a 12-lead ECG^{118,119}. PWD is therefore thought to be a predictor for atrial fibrillation. In the time domain, we propose to study atrial conduction by means of the variability of P wave duration in one ECG lead over time. We hypothesize that the variability over time in atrial conduction is associated with atrial arrhythmia and this parameter could be used as a predictor for risk of atrial arrhythmia.

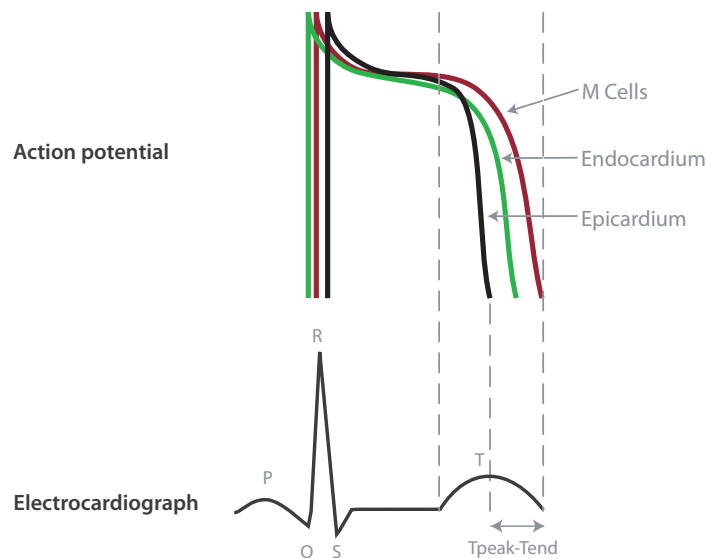


Figure 1.8. Action potentials of ventricular cell layers and the corresponding electrocardiograph.

A schematic representation of the action potentials of the different layers of the ventricular wall (top panel) on the same timeline with a schematic representation of an electrocardiograph (bottom panel). The action potential of the endocardium (green line) is initiated first, followed by the action potential of the M cells (red line) and the epicardial action potential (black line). When the first cell layer (the epicardium) completes repolarization, the potential difference between the cell layers is largest, which correlates to the peak of the T wave. As the other cell layers repolarize, the potential difference decreases, correlating to decreasing T wave amplitude. The end of the T wave occurs when the last cell layer has returned (end of the action potential of the M cells) to resting membrane potential.

Interaction effects between autonomic dysfunction and lifestyle

In addition to paralysis, secondary complications due to autonomic dysfunction may cause individuals with SCI to lead a more sedentary lifestyle. Fatigue and concentration problems associated with supine and orthostatic hypotension can limit the ability for individuals with SCI to participate in physical activity. Concurrently, the limited ability to increase heart rate and blood pressure may lead to poorer exercise tolerance even in the presence of a strong motivation to exercise. The associations between features such as a sedentary lifestyle, abnormalities in metabolism, and subsequent increases in adiposity, and risk for CVD, is well established in the able-bodied population¹²⁰⁻¹²². In a large longitudinal study following individuals with SCI from the start of rehabilitation, de Groot et al^{26,123} showed a correlation between lipid profiles and physical capacity, indicating that individuals who are better able to engage in physical activity have more favourable lipid profiles. People with higher-level lesions, less motor control and greater severity of autonomic dysfunction, who are more likely to be limited in engaging in

strenuous physical activity, would thus be more prone to adverse lipid profiles. The same group also showed a relationship between physical activity levels and CVD risk factors²³. They did not, however, examine the impact of autonomic dysfunction on these outcome measures.

Impaired glucose tolerance and type 2 diabetes are more prevalent in individuals with SCI compared to able-bodied controls¹²⁴⁻¹²⁶. Fasting glucose levels are mostly within the normal range, but individuals with SCI are shown to be more insulin resistant¹²⁵⁻¹²⁷. Interestingly, in the able-bodied population, β -adrenergic stimulation induces insulin resistance^{127,128}. Accordingly, the low resting sympathetic activity in individuals with autonomic dysfunction after SCI would be expected to be favorable in terms of insulin resistance, although the extremely high levels of sympathetic activity encountered during AD might worsen insulin resistance.

The susceptibility to adverse lipid profiles is greater for individuals with SCI. Both increased low density lipoprotein cholesterol (LDL-C) as well as reduced levels of high density lipoprotein cholesterol (HDL-C) are shown to be more prevalent in individuals with SCI, particularly in those with tetraplegia¹²⁵. Similar to the able-bodied population, a larger abdominal circumference increases the likelihood of an adverse HDL-C level^{124,129}. Physical activity also favourably impacts lipid profiles in individuals with SCI in a similar manner to the able-bodied population; regular moderate-intensity wheelchair ergometry exercise improves HDL-C¹³⁰. However, any potential relationships between lipid profiles and autonomic function after SCI remain to be investigated.

Obesity and obesity-related cardiovascular disease risk factors

As mentioned previously, individuals with SCI tend to have more sedentary lifestyles, in part due to secondary complications after injury. Also, resting metabolic rate (RMR) is decreased in individuals with SCI¹³¹. The decrease in RMR could be explained in several ways. RMR is associated with lean body mass, and individuals with SCI who experience muscle atrophy and thus lose lean mass, will consequently have a lower RMR. Another explanation entails the effect of autonomic dysfunction after SCI on the normal action of leptin. Leptin is a protein that is produced primarily by adipocytes and it regulates body adiposity by inhibiting food intake and increasing energy expenditure. Leptin acts indirectly via the central nervous system, where it causes an increase in sympathetic

activity. When supraspinal control of the sympathetic pathways is disturbed, leptin's action to regulate energy expenditure will be impaired. Jeon et al. showed an increase in leptin levels in individuals with cervical SCI, but the normal correlation between leptin and RMR was not present in the SCI group¹³². Thus, the higher plasma leptin levels in individuals with SCI did not lead to an increase in RMR as would be expected in the able-bodied, indicating an impaired action of leptin.

For these reasons, the prevalence of obesity has been thought to be elevated in this population, and studies have reported a prevalence ranging between 40 and 66%¹³³. However, it remains challenging to accurately determine obesity in individuals with SCI¹³⁴. Commonly used parameters such as body mass index (BMI) and waist circumference (WC) have limitations in practicality of use and accuracy to determine obesity¹³⁵⁻¹³⁷. Measuring BMI in individuals with SCI requires the use of wheelchair scale and an accurate measure of height (or length). Furthermore, the BMI cut-offs for obesity used in the able-bodied population are thought to underestimate obesity in individuals with SCI^{136,137}. Other direct measures of body fat mass such as dual energy x-ray absorptiometry (DEXA), hydrostatic weighing or MRI are valid methods, but are very expensive and not readily available to most individuals with SCI.

In summary, SCI remains a devastating, lifelong condition that impairs quality of life and is associated with impaired autonomic function. Cardiovascular dysfunction is a common complication of SCI, and cardiovascular morbidity and mortality is amplified and has an earlier onset and more rapid progression than in the general population²². The mechanisms underlying these consequences of SCI remain unclear. Injury to cardiovascular autonomic pathways may play an important role, but this is not well studied. The general aim of my thesis is, therefore, to explore the role of cardiovascular autonomic impairment after SCI in CVD in individuals with SCI.

Outline of this thesis

In order to address the main question of this thesis, it is important to evaluate the full extent of cardiovascular autonomic dysfunction after SCI. As explained above, autonomic function is not incorporated in the standard neurological assessment of injury. Therefore, not much is known about the prevalence or changes over time in cardiovascular dysfunction. In *Chapter 2*, the aim is to investigate the time course of changes in cardiovascular parameters and the prevalence of hypotension over time after injury.

Next, in *Chapter 3* the aim is to evaluate the relationship between cardiovascular autonomic dysfunction and risk factors for CVD following SCI, as well as the interactions between these variables and physical activity levels.

Chapters 4 and 5 aim to explore new risk assessment parameters that are specific to the SCI population, for obesity related CVD risk and for cardiac arrhythmias.

Obesity is well established as an important risk factor for CVD. In individuals with SCI, obesity is believed to be more prevalent as a result of a more sedentary lifestyle and altered metabolism due to autonomic dysfunction. However, an anthropometric marker to define obesity and obesity-related CVD risk including a cut off specific to this population is not available. *Chapter 4* aims to identify the best marker to detect adiposity and elevated CVD risk, considering practicality of use for individuals with SCI.

Modulation of cardiac electrophysiology as a consequence of autonomic dysfunction is associated with increased risk for cardiac arrhythmias after SCI. The electrocardiograph (ECG) can potentially be used to identify those individuals at risk for developing arrhythmias, particularly during episodes of AD. The aim in *Chapter 5* is therefore to evaluate the relationships between cardiovascular autonomic control and new ECG-based risk assessment parameters for the propensity to develop arrhythmias after SCI.

Chapter 2.

Prevalence and progression of cardiovascular dysfunction after spinal cord injury

Introduction

Autonomic dysfunction is common after spinal cord injury (SCI), but this has traditionally been an under-researched area compared to motor and sensory dysfunction. Indeed, these issues have only recently gained significant attention in the research arena¹³⁸. This is particularly pertinent given that two surveys have shown that individuals with SCI consider that their quality of life would improve significantly with regaining autonomic functions like bowel, bladder, sexual function^{21,139} and the resolution of cardiovascular dysfunction such as autonomic dysreflexia (AD, sudden and profound hypertension in response to sensory stimuli from below the lesion)²¹.

Cardiovascular autonomic dysfunction is a particular concern after SCI⁵². With high-level lesions (at or above the fifth thoracic level, T5), disruption of descending spinal sympathetic pathways can result in basal sympathetic hypoactivity with unopposed parasympathetic control to the heart¹⁷. This leads to impaired blood pressure control, manifested by low supine blood pressure, orthostatic hypotension (OH, further profound falls in blood pressure when upright) and bouts of extremely high blood pressure during AD⁵³. There may also be an increased risk for cardiac arrhythmia due to autonomic imbalance after SCI^{107,140}. Although AD and arrhythmia can have serious consequences, the repeated episodes of severe hypotension that occur throughout the day can be particularly troublesome in terms of quality of life, leading to feelings of general fatigue^{61,62}, difficulty participating in activities of daily living and rehabilitation⁶³, and impaired cognitive function^{64,65}.

Impairment of cardiovascular autonomic pathways is not quantified by the standard assessment of severity of SCI, the AIS assessment¹⁴¹. Resting blood pressure and heart

rate can, however, be used as relatively simple measures indicating severity of impairment to cardiovascular sympathetic pathways. A negative correlation between resting blood pressure and the level of the SCI has been shown previously^{53,138,142}. The peak heart rate that can be achieved during exercise is decreased if sympathetic control of the heart is abolished after SCI (usually only in those with lesions at or above T5), with exercise-induced increases in heart rate presumably achieved by vagal withdrawal. Indeed, the peak exercise heart rate achieved after autonomically complete SCI above T5 (approximately 105 BPM)⁴⁹ is comparable to that of able-bodied controls during complete vagal blockade (approximately 100 BPM)⁴⁸.

To the best of our knowledge it is unknown how the prevalence of hypotension and resting blood pressure and heart rate change with time after acute SCI. One recent cross-sectional study did examine cardiovascular parameters in a large cohort of individuals with SCI⁵⁴ and found that individuals with high level lesions or who had been injured for longer periods of time had lower blood pressures. Men with SCI were reported to have higher blood pressures and lower heart rates than women. However, in this study only individuals with incomplete lesions to motor and sensory pathways were examined, and any changes in cardiovascular control with time after injury, or during inpatient rehabilitation were not examined. Therefore, the objectives of our study are: (i) to determine the prevalence of hypotension in individuals with SCI during and after inpatient rehabilitation; (ii) to investigate the time course of resting blood pressure and heart rate, and peak heart rate changes with exercise, during inpatient rehabilitation and for up to 5 years after discharge; (iii) to evaluate the influence of personal (age, sex) and lesion (level and completeness) characteristics on hypotension, resting blood pressure and heart rate, and peak heart rate.

Methods

Participants

This study was part of the Dutch prospective cohort study "Physical strain, work capacity and mechanisms of restoration of mobility in the rehabilitation of persons with SCI"¹⁴³. The study comprised five measurement sessions. Ethics approval was received from the medical ethics committee of SRL/iRv Hoensbroeck for the first four measurements and the medical ethics committee of the University Medical Center Utrecht approved the

addition of the fifth measurement. This study also received ethical approval from the Simon Fraser University Department of Research Ethics for retrospective analysis of the data. All investigations were performed in association with the Declaration of Helsinki of the World Medical Association. All participants gave written informed consent.

We recruited participants from eight rehabilitation centres that are specialized in SCI rehabilitation in the Netherlands. Individuals were eligible to participate if they: had an acute traumatic or non-traumatic SCI; were between the ages of 18 and 65 years; were classified as A, B, C or D on the AIS scale¹⁴⁴; were expected to remain wheelchair dependent; did not have a progressive disease or psychiatric problem; had sufficient understanding of the Dutch language to understand the purpose of the study and the testing methods. In this study we excluded participants with known pre-existing cardiovascular disease, diabetes mellitus or cardiac diseases from our analyses. We included participants on whom we obtained data for blood pressure and supine and peak heart rate on at least two of the five occasions.

Design

Data were collected at five different occasions: at the start of active rehabilitation, when participants could sit for three-to-four hours (start of rehabilitation); three months later (3 months); at discharge from inpatient rehabilitation (discharge); one year after discharge (1 year); and five years after discharge (5 years). Trained research assistants with paramedical backgrounds collected the data using standardized procedures at the different sites.

Cardiovascular parameters

A physician recorded resting systolic (SAP) and diastolic (DAP) arterial pressure using a manual sphygmomanometer while participants were seated in their wheelchair. The measurements were recorded once, at the same time of the day during every visit. Participants were asked to eat a light meal only, to refrain from smoking and drinking caffeine or alcohol for 2 hours before testing, and to void their bladders. Participants continued taking their regular medications. We determined prevalence of hypotension using World Health Organization (WHO) criteria¹⁴⁵: for men: SAP <110 mmHg; for women: SAP <100 mmHg. Resting heart rate (HR_{rest}) and peak heart rate (HR_{peak}) were recorded using a heart rate monitor (Polar Sport tester, Polar Electro Inc., Kempele,

Finland) before and during a graded maximal aerobic capacity test. The prevalence of resting bradycardia (using two cut-offs: <60 BPM and <50 BPM) was determined, as well as the prevalence of elevated resting heart rate (>80 BPM) and tachycardia (>100 BPM). Prevalence data are reported for the SCI group as a whole, and for three subgroups defined according to lesion level.

Measures of maximal aerobic capacity were obtained during a standardized peak wheelchair exercise test on a treadmill¹⁴⁶. The test was preceded by five minutes of rest, seated in the testing wheelchair. As before, participants had a light meal before testing, abstained from caffeine and alcohol for at least two hours before the test, and emptied their bladder before the test. The HR_{rest} was determined as the mean over the last 30 seconds of this five-minute rest period (this rest period was not immediately followed by the onset of exercise, to avoid an anticipatory heart rate response to upcoming peak physical activity). During the determination of the maximal capacity the speed of the treadmill stayed constant and was set at 2 km/h for individuals with tetraplegia and at 4 km/h for individuals with paraplegia; 3 km/h was used in cases where the other options were too slow or too fast for the individual. The treadmill incline was increased by 0.36° every minute until the test was terminated due to exhaustion or inability to keep pace with the speed. The complete testing protocol and equipment used for the aerobic capacity test have been described previously by Kilkens et al.¹⁴⁶. The HR_{peak} was determined as the highest five-second average HR measured during the entire test.

Personal and lesion characteristics

Age, sex and lesion characteristics were collected at the start of rehabilitation and at discharge. A physiatrist determined lesion characteristics (level and motor/sensory completeness) using AIS criteria¹⁴⁴. Cardiac sympathetic innervation arises from spinal segments T1-T5¹⁷. Therefore, participants were divided into three subgroups according to lesion level at discharge from rehabilitation: individuals with cervical lesions (cervical); lesions at the levels T1-T5 (high thoracic) and lesions at T6 or below (low). In the cervical lesion group supraspinal control to sympathetic pathways to both the heart and vascular system can be impaired; the high thoracic group can have damage to some sympathetic pathways to the heart and the main components of the vascular system in terms of blood pressure control; the low injury level group will have spared control of

the sympathetic pathways to the heart and major vascular beds controlling blood pressure^{17,52}. We defined a lesion as complete when participants were graded as AIS A and incomplete when they were graded as AIS B, C or D at discharge from rehabilitation.

Statistical analyses

Descriptive statistics (means and standard deviations (SD)) were determined for SAP, DAP, HR_{rest} and HR_{peak} for all test occasions and per subgroup. Changes in SAP, DAP, HR_{rest} and HR_{peak} were studied using random coefficient analysis (MLwiN)^{147,148}, with three levels (centre, participant, test occasion). The dependent variables of this regression analysis were the blood pressure or heart rate variables. Firstly, SAP, DAP, HR_{rest} or HR_{peak} were modelled over time using time periods as categorical variables (dummy) with discharge as the reference, to be able to distinguish between changes during and after rehabilitation. The regression coefficient for a time dummy describes the predicted change in blood pressure or heart rate over that time period.

Secondly, the longitudinal relationships were investigated between blood pressure or heart rate with lesion and personal characteristics, using univariate analyses. Lesion level (two dummies with the low lesion group as reference and then in a subsequent analysis with the cervical lesion group as reference to be able to compare all three groups), motor completeness of the lesion (incomplete=0, complete=1), age (continuous variable), and sex (women=0, men=1) were used as independent variables in this second phase of analysis. Independent variables that showed a p-value below 0.1 were added to the multivariate model, followed by a backward elimination technique until only significant determinants remained (p<0.05). This final multivariate model, however, always included the two lesion dummy variables using the low lesion group as reference.

To study the interaction effects of personal or lesion characteristics with time, the time dummies, the personal or lesion characteristics, and their interactions were entered in models with SAP, DAP, HR_{rest} and HR_{peak} as dependent variables.

The same analyses were performed with a binomial random coefficient analysis to investigate the change in the percentage of participants that were hypotensive (no hypotension=0, hypotension=1) during and after rehabilitation. These analyses were

also performed for bradycardia (<60 BPM and <50 BPM), elevated heart rate (>80 BPM) and tachycardia (>100 BPM).

Results

Participants

A total of 197 individuals participated in the study on two or more test occasions. Participant characteristics are described in Table 2.1. The total number of participants per test/occasion (blood pressure and maximal aerobic capacity test) varied between 59 and 194 (Table 2.2). Of all participants at discharge, 40% had a cervical lesion, 19% a high thoracic and 41% a low level lesion. At discharge, 47% of the participants had a complete lesion (AIS A) and 53% had incomplete lesions (AIS B, C or D). The average age at discharge was 40.0 years (SD 14.1) and the average time since injury was 322.4 days (SD 159.7). Of our sample, 74% were male.

Table 2.1. Participant characteristics

	Start of rehabilitation (n=194)		3 months later (n=143)		Discharge from rehabilitation (n=174)		1 year after discharge (n=136)		5 years after discharge (n=110)	
	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)
Male sex	73.2		75.5		72.4		72.1		74.5	
Age (years)		40 (14)		41 (14)		40 (14)		41 (14)		44 (13)
Time since injury (days)		100 (75)		204 (75)		322 (160)		716 (172)		2379 (256)
Lesion level										
Cervical	39.7		47.6		37.9		35.3		36.4	
High thoracic	19.6		21.7		19.5		22.1		16.4	
Low level	40.7		30.7		42.4		42.6		47.2	
Lesion completeness										
AIS A	45.7		46.5		51.4		53.3		59.3	
AIS B,C,D	53.3		53.5		48.6		48.6		40.7	

Table 2.2. Cardiovascular variables at all test occasions according to lesion level

	Start of active rehabilitation		3 months later		Discharge from rehabilitation		1 year after discharge		5 years after discharge	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
All participants										
SAP (mmHg)	194	119 (16)	143	120 (16)	174	119 (16)	136	120 (20)	110	121 (20)
DAP (mmHg)	194	70 (11)	143	70 (11)	174	71 (12)	136	77 (12)	110	76 (12)
HR _{rest} (BPM)	109	85 (15)	94	80 (14)	122	77 (14)	90	78 (13)	60	72 (13)
HR _{peak} (BPM)	97	142 (26)	89	144 (29)	121	143 (29)	85	150 (31)	59	149 (31)
Participants with cervical lesions										
SAP (mmHg)	77	115 (17)	68	117 (17)	66	117 (18)	48	111 (22)	40	111 (21)
DAP (mmHg)	77	65 (10)	68	67 (11)	66	68 (12)	48	72 (12)	40	70 (13)
HR _{rest} (BPM)	27	74 (13)	29	71 (12)	36	67 (11)	18	67 (12)	15	59 (12)
HR _{peak} (BPM)	19	111 (17)	25	114 (22)	36	114 (19)	17	118 (27)	14	109 (15)
Participants with high thoracic lesions										
SAP (mmHg)	38	122 (17)	31	124 (18)	34	118 (15)	30	124 (21)	18	122 (15)
DAP (mmHg)	38	72 (12)	31	73 (13)	34	72 (12)	30	78 (12)	18	78 (7)
HR _{rest} (BPM)	24	90 (19)	23	83 (16)	21	83 (16)	21	82 (12)	13	75 (8)
HR _{peak} (BPM)	24	146 (27)	23	152 (26)	21	153 (25)	20	148 (27)	13	146 (25)
Participants with low level lesions										
SAP (mmHg)	79	121 (14)	44	121 (12)	74	122 (14)	58	125 (16)	52	127 (18)
DAP (mmHg)	79	73 (10)	44	74 (10)	74	73 (10)	58	80 (12)	52	80 (10)
HR _{rest} (BPM)	58	88 (11)	42	85 (12)	65	80 (11)	51	81 (12)	32	77 (11)
HR _{peak} (BPM)	54	151 (20)	41	159 (18)	64	157 (22)	48	161 (26)	32	167 (19)

Note Systolic (SAP) and diastolic arterial pressure (DAP), peak (HR_{peak}) and resting heart rate (HR_{rest}), on different test occasions. Data are presented for the group as a whole and for the subgroups divided according to lesion level.

Time course and determinants of systolic and diastolic arterial pressure

The time course of changes in SAP and DAP can be seen in Table 2.2. We found no significant change over time in SAP (Figure 2.1 and Table 2.3). DAP did not change during the period of rehabilitation, but increased in the first five years after discharge. Of the personal and lesion characteristics, age and lesion level were related to both SAP and DAP. For every 10-year increase in age, the SAP increased 3.6 mmHg and the DAP increased 1.7 mmHg (Table 2.3). Both SAP and DAP were significantly lower in those with cervical lesions compared to those with high thoracic and low-level lesions. Also, the DAP in the high thoracic group was significantly lower compared to the low level group (Figure 2.1A and B).

We found an interaction effect between time and lesion level for SAP but not for DAP. In those with cervical lesions, SAP decreased after discharge, whereas in those with low lesions it increased. Also, the time course of the blood pressure change between discharge and one year was different in those with cervical and high thoracic lesions; SAP in those with high thoracic lesions increased from discharge to one year afterwards (Figure 2.1A), whereas in those with cervical lesions it did not. The time course from discharge to five years after did not differ between those with cervical and high thoracic lesions. No other interaction effects between time and any personal or lesion characteristics were found.

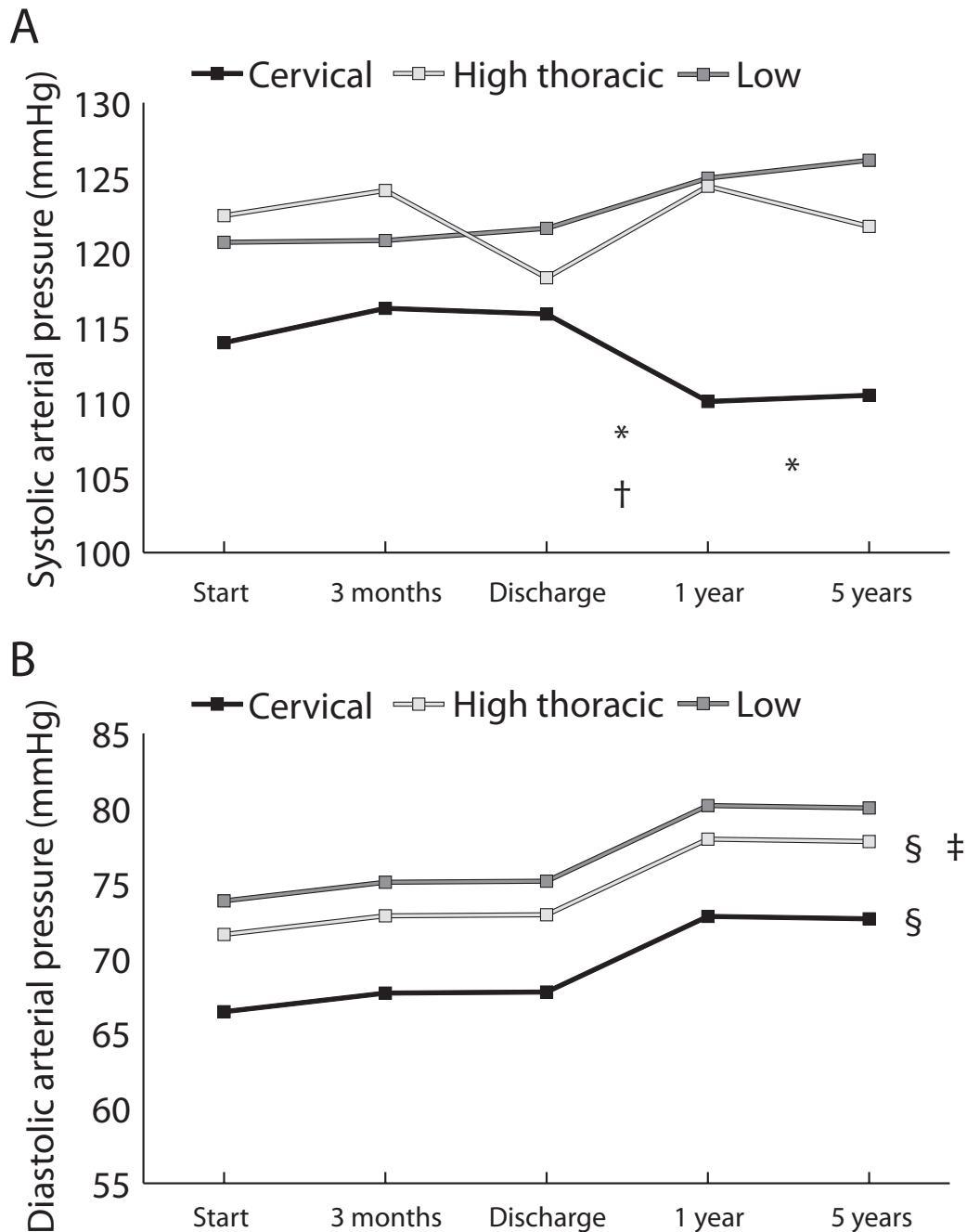


Figure 2.1. Modelled data for systolic and diastolic arterial pressure.

- A. Modelled interaction between time course and lesion level for SAP. The change over time from discharge to 1 year is significantly different ($p = 0.03$ and 0.02 respectively) between the cervical and low lesion groups (*) and between cervical and high thoracic groups (†). The change over time from discharge to 5 years is significantly different ($p = 0.02$) between cervical and low lesion groups (*). In the cervical group SAP decreases after discharge, while in the high thoracic and low level lesion group it increases.
- B. Modelled time course for DAP during and after rehabilitation. DAP is significantly lower ($p < 0.001$ and $p = 0.03$ respectively) in both cervical and high thoracic groups compared to the low lesion group (§) and significantly higher ($p < 0.001$) in the high thoracic lesion group compared to the cervical group (‡).

Time course and determinants of resting and peak heart rate

HR_{rest} and HR_{peak} over time can be seen in Table 2.2. We did not find a significant change over time in HR_{peak} after discharge, but we found an increase during rehabilitation (Figure 2.2 and Table 2.3). HR_{rest} decreased significantly during inpatient rehabilitation and decreased further from time of discharge to five years after discharge. Of the personal and lesion characteristics, only lesion level was related to both HR_{peak} and HR_{rest} after the backward elimination technique. Both HR_{peak} and HR_{rest} were lower in those with cervical lesions compared to those with low level and high thoracic lesions (Figure 2.2A and B). Also, HR_{peak} was lower in those with high thoracic lesions compared to those with low lesions (Figure 2.2B). Age was related to HR_{peak} only; HR_{peak} decreased by 5.2 BPM for every 10-year increase in age. We found no significant interaction effects between time and any personal or lesion characteristics.

Table 2.3. Blood pressure and heart rate regression analyses results

	SAP		DAP		HR _{peak}		HR _{rest}	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
Intercept	110.7 (2.41)		67.7 (1.46)		178.5 (3.92)		81.0 (1.72)	
Δ start-discharge	0.07 (1.67)	0.97	-1.15 (1.12)	0.30	-5.96 (2.84)	0.04	7.49 (1.58)	<0.001
Δ 3 month- discharge	1.72 (1.81)	0.34	0.01 (1.21)	0.99	0.55 (2.91)	0.85	3.60 (1.65)	0.03
Δ discharge-1 year	0.72 (1.83)	0.69	5.46 (1.22)	<0.001	1.18 (2.96)	0.69	0.34 (1.67)	0.84
Δ discharge -5 years	0.08 (1.95)	0.97	4.20 (1.31)	<0.001	5.77 (3.34)	0.08	-4.97 (1.92)	<0.01
Δ cervical-low level	-10.01 (1.35)	<0.001	-7.87 (0.87)	<0.001	-46.77 (2.48)	<0.001	-15.69 (1.39)	<0.001
Δ high thoracic-low level	-2.15 (1.62)	0.18	-2.23 (1.07)	0.03	-9.48 (2.53)	<0.001	-0.01 (1.45)	0.99
Completeness	-3.91 (1.25)	<0.01	NE	--	NE	--	NE	--
Age	0.36 (0.04)	<0.001	0.17 (0.03)	<0.001	-0.52 (0.07)	<0.001	NE	--

Note: Results from the random coefficient analysis for SAP, DAP, HR_{peak} and HR_{rest} after backward elimination. Shown are regression coefficients (β) and their standard errors (SE) representing the change in the outcome measure associated with an increase of one unit in the independent variable. The time and lesion level dummies indicate a change between the two test occasions and the two lesion levels respectively. NE: not entered.

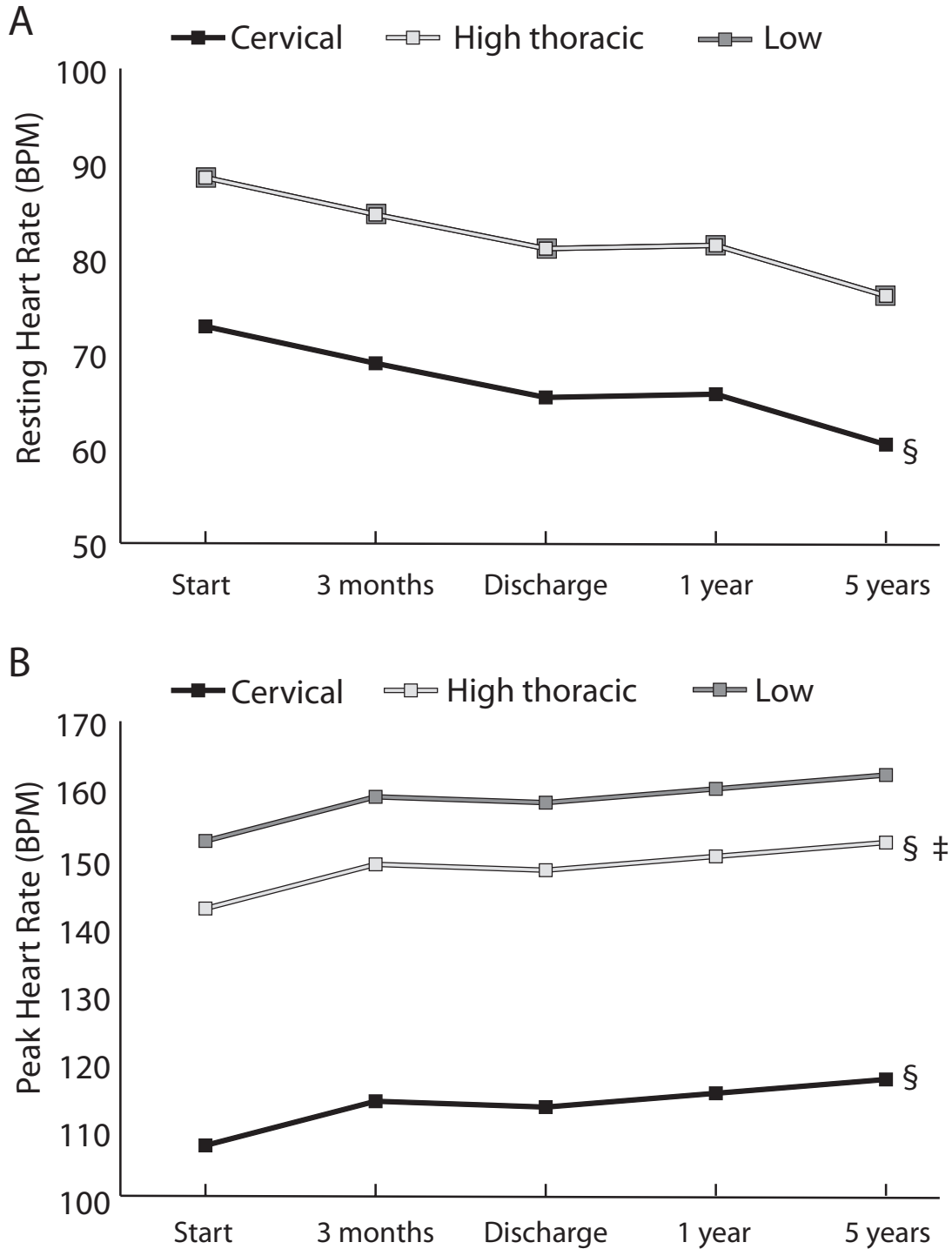


Figure 2.2. Modelled data for resting and peak heart rate

- A. Modelled time course of HR_{rest} during and after rehabilitation. There is a minimal difference between low level and high thoracic groups (the lines are overlapping). The cervical group has significantly lower ($p < 0.001$) HR_{rest} than the low lesion group (§).
- B. Modelled time course of HR_{peak} during and after rehabilitation. The cervical and high thoracic groups have significantly lower ($p < 0.001$) HR_{peak} compared to the low lesion group (§). The high thoracic group has significantly higher ($p < 0.001$) HR_{peak} compared to the cervical lesion group (†).

Prevalence of hypotension

Overall, 33% of the participants had hypotension at the start of rehabilitation according to WHO criteria¹⁴⁵. Table 2.4 shows the prevalence of hypotension at the different test occasions and for the different lesion groups.

We did not find a significant change in the prevalence of hypotension over time. Table 2.5 shows the final regression model. The odds of having hypotension were 4.18 times greater in those with cervical lesions compared to those with low lesions and 1.64 times greater in individuals with high thoracic lesions compared to those with low lesions. The odds were 2.70 times greater in individuals with cervical lesions compared to high thoracic lesions. Also, the prevalence of hypotension decreased with age; the odds were 0.75 times greater for every 10-year increase in age. Men had 2.24 times the odds of having hypotension compared to women. Completeness of injury as determined by AIS classification did not influence the odds of having hypotension.

Prevalence of bradycardia, elevated heart rate, and tachycardia

Due to the low number of positive outcomes for bradycardia (<50 BPM) and tachycardia (>100 BPM), it was not possible to run the binomial random coefficient analysis on these variables. The descriptive values for these prevalence data are shown in Table 4. We did not find a significant change in the prevalence of bradycardia (<60 BPM) over time (Table 2.5). The odds of having bradycardia were significantly greater in those with cervical lesions compared to those with low lesions, and in those with high thoracic lesions compared to those with low lesions. Age, sex and completeness of injury by AIS classification did not influence the odds for bradycardia. The prevalence of an elevated HR decreased during and after rehabilitation (Table 2.4). The odds of having an elevated HR was significantly lower in those with cervical lesions compared to low lesions. Age, sex and completeness of injury by AIS classification did not influence the odds for an elevated HR. Table 2.5 shows the final regression model.

Table 2.4. Prevalence of hypotension, bradycardia, elevated heart rate and tachycardia at all test occasions according to lesion level

	Start of active rehabilitation	3 months later	Discharge from rehabilitation	1 year after discharge	5 years after discharge
All participants					
Hypotension (%)	33.0	21.3	27.4	18.3	13.7
Bradycardia <50 BPM (%)	0	2.1	1.6	2.2	6.7
Bradycardia <60 BPM (%)	4.5	8.5	8.1	6.7	16.7
Elevated HR >80 BPM (%)	62.4	48.9	33.6	40.0	26.7
Tachycardia >100 BPM (%)	13.8	8.5	6.6	6.7	1.7
Participants with cervical lesions					
Hypotension (%)	45.6	33.0	38.0	27.8	25.3
Bradycardia <50 BPM (%)	0	3.4	5.6	11.1	26.7
Bradycardia <60 BPM (%)	14.8	24.1	27.8	27.8	60.0
Elevated HR >80 BPM (%)	37.0	27.6	8.3	11.1	6.7
Tachycardia >100 BPM (%)	0	0	0	0	0
Participants with high thoracic lesions					
Hypotension (%)	26.3	18.4	26.3	18.4	7.9
Bradycardia <50 BPM (%)	0	4.3	0	0	0
Bradycardia <60 BPM (%)	4.8	3.7	0	0	0
Elevated HR >80 BPM (%)	58.3	47.8	42.8	57.1	23.0
Tachycardia >100 BPM (%)	33.3	11.1	23.8	4.8	0
Participants with low level lesions					
Hypotension (%)	23.8	11.3	17.5	8.8	5.0
Bradycardia <50 BPM (%)	0	0	0	0	0
Bradycardia <60 BPM (%)	0	0	0	2.0	3.1
Elevated HR >80 BPM (%)	75.8	64.2	44.6	43.1	37.5
Tachycardia >100 BPM (%)	12.1	7.0	4.6	9.8	3.1

Note: The prevalence of hypotension, bradycardia (<50 BPM and <60 BPM), elevated heart rate (HR, >80 BPM) and tachycardia (>100 BPM) are shown for each test occasion. Data are presented for the group as a whole and for the subgroups divided according to lesion level.

Table 2.5. Prevalence of hypotension, bradycardia and elevated heart rate regression analyses

	Hypotension			Bradycardia (<60 BPM)			Elevated HR (>80 BPM)		
	β (SE)	OR	p	β (SE)	OR	P	β (SE)	OR	p
Intercept	-0.99 (0.38)			-5.09 (0.87)			-0.24 (0.27)		
Δ start-discharge	0.08 (0.24)	1.08	0.74	-0.47 (0.59)	0.63	0.43	1.26 (0.30)	3.53	<0.001
Δ 3 month-discharge	-0.27 (0.27)	0.76	0.30	-0.05 (0.54)	0.95	0.93	0.74 (0.30)	2.09	0.01
Δ discharge - 1 year	-0.24 (0.27)	0.79	0.39	0.14 (0.59)	1.15	0.82	0.16 (0.31)	1.17	0.61
Δ discharge - 5 years	-0.24 (0.30)	0.78	0.41	1.06 (0.59)	2.89	0.07	-0.34 (0.37)	0.71	0.36
Δ cervical - low level	1.43 (0.20)	4.18	<0.001	4.12 (0.80)	61.6	<0.001	-1.90 (0.29)	0.15	<0.001
Δ high thoracic - low level	0.49 (0.25)	1.64	0.049	0.98 (1.06)	2.66	0.36	-0.40 (0.25)	0.67	0.11
Completeness	NE	--	--	NE	--	--	NE	--	--
Age	-0.03 (0.01)	0.97	< 0.001	NE	--	--	NE	--	--
Sex	0.81 (0.22)	2.24	< 0.001	NE	--	--	NE	--	--

Note: Results of the binomial random coefficient regression analysis with backward elimination for the prevalence of hypotension, bradycardia (<60 BPM) and elevated heart rate (HR, >80 BPM). Shown are regression coefficients (β) and their standard errors (SE), and the odds ratios (OR) for the models. The time and lesion level dummies indicate a change between the two test occasions and the two lesion levels respectively. NE: not entered.

Discussion

This is the first study to investigate the time course of blood pressure, HR_{rest} and HR_{peak} over the first 5 years after SCI using a longitudinal approach, and to provide normative data in a relatively large cohort that is subdivided according to time after injury over five years, and by lesion level. We are also the first to not only identify associations between lesion or personal characteristics and cardiovascular dysfunction, but also to quantify the magnitude of increased risk associated with these traits. We found that the prevalence of hypotension did not change during and after rehabilitation: however, it was related to lesion level (positively), age (negatively) and sex. We found no change in SAP over time,

but DAP increased during the first five years after discharge. SAP and DAP were related to age (positively) and lesion level (negatively). The time course for SAP was different between the different lesion levels. HR_{peak} only changed during rehabilitation, whereas HR_{rest} decreased during and after rehabilitation. Lesion level was negatively associated with both peak and resting HR, and age was negatively associated with HR_{peak} . The prevalence of bradycardia remained the same over time, while the prevalence of elevated HR improved (decreased) during and after rehabilitation. The prevalence of bradycardia (<60 BPM) was positively correlated to lesion level, and the prevalence of elevated HR (>80 BPM) was negatively correlated to lesion level.

We found a greater prevalence of hypotension in those with cervical and high thoracic lesions compared to those with injury levels below T5. The prevalence was greatest in those with cervical lesions. This is not surprising, because individuals with cervical lesions can lose control over spinal sympathetic pathways to the heart and vasculature, and therefore a consequent reduction in resting blood pressure and a greater susceptibility to hypotension might be expected. Individuals with high thoracic lesions can retain sympathetic control over the heart, but control of the splanchnic vasculature may be impaired. This can explain the effective titration of resting blood pressure across lesion levels after SCI. Age and sex influenced the odds of hypotension after SCI, whereby older individuals had lower odds compared to younger individuals, and men had higher odds compared to women. In the able-bodied, increasing age has been shown to be related to higher blood pressures due to arterial stiffening^{149,150} and a higher prevalence of hypertension¹⁵¹, and this is in agreement with our finding of a lower prevalence of hypotension with aging after SCI. We do not know why men had higher odds of hypotension compared to women. We considered the possibility that the men in our study had more severe injuries than the women, but this was not the case. The completeness of injury based on AIS score had no effect on the prevalence of hypotension, indicating that AIS completeness is not a strong predictor for impairment to cardiovascular autonomic pathways, as previously reported by our laboratory^{53,80,152}.

The increase in SAP found in those with low level and high thoracic lesions could be related to adopting a more sedentary lifestyle after injury, as shown in a subset of this study population¹⁵³. The failure of SAP to increase with time in those with cervical SCI can be explained by the lack of sympathetic control to the heart and vasculature. In this

case, an increase in SAP due to a more sedentary lifestyle is unlikely, as this increase has been related to elevated sympathetic tone, and coordinated control of sympathetic tone after cervical SCI is often lost¹⁵⁴. This finding is in agreement with several cross-sectional studies of individuals with chronic SCI^{49,56,155-157}, which reported resting BP and HR to continue to be lower in those with high lesions compared to lower lesions in the chronic phase after injury. Some studies^{53,56} report lower SAP, DAP and HR_{rest} compared to our findings, which can be explained by the fact that their studies included a greater proportion of individuals with complete injuries compared to our mixed population. Other studies with more similar lesion characteristics to our study in terms of completeness of injuries found similar SAP, DAP and resting HR^{54,158,159}. However, a major limitation of these studies is that they do not show changes in cardiovascular parameters over time but rather a snapshot in the chronic phase of injury.

The HR_{peak} in individuals with cervical lesions was low (111 ± 17.1 BPM at the start of rehabilitation and 109 ± 15.4 BPM five years after discharge). This diminished heart rate response to exercise is most likely due to impaired supraspinal control of cardiac sympathetic outflow in this group. In the absence of coordinated sympathetic control of heart rate, the remaining heart rate response to exercise in these individuals is presumably largely mediated by vagal withdrawal. Indeed, the peak exercise heart rate in this group is comparable to that of able-bodied controls during complete vagal blockade (approximately 100 BPM)⁴⁸. The slight increase in HR_{peak} in participants with all injury levels during rehabilitation is in line with the previously reported increase physical fitness parameters, VO_{2max} and peak power output, in this cohort^{160,161}.

The variability in HR_{peak} in the group with cervical lesions is likely to be due to the fact that not all of these participants had a complete injury, and therefore some of them would have preserved cardiovascular autonomic function and higher peak heart rates.

The greater prevalence of bradycardia in those with cervical lesions underscores the vagal predominance in this group, in whom there is a greater potential for injury to descending spinal cardiac sympathetic pathways. Similarly, those in both other groups had higher resting heart rates. Interestingly, the prevalence of elevated heart rates in the low lesion group decreased with time after injury, and there are numerous factors (including the influence of rehabilitation, recovery of cardiovascular deconditioning,

improved blood volume status, medication adjustment) that could underlie this change. The prevalence of bradycardia in those with cervical injuries was a consistent feature, compatible with lack of improvement in cardiac sympathetic function over time, and this is also reflected in the failure of the impaired HR_{peak} to improve with time in this group.

There are no prior longitudinal studies that have examined changes in these cardiovascular parameters with time after injury. However, there are cross-sectional studies that predominantly focus on chronic SCI (> 1 year after injury). Prevalence of resting hypotension is not frequently reported in these studies. One study showed a prevalence of 33% in their overall sample (482 individuals with SCI) and 51% in a subset of those with AIS complete tetraplegia, but this was based on self-reported hypotension (by a questionnaire)¹⁶². The prevalence of OH is more commonly reported. OH is a variable related to resting blood pressure measured in a seated position (as it incorporates a gravitational effect), and is defined as a drop in SAP of 20 mmHg or a drop in DAP of 10 mmHg or more when moved from supine to seated/upright. We have previously reported a prevalence of OH of 50% in individuals with cervical lesions⁵³ and 47% in individuals with lesions above T5¹⁵⁹. Recently, Sisto et al.⁵⁴ reported a lower prevalence of OH of 21% in individuals with mostly cervical injuries. This lower prevalence can be explained by including only individuals with highly preserved function (AIS C and D). In these types of injuries, spinal sympathetic pathways may be more likely to remain intact, and individuals might thus be protected from the cardiovascular abnormalities that often accompany high-level injuries. Even though they did not report prevalence of resting hypotension, this group found lower resting blood pressure in those with cervical lesions compared to those with high and low thoracic injuries, suggesting a higher risk for resting hypotension in this subgroup.

Several studies have investigated cardiovascular adaptations after SCI^{44,45}. They found an increased leg vascular resistance in individuals with SCI, even though sympathetic control over the heart and vasculature was impaired, due to vascular⁴⁴ and hormonal adaptations after injury⁴⁵. These adaptations were proposed to counteract the deleterious effects of the loss of normal sympathetic regulation of blood pressure. The findings of the present study indicate that these adaptations might not be sufficient to prevent hypotension; it is tempting to speculate that without these adaptations, we might predict even more severe and prevalent hypotension.

Strengths and limitations

The main strength of the present study is the relatively large sample size, incorporating all levels and completeness of injury, and its longitudinal design. All participants were prone to cardiovascular deconditioning due to the use of a wheelchair, making level of injury and autonomic dysfunction the primary factors underlying any differences in blood pressure and heart rate at each time point. No previous studies have investigated the time course of changes in these cardiovascular variables after SCI. An additional strength of the study is the identification not only of associations between lesion or personal characteristics and cardiovascular dysfunction, but the quantification of the magnitude of increased risk associated with these traits.

One limitation of this study is that not all participants completed the test protocols at all time points. While of course this is not the ideal scenario, it is the clinical reality of these types of studies. We chose to analyse the full data set with a statistical approach that is validated for use with incomplete data sets^{147,148}. This approach has two advantages: the statistical power is greater, and it avoids the selection bias encountered by analyzing only those who performed all measurements (often healthier persons or those that are unemployed, for example). This selection bias would also not be desirable when it comes to the generalizability of the results to clinical practice. However, we also completed repeated measures analysis of variance on only those participants who completed all testing sessions (data not shown), and found no meaningful differences between the outcomes of the two approaches, further supporting the validity of our findings. Similarly, another limitation is the higher dropout rates for the maximal aerobic capacity test, leading to a lower sample size for this variable¹⁴³. The heart rate data may, therefore, be biased towards those who were willing and/or able to perform the maximal aerobic capacity test (without cardiovascular or musculoskeletal complaints; and likely excluding those using power wheelchairs), potentially overestimating HR_{peak} and HR_{rest} .

Our statistical analyses of the prevalence data for bradycardia and elevated heart rate/tachycardia were limited by the low number of positive responses. This was particularly so for the sub group analyses, and for the prevalence of bradycardia (<50 BPM) and tachycardia (>100 BPM). The analysis of the prevalence of bradycardia (<60

BPM) was also somewhat constrained by this issue, and should be interpreted with caution.

Another limitation is that some of the resting blood pressure measurements might have been obscured by episodes of AD. As it is challenging to accurately determine the occurrence of these episodes retrospectively, it is conceivable that some resting blood pressure values might be artificially elevated by episodes of AD. This could lead to an overestimation of the mean SAP and DAP in individuals with cervical and high thoracic lesions and consequently an underestimation of the prevalence of hypotension in these groups. To estimate how often this occurred, in those at risk for AD (lesions at or above T5), we qualified AD as a SAP value of 30 mmHg above the average SAP over the test occasions for that individual. Using this approach we estimated that episodes of AD occurred in a total of 23 tests out of 757 tests analyzed. As we could not be sure that these represent true episodes of AD because of the retrospective nature of this study, we included all SAP measurements in our analyses. However, it should be kept in mind that this could lead to a modest underestimation of the prevalence of hypotension in our sample.

Conclusion

At the start of active rehabilitation 33% of the participants had resting hypotension and this did not significantly improve over time, at least until 5 years after rehabilitation. Individuals with cervical lesions are most prone to resting hypotension and bradycardia compared to high thoracic and low-level injuries. In previous studies it has been shown that OH is even more prevalent (~50 %) in this population of individuals with cervical or high thoracic SCI^{53,159}. This combination of resting hypotension and OH can lead to intolerably low blood pressures, with subsequent reductions in cerebral blood flow, presyncope, syncope, cognitive impairment and fatigue¹⁵⁹. We also showed that the impaired HR responses to exercise in those with cervical lesions do not improve over time, with a prolonged negative impact on exercise tolerance and ability to participate in physical activity. These complications have a significant negative impact on quality of life in this population⁶³. The assumption that these are issues related primarily to deconditioning in the acute stage after injury and that they recover with time is incorrect and should be challenged. These findings suggest that management of (orthostatic)

hypotension and cardiac dysfunction should remain a priority for those with injuries above T5 into the chronic phase of injury.

We are the first to provide data on the time course of cardiovascular changes after injury. These data can be used to guide clinical practice, and to place changes in cardiovascular function due to interventions in perspective, relative to that expected with standard treatment alone, as has been shown for motor and sensory function¹⁶³⁻¹⁶⁵. We also identified those individuals at greatest risk for cardiovascular dysfunction considering age, sex, level and completeness of injury. These specific odds of cardiovascular dysfunction may be useful to guide targeted patient therapy.

Chapter 3.

Complex relationships between autonomic dysfunction, lifestyle changes and cardiovascular disease risk

Introduction

In addition to the search for a 'cure' for spinal cord injury (SCI), one sizeable challenge in SCI research now lies in improving the secondary complications injury. Cardiovascular disease (CVD) is one of the leading causes for morbidity and mortality after SCI, and it has an earlier onset and more rapid progression compared to the general population²². The underlying mechanisms for the elevated risk of CVD remain unclear. Lifestyle changes after SCI have classically been thought to be the main contributor to the increase in prevalence of obesity and related CVD risk. However, we propose that other factors may play an important role.

In addition to the impact of paralysis, secondary complications due to autonomic dysfunction after SCI may cause individuals to lead a more sedentary lifestyle. Damage to spinal sympathetic pathways can occur due to SCI, and this can affect many organ systems, dependent on the level of the lesion. Disruption of these pathways results in basal sympathetic hypoactivity and impaired cardiovascular autonomic control. Cardiovascular autonomic dysfunction is a particular concern in individuals with lesions above the fifth thoracic level, manifested as low resting blood pressure, orthostatic hypotension (OH, further falls in blood pressure with a change in posture), and episodes of extremely high blood pressure, autonomic dysreflexia (AD) associated with sensory stimulation below the lesion. Fatigue and concentration problems associated with resting and orthostatic hypotension^{61,62,64} can be barriers to participation in physical activity after injury. In addition, the limited ability for many individuals with SCI to increase

heart rate and blood pressure physical activity can cause poorer exercise tolerance, even in the presence of a strong motivation to exercise.

The correlation between a sedentary lifestyle, accompanied by gains in adiposity and abnormalities in metabolism, and CVD risk is well established in the general population¹²⁰⁻¹²². More recently, this association has been shown in individuals with SCI. A large longitudinal study showed a correlation between lipid profiles and physical activity, indicating that individuals with SCI who were more physically fit had more favourable lipid profiles^{26,123}. Moreover, this group showed a direct relationship between physical activity levels and CVD risk²³. However, until now, the contribution of autonomic dysfunction to these associations has not been investigated.

In addition to the described indirect effect of autonomic dysfunction on CVD risk via the effects on physical activity, there may also be a direct impact on CVD risk arising from autonomic dysfunction. There are different mechanisms that support this hypothesis. Blood pressure control problems due to autonomic dysfunction, in particular episodes of AD, can have potentially devastating consequences such as stroke, cardiac arrest, myocardial ischaemia and cardiac arrhythmia^{80,102-106}. Autonomic impairments after SCI also interfere with the action of the protein leptin that is produced by adipocytes and regulates food intake and stimulates energy expenditure. Normally, leptin acts on the arcuate nucleus in the hypothalamus, thereby increasing sympathetic activity, and stimulating energy expenditure. When supraspinal input to the spinal sympathetic pathways is blocked, this action of leptin is also lost. Indeed, the decreased resting metabolic rate (RMR) that is commonly seen in individuals with SCI, who have damage to spinal sympathetic pathways¹³¹, might be explained in part by the diminished action of leptin. Jeon et al. confirmed this by showing that leptin levels in individuals with SCI were elevated, but the correlation between leptin and RMR was lost in this group compared to able-bodied controls¹³².

Impaired glucose tolerance and diabetes mellitus are also more prevalent in individuals with SCI compared to able-bodied controls¹²⁴⁻¹²⁶, and this may be related to autonomic dysfunction. Even though fasting glucose levels are usually within the normal range, individuals with SCI are more often insulin resistant¹²⁵⁻¹²⁷. Interestingly, in the able-bodied population, β -adrenergic stimulation induces insulin resistance^{127,128}. Accordingly,

the low resting sympathetic activity in individuals with autonomic dysfunction after SCI would be expected to be favourable in terms of insulin resistance, although the extremely high levels of sympathetic activity encountered during AD might worsen insulin resistance.

As such, despite considerable research suggesting a role for autonomic impairment on CVD risk factors and potentially overall risk for CVD, no study to date has investigated the complex relationships between autonomic impairment, CVD risk factors and overall CVD risk. Therefore, one aim of this study is to first determine whether there are differences in CVD risk factors between able-bodied controls and individuals with autonomically complete and autonomically incomplete SCI. We also aimed to investigate the contribution of autonomic impairment, physical activity levels, and their interaction, on CVD risk parameters in individuals with SCI.

We hypothesize that individuals with SCI have a more adverse risk profile for CVD compared to able-bodied controls, and that individuals with autonomic impairments have an even more adverse risk profile compared to those without autonomic impairments. We hypothesize that autonomic dysfunction contributes to CVD disease risk parameters both directly, and through an interaction with physical activity levels.

Methods

This study received ethical approval from the Research Ethics Board at Simon Fraser University, and the Vancouver Coastal Health Research Institute, and was performed in association with the Declaration of Helsinki of the World Medical Association.

Participants

Studies were performed on 33 individuals with a chronic (>1 year) SCI and 19 able-bodied controls. All participants gave written informed consent, and were apparently healthy and not taking any cardiovascular medications. Individuals with SCI were recruited from posters displayed at rehabilitation centres, and through local SCI support groups. Able-bodied individuals were recruited from posters displayed at Simon Fraser University. Females were not tested during menstruation. Individuals with pre-existing (prior to SCI) cardiovascular disease were excluded from participation.

Participants made two visits to the laboratory. They were instructed to fast for 12 hours overnight, except for drinking water, before both visits. The first visit started with a brief medical history to obtain participant characteristics including age, sex, time since injury, smoking and alcohol status, and medication use. A standard assessment for neurological level and severity of injury was performed, unless the participant underwent this assessment previously and could report the results. This visit was concluded with a passive orthostatic stress test as described previously^{53,63,159}, that included 15 minutes of supine rest after which the participant was passively moved into a 90° seated position with their legs pendent for another 15 minutes or until they experienced symptoms or signs of presyncope. The second visit was composed of an oral glucose tolerance test, completion of a physical activity questionnaire, and a whole-body dual energy X-ray absorptiometry scan (DEXA).

The SCI group was subsequently divided into those with autonomically complete and incomplete injury to cardiovascular autonomic pathways (referred to as 'complete' and 'incomplete' respectively). These groups were not based on level of injury, but on the outcomes of our tests for cardiovascular autonomic impairment.

Measurements of severity of injury

Standard neurological classification

Neurological classification of level and severity of SCI was determined using the standard classification tool: the American Spinal Injury Association (ASIA) Impairment Scale (AIS). This scale assesses motor and sensory function after SCI, but does not incorporate a quantitative measure of autonomic function. AIS A score reflects complete loss of motor and sensory function; the B, C and D scores reflect gradually less severe impairments. Level of injury and impairment score together form the classification of the injury (e.g. T5A).

Cardiovascular autonomic impairment

At present there is no gold standard technique to determine autonomic impairment after SCI. Therefore, we used two techniques that have previously been used to determine cardiovascular autonomic completeness of injury, one invasive and one non-invasive technique. The first one is a resting plasma noradrenaline concentration measurement

and the second one is systolic arterial pressure variability analysis. Both measurements were determined from the passive orthostatic stress test during the first visit.

Plasma noradrenaline

A butterfly catheter was inserted into an antecubital vein and blood samples were withdrawn after 15 minutes of supine rest, and 5 minutes after the passive assumption of a seated position. The samples were centrifuged at 3°C and 3000 rpm for 10 minutes and the plasma component withdrawn for subsequent analysis. The plasma samples were then sent to the clinical laboratory of Vancouver General Hospital for determination of noradrenaline levels with high-pressure liquid chromatography (ESA Coulochem II detector, Thermo Fisher Scientific, Sunnyvale, CA, USA).

Low noradrenaline (NA) levels due to sympathetic hypoactivity, are indicative of injury to descending sympathetic pathways. Therefore, autonomic completeness was defined as a supine plasma NA level below the cut off that was determined as the lower limit of the normal range for our laboratory (<0.56 nmol/L)¹⁰⁰.

Systolic arterial pressure variability analysis

Throughout the orthostatic stress test, blood pressure was measured using finger plethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands) and a lead II electrocardiogram (ECG) was recorded using the integrated Finometer ECG. Recordings were made using an analog-to-digital converter (Powerlab 16/30, AD Instruments, Colorado Springs, CO) with a sampling frequency of 1 kHz, and stored for offline analysis using LabChart (LabChart 7, AD Instruments, Colorado Springs, CO) and Matlab (Matlab 2012b, MathWorks, MA, USA) software. Systolic, diastolic and mean arterial pressures (SAP, DAP and MAP) were determined using the peak detection function in LabChart. Heart rate (HR) was determined from the lead II ECG using the same technique. Resting SAP (SAP_{rest}) was determined as the average SAP during the supine phase and the minimum SAP (SAP_{min}) as the minimum 30 second average SAP during the seated phase. Resting HR (HR_{rest}) was determined as the average HR during the supine phase.

Beat-to-beat values for SAP, DAP, MAP and RR intervals (RRI) from the ECG were used to create a time series for these parameters for both the supine and the upright phase of the test separately. Outliers due to ectopic beats were substituted by linear

interpolation of adjacent beats and any obvious trends in the time series were removed by subtracting the best polynomial function fitted to the data. Autoregressive monovariate modelling was used to create a power spectral density plot. Low frequency (LF, 0.05-0.15 Hz), high frequency (HF, 0.15-0.3 Hz) and very low frequency (VLF, <0.03 Hz) peaks¹⁶⁶ were identified for the spectra of SAP and RRI. The power and the central frequency of each peak was calculated as well as a normalized power, calculated by dividing the power by the total variance minus the VLF power and multiplied by 100¹⁶⁶.

Dependent on the level of the lesion, some sympathetic control of blood pressure can remain, mostly in lesions between T1 and T5. Therefore, we choose to use a conservative cut-off for LF SAP power of <1.0 mmHg² was used to determine autonomic completeness, which has been shown to be the cut-off with the highest sensitivity and specificity to discriminate between SCI individuals with and without autonomic dysfunction¹⁰⁰.

Cardiovascular disease risk factors

Heart rate variability

Total HRV and very low frequency power (VLF, < 0.05 Hz) power were calculated for RRI time series using the technique described above for SAP variability.

Blood lipid profiles, insulin and glucose levels

A second venous blood sample was collected at the start of the orthostatic stress test. This sample was centrifuged as described above and sent to the clinical laboratory at Vancouver General Hospital where high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG) and glucose levels were determined by enzymatic assays (Dimension Vista system, Siemens Healthcare Diagnostics Inc, USA). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald method¹⁶⁷. TC/HDL-C was calculated from TC and HDL-C levels. Plasma insulin was determined at the same laboratory using an immunoassay, ADVIA Centaur assay (Siemens Healthcare Diagnostics Inc. USA). Insulin resistance (IR) was calculated using the homeostatic model assessment (HOMA) method¹⁶⁸.

Oral glucose tolerance

Participants came in for a second visit to the laboratory for an oral glucose tolerance test. A finger prick method was used to determine blood glucose levels (Contour, Bayer Inc., Toronto, Canada) in the fasting state and 30, 60, 90 and 120 minutes following consumption of a 75 g glucose solution (Glucodex 75 g, Rougier Pharma, Canada). The glucose level at 120 minutes after consumption was taken as the main outcome measure for glucose tolerance.

Framingham 30-year risk for cardiovascular disease score

The Framingham 30-year risk for cardiovascular disease score¹⁶⁹ was used as a measure of overall risk of CVD. This risk score is validated from the Framingham Heart study data. It incorporates the following risk factors: age, sex, smoking status, diabetes, HDL-C, TC, resting SAP, and the use of antihypertensive treatment. However, instead of including the measured SAP, we entered a SAP value of 120 mmHg into the risk score formula for all participants. This decision was based on the knowledge that SCI can impair normal blood pressure control with lesions at or above the 5th thoracic level, leading to lower resting blood pressure^{50,52}. The known relationship between SAP and CVD risk might, therefore, not exist in the same way in this population. Entering a value of 120 mmHg is neutral to the score, and therefore excludes any effect of SAP on the risk score generated. A risk score under 10% is considered low risk, a score between 10 and 20% an intermediate risk and above 20% a high risk¹⁷⁰.

Physical activity level

The Physical Activity Scale for Individuals with a Physical Disability (PASIPD) questionnaire was used to determine physical activity levels of the participants with SCI. This 13-item questionnaire asks about any physical activity during the past 7 days including, for example, household work, leisure time activity and sports. The questionnaire results in a total physical activity score in metabolic equivalent (MET) hours per day (MET hours/day). This questionnaire has been shown to correlate strongly to the gold standard outcome measure for physical fitness, maximal oxygen consumption (VO_{2max})¹⁷¹. We elected to utilize an activity scale rather than a physiological assessment because the VO_{2max} test can be technically challenging for

individuals with SCI, and the form of exercise chosen for the test might not be representative of daily activity.

Body composition

Total body fat mass in kg was determined from the whole body DEXA scan (QDR 4500, Hologic Inc., Bedford, MA, USA) and total body fat percentage was calculated by dividing the fat mass by total body mass multiplied by 100. Standardized landmarks were used to distinguish the trunk region on the DEXA scan (QDR software, Hologic Inc. Bedford, MA, USA) and determine abdominal fat mass from that region¹⁷². Abdominal fat percentage was determined as abdominal fat mass divided by total mass in the defined region multiplied by 100.

BMI was calculated from weight (kg), determined from the DEXA scan, divided by height squared (m^2), determined using an electronic ruler (Matlab 2012b, MathWorks, MA, USA) on the DEXA images. Waist circumference (WC) was measured in cm at the narrowest part of the waist after a normal expiration, while lying supine¹³⁵.

Statistical analyses

All statistical analyses were performed using JMP (JMP 10, SAS Institute Inc., Cary, NC, USA). Descriptive statistics (means and standard deviations [SD]) were determined for participant characteristics, and all dependent and independent variables, for the three different groups (able-bodied controls, the autonomically complete SCI group and the autonomically incomplete SCI group). The sample sizes within the three groups were relatively small, and therefore, a non-parametric alternative for ANOVA, the Kruskal-Wallis test, was used to determine differences between the three groups. Steel-Dwass post-hoc tests were used to determine differences between pairs, when an initial significant outcome for the Kruskal-Wallis test was found. We used one-sided hypothesis testing (for $\alpha = 0.05$), to evaluate whether the SCI groups had worse outcomes compared to the control group, and whether the autonomically complete group had worse outcomes compared to the autonomically incomplete SCI group.

Secondly, we investigated the effects of autonomic function and physical activity on CVD risk factors in individuals with SCI. Multiple linear regression analyses were performed to determine effects of the independent variables (autonomic function and physical activity), confounder variables (age, sex, visceral adiposity), and interactions effects of

autonomic function and physical activity on the dependent variables (Framingham Risk score and other CVD risk factors that showed significant differences between SCI groups). A backward elimination technique was used, eliminating variables that showed a p-value >0.1 , to determine the final model. Age and sex were included as confounders in the models for CVD risk factors, but not in the model for the Framingham risk score (as these factors are already incorporated within the risk score). Visceral adiposity has been shown to be an independent risk factor for CVD, and was therefore included as a confounder. Supine plasma NA level was included as the index of sympathetic autonomic function, and WC as an index of visceral adiposity¹⁷³.

Results

Participants with SCI were subdivided into an autonomically complete group (complete) and an autonomically incomplete group (incomplete). Three individuals with SCI tested positive for a urinary tract infection on the day of the orthostatic stress test. As this phenomenon is a known trigger for AD, we excluded all blood pressure data (including LF SAP) for these participants.

Participant characteristics

We found no significant differences in age and sex between the three groups. BMI was also not different between the groups. Levels of injury ranged from C5 to L3 in the incomplete SCI group, while the complete group consisted of only cervical injury levels.

LF SAP, LF SAP % and LF SAP_{nu} were all lower in the complete SCI group compared to the control group and the incomplete SCI group. Plasma NA during supine and seated positions was lower in the complete SCI group compared to the control group and the incomplete SCI group. SAP_{rest} and SAP_{min} were also lower in the complete SCI group compared to both other groups, confirming autonomic dysfunction in this group. HR_{rest} was lower in the complete compared to the incomplete SCI group. Participant characteristics per group are shown in Table 3.1.

Table 3.1. Participant characteristics

	Control	Incomplete SCI	Complete SCI
General characteristics			
Sample size	19	16	17
Age (years)	34 (3)	43 (3)	39 (3)
Sex (% males)	68.4	56.3	82.4
Lesion level range	-	C5-L3	C4-C7
Cardiovascular variables			
SAP _{rest} (mmHg)	133 (12)	126 (19)	109 (14) *†
SAP _{min} (mmHg)	118 (13)	113 (29)	88 (15) *†
HR _{rest} (BPM)	62 (10)	67 (9)	56 (5) †
Autonomic markers			
Plasma NA supine (nmol/L)	1.67 (0.72)	3.41 (2.24)	0.42 (0.23) *†
LF _{SAP} (mmHg ²)	2.73 (1.67)	3.98 (3.53)	0.66 (0.57) *†
Heart rate variability parameters			
HRV (ms ²)	6797 (6839)	5620 (5585)	3656 (3118)
VLF (ms ²)	2486 (3272)	1959 (1784)	1966 (1178)
Body composition and physical activity			
BMI (kg/m ²)	23.9 (3.4)	23.3 (4.9)	23.1 (3.9)
Fat (%)	21.1 (9.2) †	29.0 (7.2) *	26.9 (9.0)
Abdominal fat (%)	20.4 (9.5)	26.7 (7.0)	26.9 (9.6)
PASIPD (MET hours/day)		13.1 (10.0)	17.6 (12.4)
Framingham risk score	10.3 (9.9)	16.1 (7.3)	15.3 (8.5)

Note: * Significantly different ($p < 0.05$) from controls † significantly different between SCI groups. Data are shown as mean (SD). SAP: systolic arterial pressure; HR: heart rate; NA: supine noradrenaline; LF: low frequency; HRV: heart rate variability; VLF: very low frequency; BMI: body mass index; PASPID: physical activity scale for individuals with a physical disability.

Group differences in cardiovascular risk factors

We found no significant differences in the total or VLF power of HRV between the three groups (Table 3.1). Glucose tolerance (measured as 120-minute glucose) was impaired in both SCI groups compared to the control group (Figure 3.1A). We found no significant difference between the two SCI groups. We also found no significant differences in IR between any of the groups (Figure 3.1B). Fasting plasma insulin was greater in the complete SCI group compared to controls (Figure 3.1C), but fasting plasma glucose levels were not significantly different (Figure 3.1D). We found no significant differences in Framingham risk score between all three groups, however, the SCI group as a whole had a greater ($p = 0.02$) risk score (16 [7.9]) compared to the control group (10.3 [9.9]).

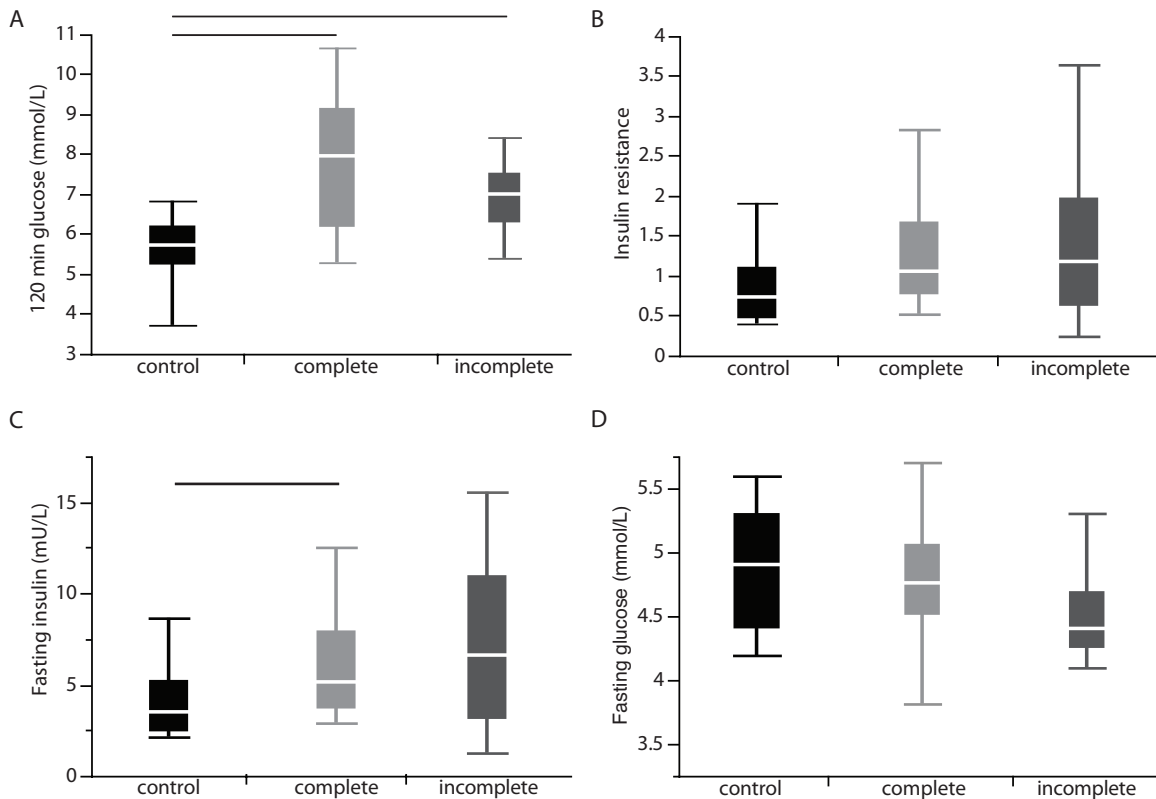


Figure 3.1. Group differences in blood glucose regulation

A. 120-minute glucose. B. Insulin resistance. C. Fasting insulin. D. Fasting glucose.

Horizontal lines indicate significant differences between groups ($p < 0.05$). The white line in the box represents the median and the full box represents the first to the third quartiles. The whiskers represent the full range of the data.

Plasma TG levels were significantly higher in those with incomplete SCI compared to the control group, but we found no significant difference between the complete SCI group and the other two groups (Figure 3.2A). TC/HDL-C ratio was significantly higher in those with incomplete, but not complete SCI, compared to the controls (Figure 3.2B). Plasma HDL-C levels were lower in both SCI groups compared to the control group, but we found no difference between the SCI groups (Figure 3.2C). We found no significant differences in plasma TC and LDL-C level between any of the groups (Figure 3.2D).

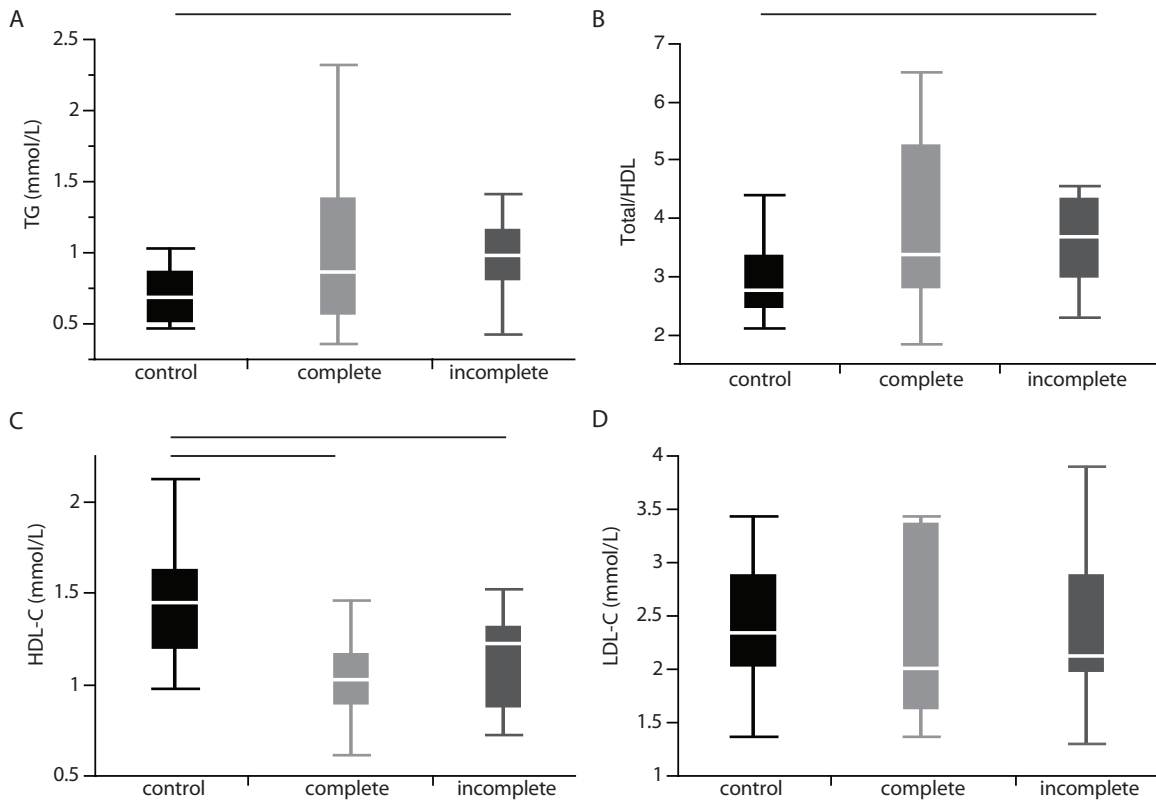


Figure 3.2. Group differences in plasma lipid variables

A. Triglyceride level (TG). B. Total cholesterol to high-density lipoprotein cholesterol ratio (TC/HDL-C).
C. HDL-C level. D. Low density lipoprotein cholesterol (LDL-C) level.

Horizontal lines indicate significant differences between groups ($p < 0.05$). The white line in the box represents the median and the full box represents the first to the third quartiles. The whiskers represent the full range of the data.

Multiple linear regression models for cardiovascular risk factors

After backward elimination in the multiple regression analyses including the independent variables (plasma NA and PASIPD score), the interaction between them, and confounder variables (age, sex and waist circumference), we found significant effects of the independent variables on the following outcome variables: Framingham risk score, 120-minute glucose, IR and plasma insulin. We did not find significant effects of autonomic function from the regression models for TG, HDL-C and TC/HDL-C ratio, but only significant effects of the confounder variables (Table 3.2).

For the Framingham risk score, we found a direct effect of WC and an interaction effect of autonomic function with physical activity. An increase in WC was related to a higher risk score, and a lower NA in combination with lower physical activity was also related to

a higher risk score. The final regression model explained 57% of the variance in the data ($R^2 = 0.57$). When the original Framingham risk score (including systolic arterial pressure) was used as the outcome measure, the same variables were included in the final regression model, and the variance explained was similar ($R^2 = 0.53$).

We found a significant effect of age and autonomic function on 120-minute glucose. 120-minute glucose increased with age and decreased with increases in plasma NA (and thus impaired autonomic function). The final regression model for 120-minute glucose explained 54% of the variance in the data ($R^2 = 0.54$). Also, we found a significant interaction effect between plasma NA and PASIPD score on both IR and plasma insulin. A lower plasma NA level in combination with a lower physical activity level was related to increases in IR and plasma insulin. The final regression models for IR and plasma insulin explained 21% and 26% of the variance in the data respectively.

For the plasma lipid variables, we did not find significant effects of autonomic function, physical activity or their interaction. We did find significant effects of age and WC on the plasma TG level. Plasma TG level decreased with age and increased with greater WC. The final regression model for TG explained 37% of the variance in the data. In addition, we found significant effects of age and WC on both plasma HDL-C and TC/HDL-C ratio. HDL-C decreased with greater WC and younger age, while TC/HDL-C ratio increased. The final regression models for HDL and TC/HDL explained 50% and 57% of the variance in the data respectively.

Table 3.2. Cardiovascular disease risk factor regression analyses results

	Framingham risk score		120 min glucose		IR		Insulin		TG		HDL		TC/HDL	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
Intercept	-27.7 (10.4)		4.1 (1.0)		1.4 (0.2)		7.0 (0.7)		-0.58 (0.6)		1.32 (0.4)		-1.28 (1.5)	
Noradrenaline	NE	--	-0.37 (0.12)	<0.01	NE	--	NE	--	NE	--	NE	--	NE	--
PASIPD score	NE	--	NE	--	NE	--	NE	--	NE	--	NE	--	NE	--
Interaction noradrenaline & PASIPD score	-0.17 (0.08)	0.04	NE	--	0.02 (0.01)	0.04	0.1 (0.04)	0.02	NE	--	NE	--	NE	--
Age	NE	--	0.1 (0.02)	<0.001	NE	--	NE	--	-0.02 (0.01)	0.05	0.02 (0.01)	0.003	-0.04 (0.02)	0.04
Sex	NE	--	NE	--	NE	--	NE	--	NE	--	NE	--	NE	--
Waist circumference	0.47 (0.12)	<0.001	NE	--	NE	--	NE	--	0.03 (0.01)	0.003	-0.01 (0.005)	0.05	0.08 (0.02)	<0.001

Note: Results from the regression analyses after backward elimination for Framingham risk score, 120 min glucose, insulin resistance (IR), triglycerides (TG), high-density lipoprotein (HDL), and total cholesterol to HDL ratio (TC/HDL). Shown are regression coefficients (β) and their standard errors (SE) representing the change in the outcome measure associated with an increase of one unit in the independent variable. NE: not entered.

Discussion

This is the first study to explore the complex relationships between autonomic dysfunction after SCI and overall CVD risk using regression modelling to identify contributions of autonomic impairment, physical activity, and interactions between these parameters. We first determined differences between able-bodied controls and two groups of individuals with SCI divided according to cardiovascular autonomic impairment. We found poorer glucose tolerance and reduced HDL-C in both SCI groups compared to the control group. Plasma insulin was elevated only in those with complete SCI compared to the control group. On the other hand, TG level and TC/HDL-C ratio were only significantly elevated in those with incomplete SCI compared to the control group.

The regression modelling gave more insight into the complexity of parameters contributing to CVD risk factors. Significant effects of autonomic function were found on the Framingham risk score, 120-min glucose, IR, and plasma insulin concentration. Factors contributing to an increased Framingham risk score were the interaction between impaired autonomic function and decreased physical activity as well as greater WC, but not autonomic dysfunction per se. We found impaired autonomic function and older age to be contributors to impaired glucose tolerance (increase in 120-minute glucose). The interaction between impaired autonomic function and decreased physical activity was the only factor that significantly contributed to both IR and plasma insulin concentration.

The subdivision of the SCI participants according to autonomic completeness was based on the normal range of plasma noradrenaline, and a cut-off for LF SAP shown to have the highest sensitivity and specificity to discriminate between individuals with and without autonomic dysfunction, as previously determined by our laboratory¹⁰⁰. The accuracy of the grouping was confirmed by the clear group differences in cardiovascular parameters such as resting blood pressure and heart rate and blood pressure during orthostatic stress. Resting heart rate was found to be lower in the autonomically complete SCI group compared to the incomplete group, but was not different from the controls. A potential explanation could be that the resting heart rate in the control subjects was somewhat low, likely related to higher physical activity levels and an

associated increase in resting cardiac parasympathetic outflow. Previous studies found comparable resting heart rates in individuals with cervical SCI compared to our complete SCI group, but found slightly higher heart rates (65 bpm compared to 61 bpm) for able-bodied controls^{53,100}, suggesting that the lack of difference in this study is probably due to a lower heart rate in the control group, rather than a higher heart rate in our SCI group.

The regression models for the Framingham risk score and 120-minute glucose showed contributions of one or both of the independent variables, while we did not find clear differences between the SCI groups with the Kruskal-Wallis tests. The latter analysis method is a more crude way to determine effects of autonomic function on our outcome variables. The grouping was purely based on autonomic function and did not strictly take the hypothesized independent variable physical activity and confounders such as age and sex into account. Even though these variables were not significantly different between the groups, the regression analysis is more sensitive in finding effects of multiple variables in combination or interaction effects. The relatively small sample size can attribute to this discrepancy, as finding significant differences between multiple pairs in any ANOVA test generally requires large sample sizes.

The regression model for the Framingham risk score showed contributions from the interaction between physical activity and autonomic function, but not autonomic function per se. The confounder variable WC as a marker for visceral adiposity had a large effect on the risk score, in line with many previous studies in the general population as well as in those with SCI¹⁷³⁻¹⁷⁵.

The regression models for glucose handling parameters (120-minute glucose, insulin and IR) gave a clear insight indicating that autonomic impairments play a role in worsening of glucose tolerance and IR, but a complete picture cannot yet be drawn from these results. The model did not explain all variance in the outcome measures, so there must be other factors that we did not include in the model contributing as well.

Previously, glucose tolerance and IR have been shown to be more prevalent in the SCI population^{126,176,177} than in able-bodied controls, and various explanations have been proposed. One explanation for impairments in glucose handling is the dramatic loss of muscle mass after injury, as muscle mass is the primary tissue contributing to insulin-

induced glucose uptake. This is supported by studies showing that glucose tolerance and IR were more pronounced in individuals with tetraplegia compared to paraplegia, who lose muscle mass over a larger region of the body^{124,178}. However, differences in autonomic function can also be proposed as a mechanism explaining these changes in glucose handling. Sympathetic activity induces IR and reduced uptake of glucose in muscles¹²⁸, but the reduction in blood flow due to sympathetically mediated vasoconstriction within the musculature has also been proposed as a mechanism explaining reduced insulin sensitivity¹⁷⁹. As mentioned previously, resting sympathetic activity is lower in individuals with autonomic dysfunction after SCI, which would be associated with less IR. In contrast, during episodes of AD, sympathetic activity is extremely high, which could potentially explain IR in these individuals. This explanation was also proposed by Karlsson et al., who showed impaired insulin sensitivity in individuals with tetraplegia, while measuring plasma noradrenaline for 24 hours that showed frequent episodes of high sympathetic activity (e.g. AD)¹²⁷. They did not show impaired glucose tolerance, in contrast to our findings. One explanation might be that they found no difference in resting plasma noradrenaline between SCI and control groups, suggesting that not all individuals with SCI had autonomic impairments. In combination with a very small sample size (n=7), this might explain the lack of a significant difference.

We did not find any significant effects of autonomic function, physical activity or the interaction between these variables on any of the plasma lipid variables. This suggests that lipid profiles are not affected by these independent variables, but we did show differences between the SCI groups and the control group, suggesting a more general effect of deconditioning after SCI on adverse lipid profiles. This is in line with previous findings of decreased HDL-C and increased TG in both paraplegic and tetraplegic participants^{180,181}. LDL-C concentrations were similar between all three groups, which is in accordance with previous findings that show no differences between individuals with SCI and able-bodied controls¹⁸².

Strengths and limitations

The main strength of the present study is the specific measurement of autonomic impairment of individuals with SCI, in contrast to using AIS assessments to characterize level and completeness of injury. It has been shown that AIS completeness does not necessarily correlate with completeness of injury to autonomic pathways⁸⁰. We are the first to explore the complex relationships between autonomic dysfunction after SCI and overall CVD risk using regression modelling to identify contributors to this relationship. Previously, most studies determined differences between SCI groups divided by level of injury for CVD risk factors, which would fail to capture the interactions with autonomic impairment. Also, in addition to investigating individual risk parameters, we included the Framingham risk score to explore the overall risk of CVD.

One limitation of our study is that the sample size was relatively small. Unfortunately, this is inherent to research in this population. This might in part explain why we did not find significant differences between the SCI groups for every parameter that did show an effect of autonomic function in the regression analysis. Another reason for these discrepancies could be that other factors that were not constant between the two SCI groups masked the effects of autonomic function on our outcome measurements.

Another limitation is that we did not use a direct measurement of aerobic capacity such as VO_{2max} , or measurements of physical activity from accelerometers, but instead used a recall questionnaire, the PASIPD, to determine physical activity levels. Although this score has been validated against VO_{2max} , it does require an individual to subjectively assess the intensity of an activity and so may be subject to recall bias.

We excluded individuals from this study with known pre-existing (prior to injury) CVD, because we were interested in the risk of CVD associated with SCI. The average risk score for all participants with SCI was 16 (7.9) indicating a moderate risk for the group as a whole, which was significantly different from the control group with a score of 10 (9.9). This elevated risk score fits with the known high morbidity and mortality due to CVD after SCI, and the fact that individuals with SCI experience an earlier onset and faster progression of CVD than in the general population^{22,183}. However, it is likely that this is an underestimation of the true CVD risk in a larger cohort of individuals with SCI,

incorporating those with known pre-existing cardiovascular disease. Underestimations would be expected for adverse values for the individual risk factors as well.

Conclusion

We provide evidence that autonomic function contributes to overall CVD risk, and that it seems to play a particularly important role in impaired glucose regulation after SCI. The regression models for glucose regulation parameters all showed an effect of autonomic function, although whether there was a direct effect or an interaction effect with physical activity was not the same across the different variables. The present study explored effects on many different risk factors and, to minimize the burden on the participants, we chose to use minimally invasive and less time-consuming tests in order to collect information on as many variables as possible in as many participants as possible. The insight gained from this study can now be used to explore the identified relationships in a larger population, potentially including those individuals with SCI and known pre-existing CVD. The use of a more sophisticated technique to determine glucose tolerance and insulin sensitivity, such as the euglycemic hyperinsulinemic clamp, could give insight into the interactions between glucose and insulin and their relationships with autonomic dysfunction. Also, instead of a retrospective index for physical activity, a test for aerobic capacity or accelerometers could be used to explore these relationships in more depth.

Clinical implications resulting from these findings are that a sedentary lifestyle and associated obesity is not the only factor contributing to impaired glucose tolerance, insulin resistance, and overall CVD risk. More attention should be paid to the fact that individuals with autonomic dysfunction after SCI, even those who are lean, can have an elevated risk for CVD.

Chapter 4.

Waist circumference is the best index for obesity-related cardiovascular disease risk in individuals with spinal cord injury

Introduction

Great progress has been made in surgical techniques and initial care for individuals who sustain a spinal cord injury (SCI), but secondary complications after injury remain a major concern. Cardiovascular disease (CVD) is one of these secondary complications, and is the leading cause of morbidity and mortality in this population, with SCI patients experiencing an earlier onset and faster progression of CVD than in the general population^{22,183}. Obesity is an important and well-known risk factor for CVD, but it remains challenging to accurately determine obesity in this population¹³⁴. This is particularly important because following a SCI, individuals undergo changes in body composition, metabolic rate, and autonomic function. These alterations, coupled with a more sedentary lifestyle after injury, may lead to higher prevalence of obesity in this population.

A first step to address the issue of obesity after SCI is to find an accurate, simple, and meaningful assessment tool that can be used to identify obesity in this population. Two characteristics that are important in the assessment tool are the practicality and the accuracy of the tool. BMI (weight/height²) has been used worldwide and is promoted by the WHO as a simple indicator of obesity in the general population^{184,185}. Although BMI does not specifically measure fat mass, it has been shown to correlate well with measures of body fat at a population level¹⁸⁵. However, in the SCI population, BMI is not a very practical or accurate measure of obesity. Measuring height and weight is not possible in individuals with SCI without sophisticated tools. For example, to measure weight, a wheelchair scale is needed. Height can be inferred from length, while lying

supine with straight legs and flexed feet, but this position can be challenging due to contractures and spasticity. Not only is the use of BMI as a measure of obesity impractical in a SCI population; the proposed cut-offs have been shown to underestimate obesity in this population¹³⁵⁻¹³⁷. This is likely due to decreases in muscle mass below the lesion after injury, such that individuals with SCI with the same height and weight (and thus BMI) as an able-bodied individual will have greater fat mass.

Four other anthropometric measures that have been used as surrogate markers for obesity in the able-bodied population are waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and neck circumference (NC). WC is simply measured in a standing position, using a tape measure around the abdomen after normal expiration. Although some groups advocate variations in the exact measurement location for WC, the strength of the correlations between WC and both fat percentage and CVD risk factors have been shown to be equally strong for several locations of WC measurements¹⁸⁶. In the able-bodied, WC has been shown to be a strong predictor for both total body fat and visceral adipose tissue^{174,187-189} and is highly correlated with CVD risk factors directly^{187,190,191}. In contrast to the standard in the able-bodied population, WC is measured in the supine position in individuals with SCI¹³⁵. This is because in the supine position WC is less dependent on abdominal muscle tone, which may be impaired in individuals with SCI. Several studies suggest strong correlations between WC and adiposity, and SCI and CVD risk¹⁹²⁻¹⁹⁴, and one study has shown correlations between visceral adiposity, WC and metabolic risk factors for CVD¹⁷³. However, no study to date has examined the relationships between multiple candidate markers of obesity, adiposity, and overall risk for CVD to determine the best index for obesity-related CVD in a SCI cohort. Although WC has been shown to be highly correlated to visceral fat in both the able-bodied population and in individuals with SCI, the latter have 42% more visceral adipose tissue at the same WC, suggesting the cut-off criteria for obesity should be lowered in this population¹⁷³.

The disadvantage of WC is that it assumes that CVD risk is the same for everyone with the same WC regardless of their height, or body shape, and this might not be the case^{195,196}. Accordingly, WHR and WHtR are two parameters that are commonly used to "correct" WC to account for these differences. In the able-bodied, both have been

shown to be better predictors of CVD than WC in a large meta-analysis, with WHtR being the strongest predictor¹⁹⁷. However, WHR is arguably more suitable for an SCI population as the difficulties with measuring height are avoided. To date, only one study has examined the validity of the corrected WC markers in the SCI population¹⁹³. They showed that WHR is strongly correlated with visceral adiposity in individuals with SCI, but not how this relates to the presence of other adverse CVD risk factors¹⁹³.

Finally, several recent studies in the able-bodied identified a strong correlation between NC and visceral adipose tissue, as well as a correlation with CVD risk above and beyond the risk related to visceral fat¹⁹⁸⁻²⁰¹. Whether NC is also a predictor for obesity or other adverse cardiovascular risk factors in individuals with SCI is unknown.

Thus, it remains unclear, which of these suggested markers is the strongest predictor of obesity in individuals with SCI. Furthermore, since arguably the more relevant information is not just whether a marker can detect obesity, but whether this increased adiposity is associated with increased risk of CVD, additional studies examining their relationships with CVD risk factors after SCI are warranted. The aim of this study was, therefore, to identify the best marker for obesity related CVD risk profile in individuals with SCI, considering both practicality of use, and ability to detect: (i) adiposity and (ii) CVD risk factors.

Methods

This study received ethical approval from the Research Ethics Committee at Simon Fraser University, and the Vancouver Coastal Health Research Institute, and was performed in association with the Declaration of Helsinki of the World Medical Association.

Participants

Studies were performed on 27 individuals with chronic SCI (> 1 year), who gave written informed consent, had no known pre-existing (prior to injury) cardiovascular disease, and were not taking any cardiovascular medications. Individuals with SCI were recruited from posters displayed at rehabilitation centres, and through local SCI support groups. All participants abstained from drinking alcoholic beverages from the night before testing

and from caffeine on the morning of testing. They did not participate in vigorous exercise on the morning of testing. Females were not tested during their menstrual period. Neurological classification of the level and severity of the injury was determined using the AIS assessment¹⁴¹. An AIS A score reflects complete loss of motor and sensory function; the B, C and D scores reflect progressively less severe impairments. Level of injury and impairment score together form the classification of the injury (e.g. T5A). Smoking status was recorded.

Anthropometric variables

BMI was calculated from weight (kg), determined using a Dual Energy X-ray Absorptiometry (DEXA) whole body scan (QDR 4500, Hologic Inc., Bedford, MA, USA), divided by height squared (m^2), determined using an electronic ruler (Matlab 2012b, MathWorks, MA, USA) on the DEXA images. Length as determined using a similar method has been shown to correlate well with measured height in the able bodied population (Pearson's correlation coefficient of 0.996)¹³⁷. In cases where participants could not fully stretch their legs due to contractures or spasticity, a self-reported measure of height was used (n=4). WC was measured in cm at the narrowest part of the waist after a normal expiration¹³⁵. This site was chosen for ease of localization and measurement when the participant was lying supine. Hip circumference (HC) was measured in cm around the widest portion of the buttocks. Both WC and HC were measured while lying supine on the DEXA scanner bed, and using a stretch-resistant measuring tape¹³⁵. WHR was determined by dividing the measured WC by the measured HC. WHtR was determined by dividing the measured WC by height. NC in cm was measured at the middle of the neck.

Body composition

Total body fat mass in kg and total body fat percentage were determined using the whole body DEXA scan. Abdominal fat was determined using standardized landmarks to distinguish the trunk region¹⁷², and abdominal fat percentage was determined as abdominal fat mass divided by total mass in the defined region multiplied by 100.

Fasting plasma levels of lipids, glucose and insulin

A venous blood sample was collected following a 12-hour overnight fast (excluding water). Samples were centrifuged immediately at 3 °C and 3,000 rpm for 10 minutes and the plasma component withdrawn for subsequent analysis. The plasma samples were sent to the clinical laboratory at Vancouver General Hospital where high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG) and glucose levels were determined by enzymatic assays (Dimension Vista system, Siemens Healthcare Diagnostics Inc, USA). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald method¹⁶⁷. TC/HDL-C was calculated from TC and HDL-C levels. Plasma insulin was determined at the same laboratory using an immunoassay, ADVIA Centaur assay (Siemens Healthcare Diagnostics Inc. USA). Insulin resistance was calculated using the homeostatic model assessment (HOMA) method¹⁶⁸.

Oral glucose tolerance test

Participants fasted for 12 hours overnight prior to the test, with the exception of drinking water. A finger prick method was used to determine blood glucose levels (Contour, Bayer Inc., Toronto, Canada) in the fasting state and 30, 60, 90 and 120 minutes following consumption of a 75 g glucose solution (Glucodex 75 g, Rougier Pharma, Canada).

Framingham 30-year risk for cardiovascular disease score

We used the Framingham 30-year cardiovascular disease risk score¹⁶⁹ as a measure of overall risk of CVD. This risk score incorporates the following risk factors: HDL-C, TC, age, sex, systolic arterial pressure (SAP) at rest, smoking status, diabetes, and antihypertensive treatment. However, instead of including the measured SAP, we entered a SAP value of 120 mmHg into the risk score formula for all participants. This decision was based on the knowledge that SCI can impair normal blood pressure control with lesions at or above the 5th thoracic level, leading to lower resting blood pressure^{50,52}. The known relationship between SAP and CVD risk might, therefore, not exist in the same way in this population. Entering a value of 120 mmHg is neutral to the score, and therefore excludes any effect of SAP on the risk score generated. A risk score under 10% is considered low risk, a score between 10 and 20% an intermediate risk and above 20% a high risk¹⁷⁰.

Statistical analyses

All statistical analyses were performed using SigmaPlot version 12 (Systat Software Inc., San Jose, CA). Data were tested for normality using the Shapiro-Wilk test. Correlations were performed using Pearson Product Moment analyses (parametric data) or Spearman Rank Order tests (nonparametric data) to examine the relationships between the anthropometric parameters and abdominal fat percentage, individual risk factors, or the Framingham risk score. Receiver Operator Characteristic (ROC) curves were generated for the anthropometric measures that were significantly correlated with both abdominal fat percentage and the Framingham risk score. A Framingham 30-year risk score of more than 10% was considered a positive outcome. Significance was assumed at $p < 0.05$; data are reported as mean \pm SD.

Results

Participants

Twenty-seven individuals (19 men and 8 women) with a range of injury levels from the 4th cervical level to the 12th thoracic level, including both complete and incomplete injuries, participated in this study. Fifteen individuals had a cervical lesion and 12 had a thoracic lesion. There were 14 individuals with AIS A scores, 6 with AIS B, 5 with AIS C and 2 with AIS D. The average age was 39.8 ± 10.8 years. Participant characteristics are shown in Table 4.1.

Anthropometric measures and body composition

The anthropometric parameters BMI, WC, and WHtR were significantly and positively correlated with both abdominal fat percentage (Figure 4.1A-C) and with total body fat percentage. The correlations between both WHR (Figure 4.1D, $r = 0.35$ $p = 0.08$) and NC ($r = 0.39$ $p = 0.08$) with abdominal fat percentage did not quite achieve statistical significance. WHR and NC were not significantly correlated with total body fat percentage ($p = 0.52$ and $p = 0.11$ respectively).

Table 4.1. Participant characteristics.

	Mean (SD)	Number of participants
Age (years)	40 (11)	27
BMI (kg/m ²)	23.7 (4.4)	27
Waist circumference (cm)	87 (12)	27
Waist-to-height ratio	0.51 (0.07)	27
Waist-to-hip ratio	0.87 (0.10)	27
Neck circumference (cm)	39 (3)	22
Total cholesterol (mmol/L)	3.80 (0.79)	23
High density lipoprotein cholesterol (mmol/L)	1.13 (0.32)	23
Low density lipoprotein cholesterol (mmol/L)	2.23 (0.73)	23
Triglycerides (mmol/L)	0.96 (0.44)	23
Total / high density lipoprotein cholesterol ratio	3.60 (1.19)	23
Insulin (mU/L)	6.48 (3.39)	23
Glucose (mmol/L)	4.5 (0.46)	20
Insulin resistance	1.28 (0.66)	23
120 min glucose (mmol/L)	7.4 (1.39)	27
Framingham risk score	15 (8)	23

Anthropometric measures and cardiovascular disease risk factors

The correlation coefficients between the anthropometric variables and CVD risk factors are shown in Table 4.2. BMI was positively and significantly correlated with only one of the CVD risk factors; fasting TG level. WC was positively and significantly correlated with five CVD risk factors: fasting glucose; TG; TC; LDL-C; and TC/HDL-C ratio. WHR correlated positively with four CVD risk factors: fasting glucose; TC; LDL-C; and TC/HDL-C ratio. WHtR was positively correlated with four CVD risk factors: TC; TG; TC/HDL-C ratio; and 120-minute glucose. NC was not significantly correlated with any of the CVD risk factors.

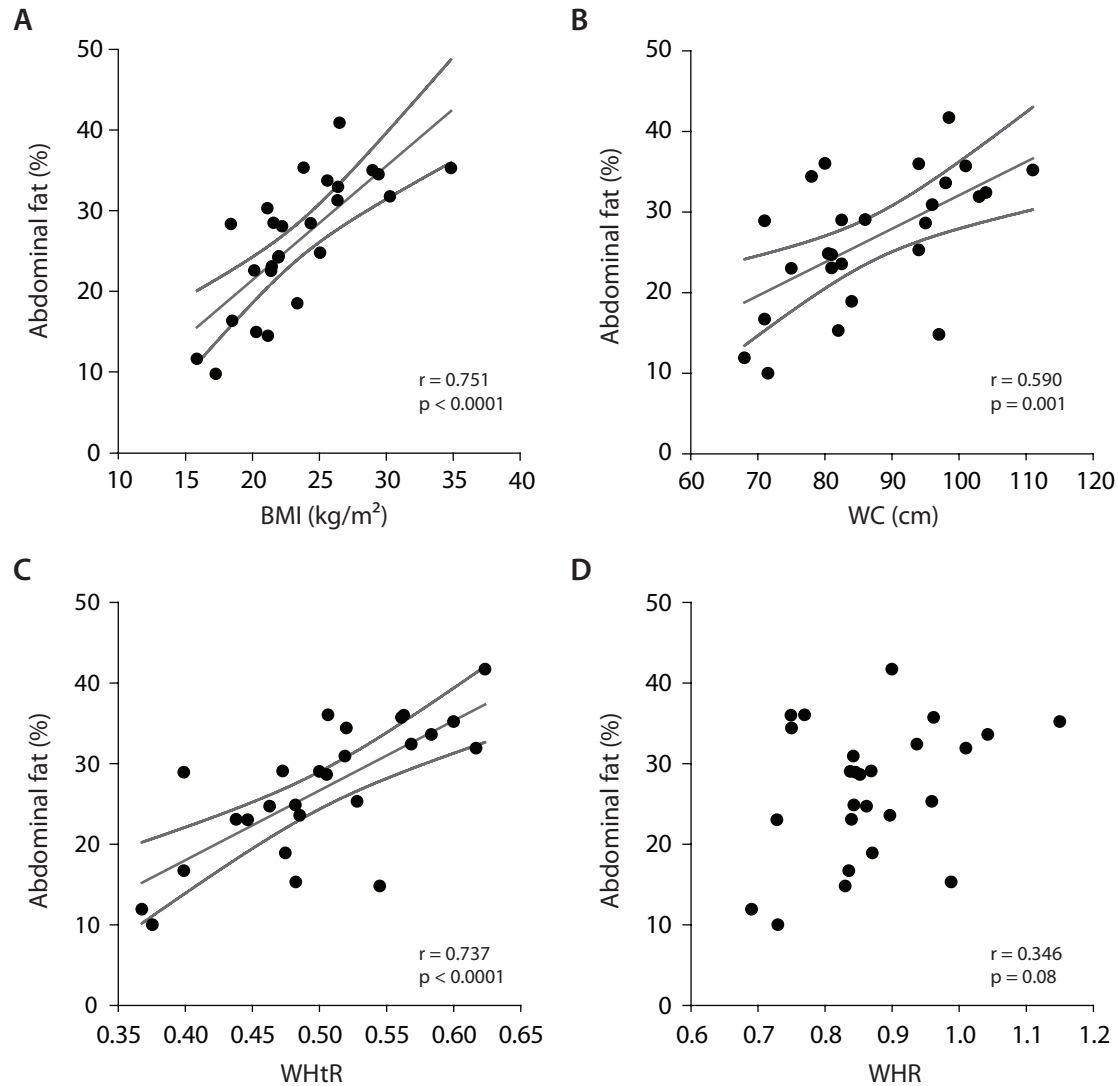


Figure 4.1. Correlations between abdominal fat percentage and anthropometric markers for obesity

- A. Body mass index (BMI) was significantly correlated to abdominal fat percentage.
 - B. Waist circumference (WC) was significantly correlated to abdominal fat percentage.
 - C. Waist-to-height ratio (WHtR) was significantly correlated to abdominal fat percentage.
 - D. Waist-to-hip ratio (WHR) was not significantly correlated to abdominal fat percentage.
- Regression lines and confidence intervals are shown for significant ($p < 0.05$) correlations.

Table 4.2. Correlations between anthropometric variables and individual risk factors

		Insulin	Glucose	TG	TC	HDL-C	LDL-C	TC/HDL ratio	120 min glucose
BMI	r	0.286	0.402	0.421	0.386	0.146	0.201	0.218	0.143
	p	0.22	0.06	0.045	0.07	0.51	0.35	0.32	0.48
WC	r	0.247	0.463	0.461	0.575	-0.118	0.429	0.564	0.318
	p	0.29	0.03	0.03	0.004	0.59	0.04	0.005	0.11
WHR	r	0.403	0.503	0.402	0.610	-0.248	0.470	0.622	0.300
	p	0.08	0.01	0.056	0.002	0.25	0.02	0.002	0.13
WHtR	r	0.349	0.360	0.409	0.532	0.077	0.321	0.424	0.394
	p	0.13	0.09	0.052	0.009	0.73	0.13	0.04	0.04
NC	r	-0.174	0.409	0.342	0.306	-0.098	0.388	0.352	0.332
	p	0.49	0.07	0.14	0.18	0.67	0.08	0.12	0.13

Note: WC: waist circumference; WHtR: waist-to-height ratio; WHR: waist-to-hip ratio; NC: neck circumference; TG: triglyceride; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. Data in bold text represent significant correlations.

Anthropometric measures and the Framingham 30-year risk score

Of the anthropometric measures, only BMI was not significantly correlated to the Framingham risk score. WC had the strongest correlation ($r = 0.66$, $p < 0.001$) with the risk score, but WHtR ($r = 0.60$, $p < 0.01$), WHR ($r = 0.56$, $p < 0.01$) and NC ($r = 0.51$, $p = 0.02$) also showed significant correlations. ROC curves were generated for WC and WHtR as those measures were correlated with both the Framingham risk score, and abdominal and total fat percentages. The area under the curve (AUC) for WC was 0.92 and the optimal cut off was determined at 94 cm (95% confidence interval [CI] 0.72-0.99 and $p < 0.0001$), with a specificity of 100% and a sensitivity of 79%. For WHtR, the area under the curve was 0.87 (95% CI 0.66-0.97 and $p < .0001$) and the optimal cut off was determined at 0.53, with a specificity of 100% and a sensitivity of 71%. The ROC curves for a subgroup of only male participants showed the optimal cut off for WC at 94 cm and 0.51 for WHtR. Correlations and ROC curves for WC and WHtR with the Framingham risk score are shown in Figure 4.2.

When the original Framingham risk score (including systolic arterial pressure) was used as the outcome measure, WC remained to have the strongest correlation with the risk score ($r=0.55$, $p=0.006$) and the ROC for WC had an AUC of 0.92 and an optimal cut-off at 94 cm.

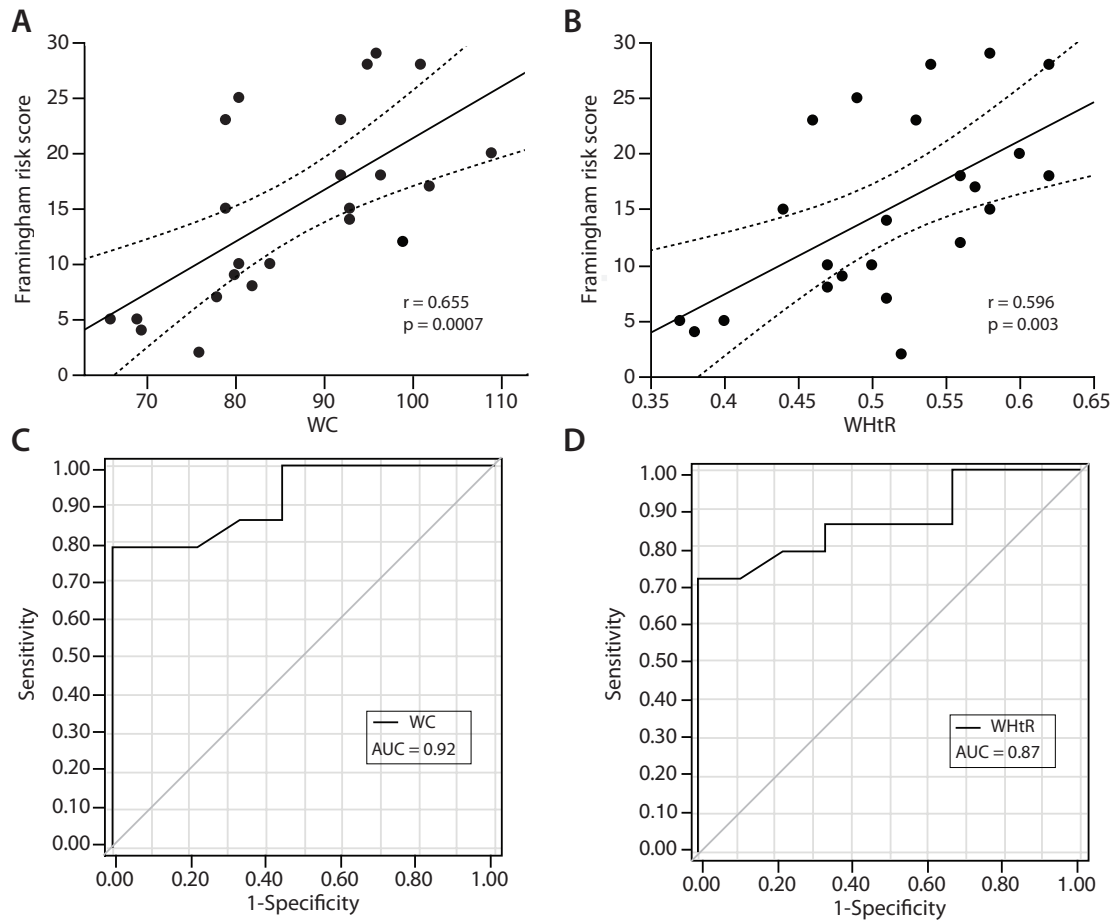


Figure 4.2. Correlations between anthropometric measures and Framingham risk score and corresponding receiver operator characteristic curves

- A. Correlation with waist circumference (WC);
- B. Correlation with waist-to-height ratio (WHtR);
- C. Receiver operator characteristic (ROC) curve for WC;
- D. ROC curve for WHtR.

A regression line and confidence intervals are shown for the significant ($p < 0.05$) correlations. The areas under the curves (AUC) of the ROC curves are shown.

Discussion

The main findings of this study are that BMI, WC and WHtR were correlated with both total and abdominal fat percentage. In addition, we found WC and its normalized versions, WHtR and WHR, to be correlated with several CVD risk factors. Two of these measures, WC and WHtR, were significantly correlated with both abdominal adiposity and the Framingham 30-year cardiovascular disease risk score. Of these two measures, WHtR is the least practical measure for individuals with SCI, because height is challenging to accurately determine in those with contractures or spasticity. We, therefore, suggest that WC is the best marker for obesity-related CVD risk in this population. The stronger correlation with the Framingham risk score and the slightly higher AUC of the ROC curve further support this conclusion. We showed that a cut-off for WC at 94 cm was optimal to predict a positive outcome of >10 % risk on the 30-year risk score.

In the present study, we found that BMI was strongly related to body fat percentage, explaining 63% of the variance in abdominal fat percentage and 52% of the variance in total body fat percentage. Previously, studies in the SCI population showed that only 35-36% of the variance in total body fat percentage could be explained by BMI^{135,202}. Our finding is more similar to the 46-79% described in the able-bodied population¹³⁵. This might be because BMI was determined by length and weight measured from the DEXA scan in the present study, which may be more robust than using self-reported height or weight as in previous studies in SCI individuals¹³⁷. Indeed, we compared self-reported height and weight to height and weight determined from the DEXA scan (data not shown). Although height from these two methods correlated well ($r = 0.912$, $p < 0.001$), Bland-Altman analysis showed there was a bias of about 2 cm, where self-reported height was greater than that measured from DEXA. Self-reported weight also correlated well with weight from the DEXA scan ($r = 0.945$, $p < 0.05$), but again Bland-Altman analysis showed a bias of 1.7 kg, where self-reported weight was greater than that measured from DEXA.

Although BMI was strongly related to abdominal fat percentage, it was not correlated with the CVD risk factors studied, or the Framingham risk score. This discrepancy has been documented previously^{124,135,203,204}. It could be due to errors in the calculation of BMI, or a lack of information about the distribution of abdominal fat using this measure. In our case the first explanation would be unlikely, because BMI determined by length and weight via the DEXA scan has been shown to be highly accurate¹³⁷. The second explanation is more likely, because despite being correlated with abdominal fat percentage, BMI provides no information about the distribution of adiposity within the abdomen. Even the “gold standard” of DEXA, which does provide specific fat percentages for the abdominal area, does not have the ability to differentiate between subcutaneous and visceral fat. This is important because visceral fat, rather than overall abdominal fat, is thought to be the main contributor to CVD risk, and cannot be discerned from either BMI or DEXA measurements¹⁷⁴. Our results show a stronger correlation between BMI and abdominal fat than WC and WHtR, however, this may or may not represent a strong relationship with the parameter of greatest interest, visceral fat. Unfortunately, we did not have access to techniques to quantify visceral fat. Previous studies that measured visceral fat using MRI or computed tomography in the able-bodied showed that WC and WHtR are strongly associated with visceral fat, and so are better predictors of CVD risk^{174,205}.

All three measures of waist circumference (WC, WHtR and WHR) were strongly correlated with the individual risk factors. However, based on the individual risk factors, none of these measures emerged as a superior predictor of an adverse CVD risk profile. Subsequently, we found that these three measures were also correlated with the Framingham 30-year risk score. Only WC and WHtR were correlated to both adiposity and the risk score, suggesting these represent stronger markers for obesity-related CVD risk. WC was more strongly correlated with all the cardiovascular risk factors and the Framingham risk score than WHtR, but had a slightly weaker (although still highly significant) correlation with abdominal adiposity. On balance, and incorporating practicality of use, we conclude that WC is the best marker for the presence of obesity-related CVD factors in individuals with SCI.

The Framingham risk score is a validated score from the Framingham Heart study, predicting 30-year risk of CVD¹⁶⁹. The score includes the following risk factors: HDL-C, TC, age, sex, systolic arterial pressure at rest, smoking status, diabetes, and antihypertensive treatment. We adapted the use of the score slightly by entering a neutral value for SAP (120 mmHg) for all participants. In this way, we eliminated the effects of blood pressure on the risk score. Ordinarily, a SAP above 120 mmHg would increase the risk score and below this level would lower the risk score. This incorporates the relationship between hypertension, a component of metabolic syndrome, and its known contribution to the increased CVD risk associated with obesity²⁰⁶. However, SCI can affect resting blood pressure levels as well as the normal regulation of blood pressure. Individuals with an injury at or above the 5th thoracic level may have loss of descending spinal sympathetic drive to the heart and blood vessels of the skeletal muscles and the gut⁵⁰. The sympathetic hypoactivity leads to low resting blood pressure and impairs blood pressure control^{50,52}. Given the effect of SCI on normal blood pressure control, the presence or absence of hypertension might not be as tightly related to risk of CVD in those with SCI as in the able-bodied population. The lowered SAP in individuals with damage to these autonomic pathways might be hypothesized to have a 'protective' effect in the risk score, but the associated blood pressure dysregulation also manifests in abnormal cardiovascular reflex control^{53,66} that may be detrimental in terms of CVD risk²², and this delicate balance would not be captured using the Framingham risk score. Therefore, it is unclear whether hypotension is necessarily cardioprotective in the SCI population. We, therefore, decided to exclude effects of SAP on the CVD risk score entirely.

We excluded individuals from this study with known pre-existing (prior to injury) CVD, because we were interested in the risk of CVD associated with SCI. The average risk score for our sample was 15 (8) indicating a moderate risk for the group as a whole. This elevated risk score fits with the known high morbidity and mortality due to CVD after SCI, and the fact that individuals with SCI experience an earlier onset and faster progression of CVD than in the general population^{22,183}. However, it is likely that this is an underestimation of the true CVD risk in a larger cohort of individuals with SCI, incorporating those with known pre-existing cardiovascular disease.

The optimal cut-off for WC was determined to be 94 cm. This cut-off is slightly lower than the 102 cm cut off for males used in the able-bodied population¹⁷⁰. We did not provide separate criteria for males and females, due to the low number of women in our study; the majority of our sample was male. This is representative of the population of individuals with SCI as a whole, in whom the incidence of injury is known to be higher in men than women¹. The ROC curve for a subgroup of just the males revealed the same optimal cut off of 94 cm for WC, indicating that the cut off is not artificially lower due to inclusion of women in the sample.

The lower cut off for WC is in line with studies that have shown that general cut-offs for BMI and WC underestimate obesity in individuals with SCI^{135,136,173}. This underestimation is probably due to the fact that individuals with SCI lose muscle mass, gain fat mass after injury, and thus have a different body composition with the same WC. This has been confirmed using computed tomography to determine visceral fat, where individuals with SCI had more visceral adipose tissue compared to able-bodied controls matched for WC, BMI, weight and total abdominal tissue. This supports the need for a lower cut off for WC in this population. In addition, the WC criteria might be expected to be slightly smaller in our study because we conducted measurements in the supine position instead of the upright position as in the general population, and this would be associated with reduced gravitational effects on abdominal distension. The concept of using a population-specific cut-off is not new, and in fact has already been proposed for different ethnicity^{205,207}, sex, and age categories²⁰⁸.

The main limitation of this study is the fairly small sample size in this population. Studies on obesity indices in the able-bodied population are usually very large, but in a specific population such as this one, the sample size is restricted. However, we were still able to detect substantial correlations between body composition, CVD risk factors, and our anthropometric measures. The main limitation of the small sample size is that we were not able to differentiate our optimal cut-off for men and women, or specific age categories. Another limitation is that we used the presence of an adverse cardiovascular risk profile as the main outcome measure for correlation with our obesity-markers, instead of the presence of CVD itself. We, therefore, provide a tool to predict the presence of an adverse risk profile and not risk of CVD per se.

Conclusion

We propose that WC can provide a simple, and more sensitive alternative to BMI that is easy to use in general medical practice, research, or at home. This measure is well suited for use in individuals with SCI because it does not require measures of height or weight that are more challenging to determine in this population. We also provide an optimal WC cut-off for determining the presence of an adverse cardiovascular risk profile at 94 cm. This cut-off provides a simple tool to predict adverse CVD risk profiles related to obesity, that can be used to guide risk management, and as a practical aid for individuals with SCI to maintain a healthy body composition.

Chapter 5.

Electrocardiogram-based predictors for cardiac arrhythmia are related to autonomic impairment after spinal cord injury

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in individuals with spinal cord injury (SCI)^{22,209}. Furthermore, CVD after SCI has an earlier onset and more rapid progression than in the able-bodied²².

One possible cause of this increased risk for CVD is altered autonomic function after SCI, with associated modulations in cardiac electrophysiology and increased risk of cardiac arrhythmias¹⁰⁸. Rats with high thoracic lesions demonstrate changes in protein expression that increase sarcoplasmic reticulum calcium load and lead to ectopic activity¹⁰⁸. These changes are associated with a decreased electrical stimulation threshold to induce ectopics¹⁰⁸.

The extent of cardiovascular autonomic dysfunction is related to the level and severity of injury to spinal cardiovascular sympathetic pathways²¹⁰. Injuries above the fifth thoracic level (T5) can impair sympathetic regulation of the heart and splanchnic vasculature (a key site for blood pressure regulation) with particularly pronounced effects on cardiovascular control²¹⁰. Cardiovascular parasympathetic pathways do not pass through the spinal cord, and thus are not affected by SCI. As such, the normal coordinated pattern of activity of the autonomic nervous system can become dyssynchronous, with devastating effects on cardiovascular function^{80,113,117}.

Case reports in humans^{71,105,211} as well as studies in rodents²¹² have documented ventricular and/or atrial arrhythmias after SCI, particularly during episodes of autonomic dysreflexia (AD), sudden onset of profound hypertension triggered by sensory stimuli

below lesion). During AD the precipitating stimulus triggers massive sympathetic discharge through a spinal reflex that is no longer subject to regulation by the injured descending spinal autonomic pathways. This leads to extreme hypertension, and increases the spatial dispersion of ventricular repolarization, increasing the likelihood of re-entry arrhythmias¹¹³. In addition, unique to SCI, this elevated cardiac sympathetic activity is coupled with elevated cardiac vagal activity during AD, through hypertension-induced stimulation of the arterial baroreflex and subsequent increases in efferent parasympathetic activity mediated via the cardiac vagus nerves⁶⁹, and this may be particularly proarrhythmogenic.

The electrocardiograph (ECG) can potentially be used to identify those individuals at risk of developing arrhythmias during episodes of AD. Prolonged transmural dispersion of ventricular repolarization has been shown to be a substrate for ventricular arrhythmias, specifically Torsade des Pointes¹¹⁵. Transmural dispersion of repolarization corresponds to the difference in timing and duration of repolarization in the different layers of the ventricular wall¹¹⁴ which is reflected by the $T_{\text{peak}}-T_{\text{end}}$ interval of the ECG¹¹⁴. Hence, $T_{\text{peak}}-T_{\text{end}}$ can be used as a risk assessment parameter for ventricular arrhythmias^{114,213}. Another ECG-derived parameter that is believed to identify increased risk for ventricular arrhythmias is the QT variability index (QTVI)²¹⁴.

In addition to an increased risk of ventricular arrhythmias, atrial arrhythmias such as atrial fibrillation are common in individuals with SCI¹¹⁷. Prolongation of intra-atrial conduction time is associated with atrial fibrillation attributable to inhomogeneous propagation of sinus impulses in the atria¹¹⁸. It has been proposed that this could be identified by variation in P-wave duration in differently oriented surface ECG leads, or P-wave dispersion (PWD)¹¹⁹. Another approach would be to study P-wave variability in the time and frequency domain (by assessing the P-wave duration in the same ECG lead over time), and we propose that this parameter could also be a predictor for atrial arrhythmias.

A previous report in humans has investigated differences in ECG parameters²¹⁵ between individuals with high and low level SCI, but failed to account for injury to cardiovascular autonomic pathways, which would be expected to influence both the ECG characteristics, and susceptibility to arrhythmia. Accordingly, our goal was to explore the

proposed ECG predictors for ventricular and atrial arrhythmias in individuals with chronic SCI with different levels and severities of injury to cardiovascular autonomic pathways. We hypothesized that those with severe autonomic injury would have increased $T_{peak-T_{end}}$, QTVI, PWD and P-wave variability compared to those with incomplete autonomic injury and able-bodied controls.

Methods

The study received ethical approval from the Simon Fraser University and the University of British Columbia Research Ethics Committees, and was performed in association with the Declaration of Helsinki of the World Medical Association.

Participants

Studies were performed on 28 individuals with chronic (>1 year) SCI, and 27 able-bodied controls. All participants gave written informed consent, and were apparently healthy and not taking any cardiovascular medications. Females were not tested during their menstrual period. Participants abstained from drinking alcoholic beverages starting the night before testing and drinking caffeine and smoking on the morning of testing.

Measures of completeness of injury

Motor and sensory impairment

Neurological classification of level and severity of SCI was determined from the AIS. This scale assesses motor and sensory function after SCI^{141,216}, but does not incorporate a quantitative measure of autonomic function⁸¹. An AIS A score reflects complete loss of motor and sensory function; the B, C and D scores reflect gradually less severe impairments. Level of injury and impairment score together form the classification of the injury (e.g. T5A).

Autonomic impairment

At present there is no gold standard for the quantitative assessment of autonomic completeness of SCI. Therefore, to assess severity of injury to spinal autonomic pathways in individuals with SCI we used three methods: sympathetic skin responses (SSR); plasma noradrenaline levels; and low frequency power of systolic arterial pressure (LF SAP). The SSR scores were used to subdivide the SCI group for the

categorical analyses, while plasma noradrenaline and LF SAP were used as continuous variables for the correlation analyses.

Sympathetic skin responses

This test assesses sympathetic cholinergic pathways, which are dependent on central and peripheral sympathetic input⁸⁸. Impaired supraspinal control of sympathetic pathways leads to an impaired SSR, and therefore this can be used to evaluate autonomic injury following SCI. Recordings were conducted simultaneously and bilaterally from both hands in response to a pulse applied ten times to the left median nerve⁸⁰. Each stimulus was a single 0.2 ms duration pulse and had an intensity of 8-10 mA. The time delay between stimuli was variable in order to eliminate habituation to the stimuli. The outcome measurement is a sum score of the normal elicited SSR with a maximum response of 10 for each stimulation site. The maximum score (20) is considered a normal SSR. For categorical analyses, autonomic completeness of injury was determined based on the SSR⁵³.

Plasma noradrenaline levels

We used an orthostatic stress test⁵³ to assess severity of cardiovascular autonomic dysfunction from plasma noradrenaline responses to orthostasis. Low noradrenaline levels, due to sympathetic hypoactivity, are indicative of injury to descending sympathetic pathways⁵⁰. A butterfly catheter was inserted into an antecubital vein and samples were withdrawn after 15 minutes of supine rest, and then five minutes after the passive assumption of a seated position. Samples were centrifuged at 3°C and 3000rpm for 10 minutes and the plasma component withdrawn for subsequent analysis. The plasma samples were then sent to the clinical laboratory of Vancouver General Hospital for determination of noradrenaline levels with high-pressure liquid chromatography (ESA Coulochem II detector, Thermo Fisher Scientific, Sunnyvale, CA, USA).

For correlative analyses we used the absolute noradrenaline level, as described by our group previously⁵³.

Low frequency power of systolic arterial pressure

The power of LF SAP variability reflects sympathetic drive to the resistance vessels⁵⁰, and is markedly reduced in individuals with autonomically complete SCI²¹⁷. Continuous beat-to-beat blood pressure was recorded using finger plethysmography (Finometer, Finapres Medical Systems BV, Arnhem, The Netherlands) during 15 minutes of supine rest. We fitted autoregressive monovariate models to the time series of the beat-to-beat systolic arterial pressure signal and identified the low frequency peak (at ~0.1Hz) from the power spectrum. We calculated central frequency, attributed power, percentage power and power in normalized units:

$$LF_SAP_{nu} = \frac{LF_SAP_{power}}{Total_{power} - VLF_SAP_{power}} \times 100$$

For correlative analyses we used the absolute value of the LF SAP, as described by our group previously⁵³.

Continuous electrocardiogram

A resting lead II ECG (Finometer ECG module, Finapres Medical Systems BV, Arnhem, The Netherlands) was continuously recorded in the supine position for 15 minutes. Data acquisition was performed with a sampling frequency of 1KHz using an analog-to-digital converter (Powerlab 16/30, AD Instruments, Colorado Springs, CO).

Electrocardiogram interval detection

ECG parameters (RR interval, QT, QTc, using the method of Bazett²¹⁷, $T_{peak}-T_{end}$, QTVI and P-wave duration) were determined for every beat using customized software (LabView 2009, National Instruments) (Figure 5.1).

The location and amplitude of the R-wave was determined from the point of zero slope at the maximum amplitude of the derivative of the signal. The stable T-P section was identified by linear regression of the signal between two consecutive R-waves. The T_{peak} was determined from the maximum value of a 3rd order polynomial applied 100ms after the preceding R up until the midpoint of the T-P section. The Q-wave was identified from the minimum value of a 3rd order polynomial applied to the 50ms of data preceding the R-wave. The P_{peak} was determined from the maximum point of a polynomial regression of the 20ms of data preceding the Q-wave. The T_{end} was determined from

the point at which two regression lines intersect, applied from the T_{peak} forwards and from the middle of the T-P section backwards. Finally, the P_{end} was identified from the point at which regressions applied to the section between the P_{peak} and Q-waves intersect.

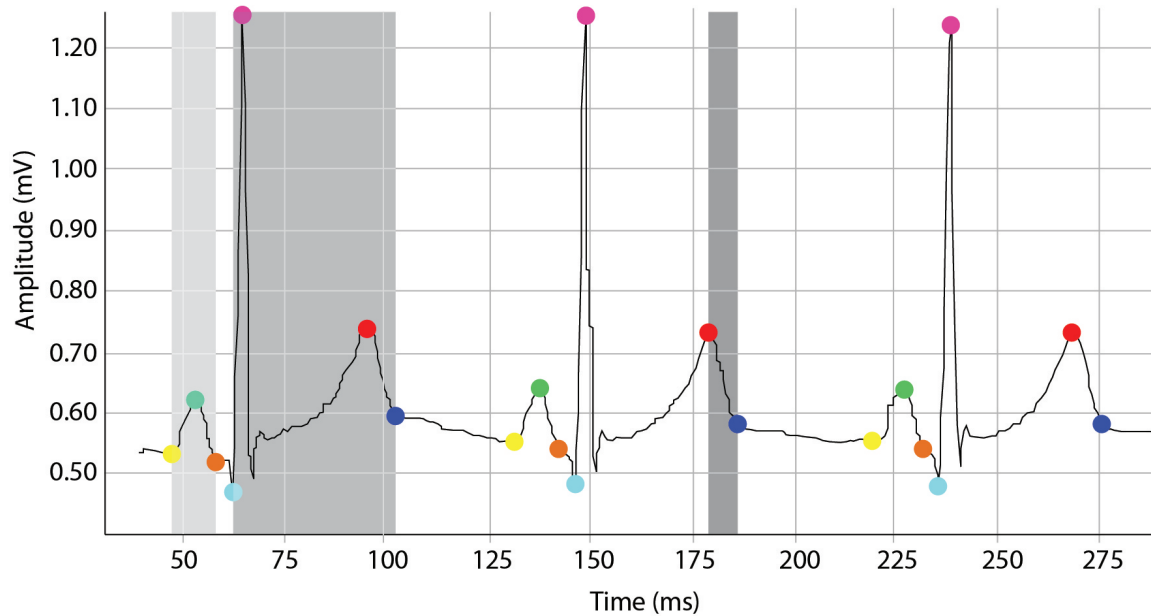


Figure 5.1. Example graphical representation of the customized ECG interval detection.

Different coloured markers are used for the different points of interest. The program outputs time and amplitude of coordinates for each point of interest as well as the corresponding intervals. The shaded areas represent P-wave duration (light grey), QT interval (grey) and $T_{peak}-T_{end}$ duration (dark grey).

Variability analyses

We determined variability for each of the ECG intervals ($T_{peak}-T_{end}$ and P-wave duration) using autoregressive spectral analysis. QTVI was determined using the following equation:

$$QTVI = \log_{10} \frac{(QT_v / QT_m)^2}{(RR_v / RR_m)^2}$$

Where QT_v is the QT variability, QT_m the mean QT interval, RR_v the variability of RR interval, and RR_m the mean RR interval²¹⁴. QTVI is typically a negative value, with values closer to zero being considered abnormal¹¹⁵.

12-lead electrocardiogram

To determine PWD, we collected 12-lead ECG's during seated rest from seventeen participants with cervical SCI. In fifteen age and sex matched able-bodied controls a 12-lead ECG (Burdick Atria 6100, Cardiac Science Corporation, Hannover, Germany) was recorded during supine (n=14) and seated (n=10) rest. Digital calipers were used to measure the P-wave duration in every lead from the signal averaged 12-lead ECG's scanned in high resolution (600 dpi) from a paper print. According to convention¹¹⁸, P-wave duration in a single lead was discarded when the beginning and end of the P-wave could not be accurately determined. PWD was calculated for every ECG with more than 9 measurable leads¹¹⁸.

Statistics

Statistical analyses were performed using SigmaPlot version 11 (Systat Software Inc, San Jose, CA). Data were tested for normality using the Shapiro-Wilk test. Comparisons between two groups were performed using unpaired Student's t-tests or Wilcoxon tests for parametric and nonparametric data respectively. One-way ANOVA or Kruskal-Wallis test with Tukey or Steel-Dwass post-hoc tests were performed for comparisons between three groups. Correlations between variables were performed using Pearson Product Moment analyses or Spearman Rank Order tests. Possible differences in sex and the number of discarded leads in each group (PWD analysis) were determined using the Pearson Chi-squared test. All data are reported as mean \pm SD. Statistical significance was assumed at the level of $p < 0.05$.

Results

Participants with SCI were subdivided into different groups for analyses: (1) by region of injury - cervical or thoracic; (2) by level of injury – below or above (and at) T5; (3) by autonomic impairment as determined from the SSR – complete (score of zero) or incomplete (score greater than zero); (4) by motor and sensory impairment according to the AIS score – complete (AIS A) or incomplete (AIS B, C or D).

Participant characteristics

We found no significant age or sex differences between groups. Levels of injury in the cervical SCI group ranged from C4 to C7 and motor/sensory impairment defined by AIS grades¹⁴¹ was as follows: grade A, n=7; grade B, n=5; grade C, n=2; and grade D, n=1. Levels of injury in the thoracic group ranged from T3 to T11 and motor/sensory impairment was as follows: grade A, n=11; grade B, n=1; and grade D, n=1. Not all participants opted to complete all test procedures. Participant characteristics can be found in Table 5.1.

Table 5.1. Participant characteristics.

	Age (years)	Sample size	Males
Able-bodied Controls	30 (10)	27	14 (52%)
All SCI Participants	35 (7)	28	21 (75%)
SCI subdivided by level of injury			
Cervical SCI	34 (7)	15	12 (80%)
Thoracic SCI	35 (8)	13	9 (69%)
SCI subdivided according to injury above or below T5			
SCI Above T5	34 (6)	22	16 (73%)
SCI Below T5	37 (10)	6	5 (83%)
SCI subdivided according to autonomic completeness of injury			
Autonomically Complete SCI	35 (7)	13	11 (85%)
Autonomically Incomplete SCI	35 (7)	15	10 (67%)

Note: Data are shown as mean (SD).

Continuous electrocardiogram

Electrocardiogram interval analyses

Resting RR interval was significantly longer in the cervical SCI group compared to controls ($p=0.04$), consistent with modest bradycardia. RR interval was significantly shorter in the thoracic SCI group compared to the cervical SCI group ($p=0.02$). There were no differences between groups for all other parameters (QT, QTc, $T_{peak}-T_{end}$ and P-wave duration). Absolute values for all intervals are summarized in Table 5.2.

Variability analyses

$T_{\text{peak}}-T_{\text{end}}$ variability was significantly greater in the SCI group compared to controls ($p=0.04$, Figure 5.2A). Level of injury influenced $T_{\text{peak}}-T_{\text{end}}$ variability, with significantly greater $T_{\text{peak}}-T_{\text{end}}$ variability only in those with lesions above T5 compared to controls ($p=0.02$, Figure 5.2B). $T_{\text{peak}}-T_{\text{end}}$ variability was also significantly greater in those with autonomically complete injuries compared to controls ($p=0.02$, Figure 5.2C). We found no significant differences in $T_{\text{peak}}-T_{\text{end}}$ variability between SCI groups and controls when grouped according to AIS scores (Figure 5.2D).

Table 5.2. ECG intervals: RRI, QT, QT_c, T_{peak}-T_{end} and P-wave duration.

	RRI (ms)	QT (ms)	QT _c (ms)	T _{peak} -T _{end} (ms)	P-wave duration (ms)
Able-bodied Controls	951 (109)	373 (22)	384 (22)	69 (7)	100 (15)
All participants with SCI	991 (137)†	379 (29)	382 (22)	69 (7)	102 (14)
SCI subdivided by level of injury					
Cervical SCI	1047 (112)†	389 (25)	381 (20)	68 (8)	
Thoracic SCI	925 (138)‡	367 (30)	383 (28)	69 (7)	
SCI subdivided according to injury above or below T5					
SCI Above T5	1006 (124)	381 (29)	381 (20)	68 (7)	
SCI Below T5	933 (179)	371 (33)	388 (36)	70 (10)	
SCI subdivided according to autonomic completeness of injury					
Autonomically Complete SCI	1039 (131)	392 (24)	387 (19)	69 (7)	
Autonomically Incomplete SCI	949 (133)	367 (29)	378 (26)	68 (8)	

Note Data are shown as mean (SD). † denotes $p<0.05$ compared to controls; ‡ denotes $p<0.05$ compared to the cervical SCI group

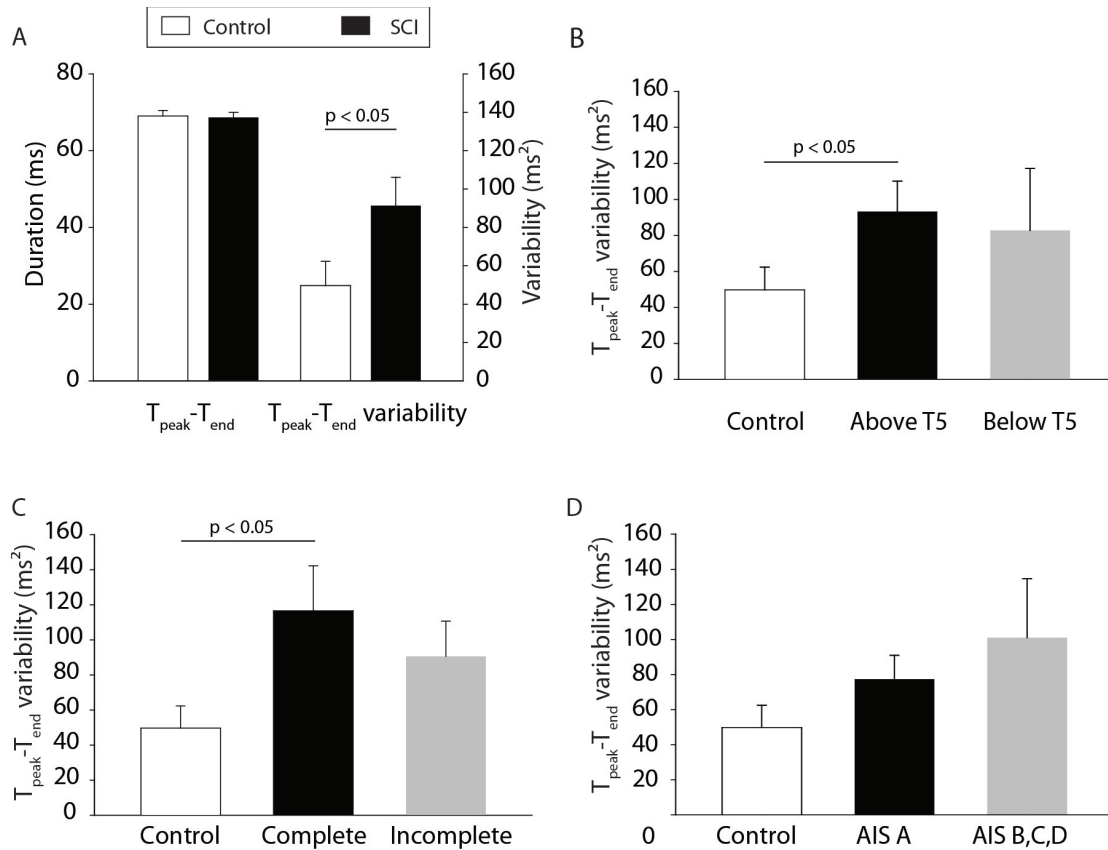


Figure 5.2. $T_{\text{peak}}-T_{\text{end}}$ and $T_{\text{peak}}-T_{\text{end}}$ variability compared between groups.

- $T_{\text{peak}}-T_{\text{end}}$ and variability $T_{\text{peak}}-T_{\text{end}}$ compared between the SCI and control groups.
- $T_{\text{peak}}-T_{\text{end}}$ variability compared between SCI groups above and below T5 and the controls group.
- $T_{\text{peak}}-T_{\text{end}}$ variability compared between autonomically complete and incomplete SCI groups and the control group.
- $T_{\text{peak}}-T_{\text{end}}$ variability compared between SCI groups divided by AIS score and the control group.

The bars represent the groups mean and the error bars represent SEM.

QTVI was significantly greater (closer to zero) in the SCI group compared to controls ($p=0.02$). Again, this was related to the level of injury, with greater QTVI in those with lesions above T5 compared to controls ($p=0.02$, Figure 5.3A). We found no significant difference between those with lesions below T5 and controls. Similarly, QTVI was significantly greater in those with autonomically complete injuries compared to controls ($p=0.01$). Responses in those with autonomically incomplete injuries were not significantly different from both other groups (Figure 5.3B). We found no significant differences in QTVI between control and SCI groups when grouped according to AIS scores (Figure 5.3C). In addition, QTVI and $T_{\text{peak}}-T_{\text{end}}$ variability were significantly correlated with each other ($r=0.734$, $p<0.001$, Figure 5.3D).

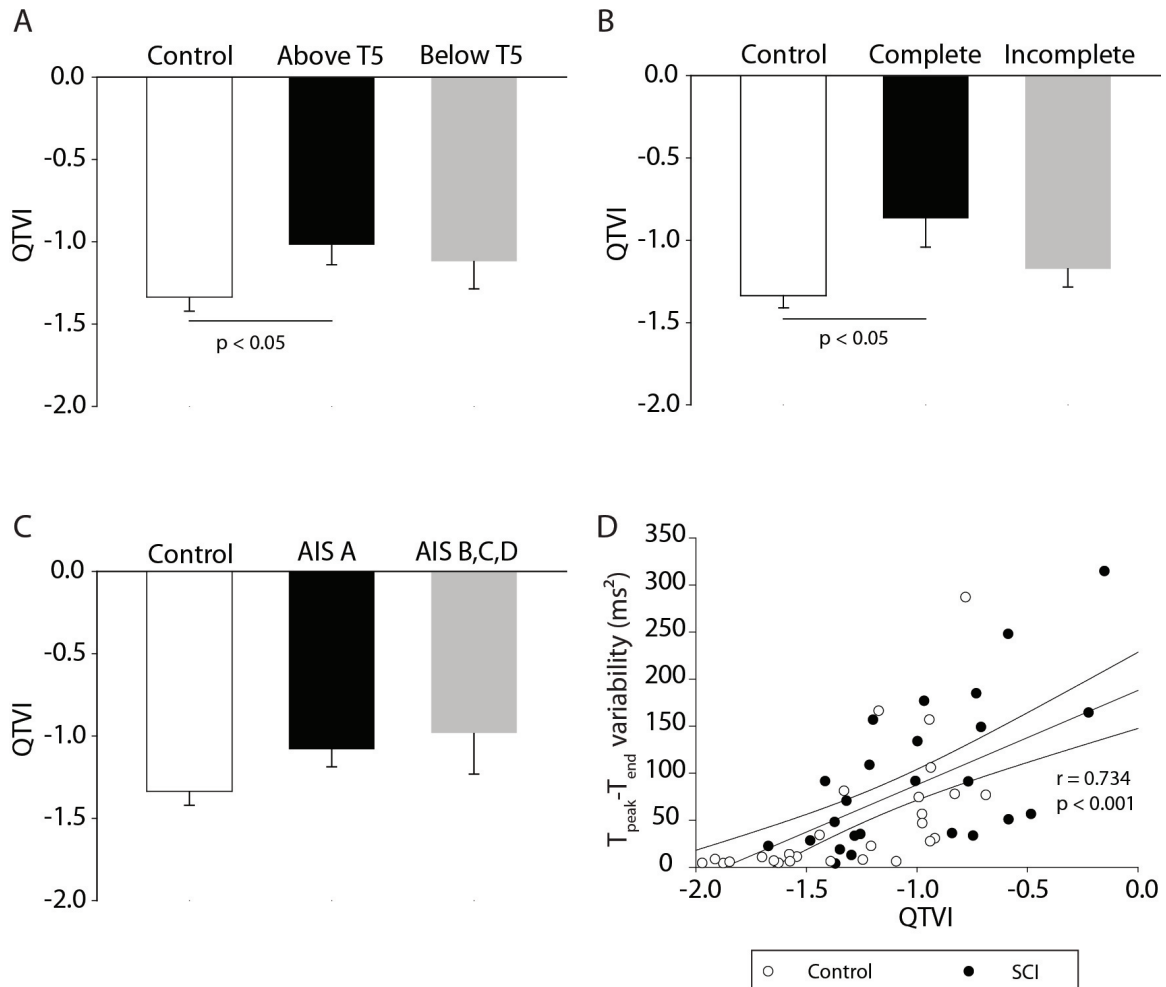


Figure 5.3. QTVI compared between groups

- QTVI compared between SCI groups above and below T5 and the controls group.
- QTVI compared between autonomically complete and incomplete SCI groups and the control group.
- QTVI compared between SCI groups divided by AIS score and the control group.
- A correlation between QTVI and $T_{\text{peak}}-T_{\text{end}}$ variability.

The bars represent the groups mean and the error bars represent SEM.

P-wave duration variability was greater in SCI individuals compared to controls ($p < 0.01$, Figure 5.4A). ANOVA testing with the different SCI subgroups was not conducted for this parameter due to a smaller sample size for this measure (control $n=17$ and SCI $n=16$). P-wave variability was correlated with both $T_{\text{peak}}-T_{\text{end}}$ variability ($r=0.608$, $p < 0.001$, Figure 5.4B) and QTVI ($r=0.421$, $p=0.01$). There was no correlation between P-wave variability and the average cycle length (RR interval) ($r=0.074$, $p=0.07$). All variability data are summarized in Table 5.3.

Table 5.3. Variability parameters: $T_{\text{peak}}-T_{\text{end}}$, QTVI and P-wave variability.

	$T_{\text{peak}}-T_{\text{end}}$ variability (ms ²)	QTVI	P-wave variability (ms ²)
Able-bodied Controls	45 (66)	-1.31 (0.41)	29 (19)
All SCI Participants	91 (78) †	-1.03 (0.53) †	105 (63) †
SCI subdivided by level of injury			
Cervical SCI	92 (88)	-0.97 (0.62)	
Thoracic SCI	90 (71)	-1.09 (0.41)	
SCI subdivided according to injury above or below T5			
SCI Above T5	94 (79) †	-1.02 (0.56) †	
SCI Below T5	83 (84)	-1.12 (0.43)	
SCI subdivided according to autonomic completeness of injury			
Autonomically Complete SCI	103 (89) †	-0.87 (0.62) †	
Autonomically Incomplete SCI	80 (70)	-1.17 (0.40)	
SCI subdivided according to AIS score			
AIS A	77 (58)	-1.08 (0.43)	
AIS B, C or D	101 (100)	-0.98 (0.63)	

Note: † denotes $p < 0.05$ compared to controls. Data are shown as mean (SD).

Correlations with autonomic impairment

We found a significant negative correlation between upright noradrenaline and P-wave variability ($r = -0.496$, $p = 0.04$, Figure 5.4C). In addition, $T_{\text{peak}}-T_{\text{end}}$ variability ($r = -0.341$, $p = 0.01$) and P-wave variability ($r = -0.497$, $p < 0.01$) were significantly correlated with the severity of autonomic impairment, as determined from the LF SAP_{nu} (Figure 5.4D).

12-lead electrocardiogram

We found no significant differences in PWD between the SCI group (37 ± 13 ms) and controls (32 ± 13 ms, $p = 0.35$). In more individuals with SCI ($n = 15$; 88%) one or more leads were rejected for analysis than in controls ($n = 2$; 14%) ($X^2 = 2.26$ and $p < 0.001$). Since the 12-lead ECG was recorded while seated in SCI participants, but while supine in controls according to convention, we evaluated whether alterations in autonomic tone associated with postural changes could have influenced our results, in a subset ($n = 10$) of controls. We found no significant differences in supine and seated PWD in controls (32 ± 13 ms and 29 ± 5 ms respectively, $p = 0.61$). Also, when compared to the SCI group, the seated PWD in controls remained similar ($p > 0.05$).

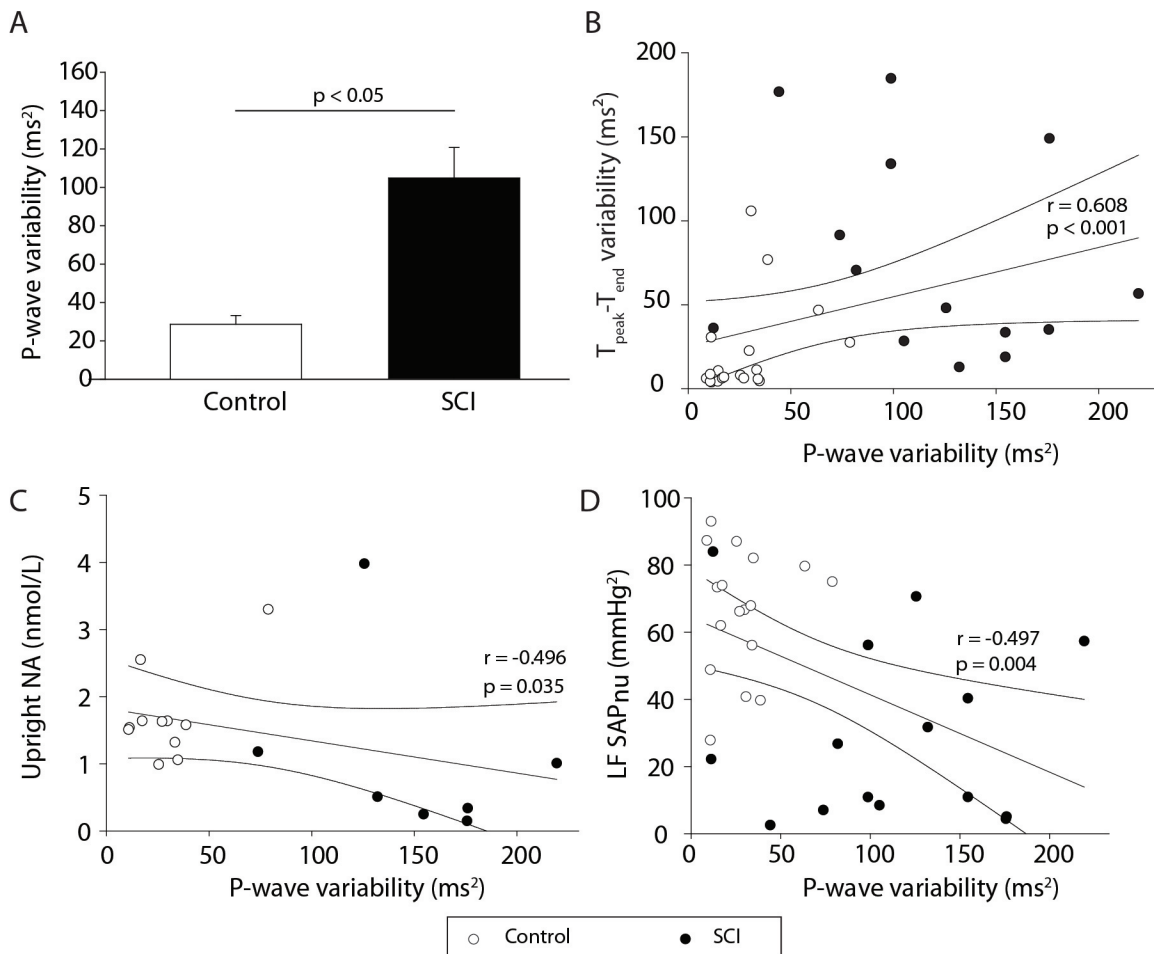


Figure 5.4. P-wave variability over time compared between groups.

- A. P-wave variability compared between the SCI group and the control group.
- B. A correlation between P-wave variability and T_{peak}-T_{end} variability.
- C. A correlation between P-wave variability and upright noradrenaline (NA).
- D. A correlation between P-wave variability and LF SAP_{nu}.

Discussion

We evaluated different ECG-based parameters that might be used as risk assessment tools for the propensity to develop cardiac arrhythmias after SCI. Not only did we observe differences in these parameters between individuals with SCI and able-bodied controls, but we also found correlations between these measures and the severity of autonomic impairment after injury. Our main finding was that $T_{\text{peak}}-T_{\text{end}}$ variability and QTVI were greater in the SCI group, particularly in those with high-level and autonomically complete lesions. The relationship between the severity of autonomic impairment and increased variability in the $T_{\text{peak}}-T_{\text{end}}$ and QT intervals suggests that autonomic impairment could underlie the increased risk for ventricular arrhythmias after SCI. The abnormal variability of $T_{\text{peak}}-T_{\text{end}}$ and QTVI was independent of the AIS score, confirming that completeness of injury to motor or sensory pathways does not necessarily correlate with completeness of injury to autonomic pathways. This is consistent with our earlier findings⁸⁰ and highlights the need for quantitative autonomic function testing after SCI.

Our data are supported by a recent study that reported increased QTVI in individuals with SCI²¹⁸. However, their data did not show differences in QTVI between different SCI groups. The grouping strategy in this study was purely based on injury level, not taking into account completeness of injury in general terms or specifically to cardiovascular autonomic pathways. The lack of difference in QTVI between their groups could reflect this failure to account for completeness of injury to cardiovascular autonomic pathways, as we found that QTVI was correlated to measures of autonomic function.

P-wave duration variability in a continuous ECG recording was increased in the SCI group compared to controls. The increase in P-wave variability was significantly correlated with measures of severity of injury to autonomic pathways. Unfortunately, due to a smaller amplitude of the P-wave in general, occasional background noise, and axis shifts, faithful detection of P-wave duration was difficult, reducing the sample size for this measure (SCI $n=16$; control $n=17$), and rendering subanalyses invalid. Nevertheless, we can exclude the possibility that the increased P-wave variability in the SCI group was artefactual due to increased background noise in this group because we quantified the noise levels at the isoelectric T-P sections of the ECG and they were not

significantly different between groups (control $0.0002 \pm 0.0001 \text{ms}^2$ and SCI $0.0007 \pm 0.0001 \text{ms}^2$, $p=0.29$). In addition, these noise levels are negligible compared to the variability in P-wave duration of 29ms^2 and 105ms^2 in the control and SCI groups respectively. We propose that the increased P-wave variability observed is compatible with abnormal repolarisation properties within the atria in those with loss of the normal sympathetic modulation of the heart, and may be associated with an increased risk of experiencing atrial arrhythmia.

PWD is thought to be correlated with risk for atrial arrhythmia. In this study, we found no difference in PWD between the SCI group and controls. This can be explained in two ways: either the SCI group is not at greater risk for atrial arrhythmias, or PWD is not a good risk assessment parameter for atrial arrhythmias after SCI. In a subgroup ($n=9$) of individuals with autonomically complete SCI (and thus a high likelihood of experiencing atrial arrhythmia) the PWD remained similar to controls, making the first explanation unlikely. The second explanation is supported by some technical difficulties in measuring this parameter; more leads were discarded in the ECG of SCI individuals than in controls. This could have led to an artificial decrease in PWD in the SCI group, and may have masked any true differences between groups in this parameter. Since the determination of P-wave variability over time on a continuous ECG requires measureable P-waves in only one ECG lead, we suggest it may provide a better parameter for risk of atrial arrhythmias after SCI.

One limitation of this study is that not all parameters were collected in every participant; some participants declined to participate in all tests. A second limitation is that we could not measure a direct correlation between $T_{\text{peak}}-T_{\text{end}}$ variability, QTVI and P-wave variability and the occurrence of cardiac arrhythmias. There is good evidence, however, that AD, and thus autonomic impairment after SCI, is associated with cardiac arrhythmias^{80,117}. The relationship shown between autonomic impairment and these ECG parameters implies that a correlation between these measures and susceptibility to cardiac arrhythmias may exist, but does not confirm it. Therefore, at present we acknowledge that we have not demonstrated a link between these measures and propensity to experience arrhythmia, but rather that we have identified ECG-based characteristics in individuals with SCI that are associated with an increased risk of arrhythmia in other populations^{114,214,218}. However, preliminary data during AD from four

individuals with autonomically complete cervical SCI (in whom resting QTVI and $T_{\text{peak}}-T_{\text{end}}$ variability were already increased, similar to the present findings) reveal further marked increases in both QTVI ($+0.43 \pm 0.1$; $p < 0.05$) and $T_{\text{peak}}-T_{\text{end}}$ variability ($+300 \pm 97 \text{ms}^2$; $p < 0.05$) compared to baseline, associated with marked ventricular ectopy. These data need verification in a larger population, but do support our hypothesis that these measures are related to arrhythmia risk. Future directions would, therefore, be to determine the relationships between these ECG-based parameters at rest and the occurrence of cardiac arrhythmias during episodes of AD, when risks for arrhythmia are highest.

Conclusion

We have observed abnormal ECG characteristics in individuals with autonomically complete SCI that, at least in able-bodied populations, are associated with increased susceptibility to cardiac arrhythmia. The increases in $T_{\text{peak}}-T_{\text{end}}$ variability, P-wave variability and QTVI observed occurred only in those individuals with complete lesions to descending cardiac sympathetic pathways, who also experience episodes of AD during which risk for arrhythmia is high. Furthermore, the magnitude of impairment to cardiac autonomic pathways was directly correlated with the severity of these ECG abnormalities. We propose that these ECG characteristics provide useful measures of severity of injury to cardiovascular autonomic pathways, and may prove to be indicative of susceptibility to cardiac arrhythmia after SCI.

Chapter 6.

General discussion

In this chapter the main findings disseminated from this thesis will be placed in perspective with other studies in this field. This is followed by the implications for the clinical field and individuals living with SCI and future directions for research arising from this thesis.

Prevalence and progression of cardiovascular autonomic dysfunction after spinal cord injury

Chapter 2 outlines the prevalence and progression of cardiovascular autonomic dysfunction after SCI. At the time that individuals with SCI first started inpatient rehabilitation, 33% of them had resting hypotension. As hypothesized, the prevalence of hypotension and bradycardia was greatest in the participants with cervical lesions. Most importantly, I found that the prevalence of cardiovascular autonomic dysfunction did not significantly improve over time until 5 years after discharge from the rehabilitation clinic.

Although this was the first longitudinal study to examine cardiovascular autonomic dysfunction after SCI, there have been several cross-sectional studies in cohorts of individuals with chronic SCI (>1 year). Prevalence of resting hypotension is not frequently reported, but orthostatic hypotension (OH, additional drop in blood pressure upon a change in posture) is quite commonly reported. One study, which evaluated prevalence of (self-reported) resting hypotension, showed similar findings of 33% prevalence within their overall cohort and 51% in a subset of individuals with AIS complete tetraplegia¹⁶². The prevalence of OH reported in individuals with chronic SCI varies between 21% and 50%, and this is probably influenced by the composition of the sample in terms of the levels and severity of injury of the participants. A prevalence of OH of 50% was reported in individuals with cervical lesions⁵³, 47% in individuals with

lesions above T5¹⁵⁹, and 21% in individuals with cervical lesions⁵⁴, but restricted to those with highly preserved function (AIS C and D) in whom spinal sympathetic pathways may be more likely to remain intact.

These findings on prevalence of resting hypotension and OH in individuals with chronic SCI support the lack of improvement in cardiovascular parameters over time in the present study. Moreover, this might explain in part why individuals living with a SCI have ranked the improvement of autonomic dysfunction as a high priority for improving quality of life. With this knowledge foundation, I went on to investigate whether cardiovascular autonomic impairments have an additional and long-term effect on CVD risk.

Role of autonomic impairment on cardiovascular disease risk

In Chapter 3, evidence is provided that autonomic impairment after SCI affects overall risk of CVD, and particularly influences glucose regulation. In terms of the overall risk of CVD, it was shown that there was an interaction effect between autonomic impairment and physical activity level. As described previously, autonomic dysfunction can negatively impact physical activity both through limiting participation in physical activity as a consequence of general feelings of fatigue, and through limited exercise tolerance due to the minimal ability to increase heart rate and blood pressure during physical activity. This interaction contributes to the risk of CVD after SCI.

In addition, it was shown that variables related to glucose regulation were affected by autonomic impairments, in contrast to the plasma lipid markers, which were mostly explained by known indicators such as, age, sex and waist circumference. This suggests that adverse lipid profiles after SCI are mostly related to general effects of aging and additional effects of deconditioning and related obesity. These findings are in line with many previous studies that showed decreased HDL-C levels and increased TG levels in both paraplegic and tetraplegic participants^{125,177,181}. Significant correlations between adverse lipid profiles, WC, and physical fitness have also been shown previously^{129,177,219}. Also, exercise training improves the adverse lipid profiles in individuals with SCI¹³⁰.

The intricacies of the role of autonomic impairments on glucose regulation remain unclear. Glucose tolerance was directly affected by autonomic impairment, whereas

there was an indirect effect through physical activity level on plasma insulin and IR. Many different mechanisms by which SCI can impact insulin mediated glucose uptake have been proposed; some related to general deconditioning after SCI and some related to autonomic dysfunction (Figure 6.1). As muscle is one of the main tissues responsible for insulin mediated glucose uptake from the blood, the extreme loss of muscle mass due to SCI will impair total insulin mediated glucose uptake. Cellular defects affecting insulin receptor sensitivity have been proposed to be mediated by obesity²²⁰. As mentioned previously, increased sympathetic activity has been related to IR, and therefore IR might develop after SCI as a consequence of repeated episodes of autonomic dysreflexia (AD) when sympathetic activity is extremely high. One way that AD might impair the effect of insulin is through a reduction in blood flow due to vasoconstriction impairing glucose uptake by tissues¹⁷⁹. In addition, it is proposed that sympathetic activity during AD promotes IR by activating α_1 receptors in adipose tissue, increasing protein-kinase C concentrations, which interferes with insulin signalling²²¹.

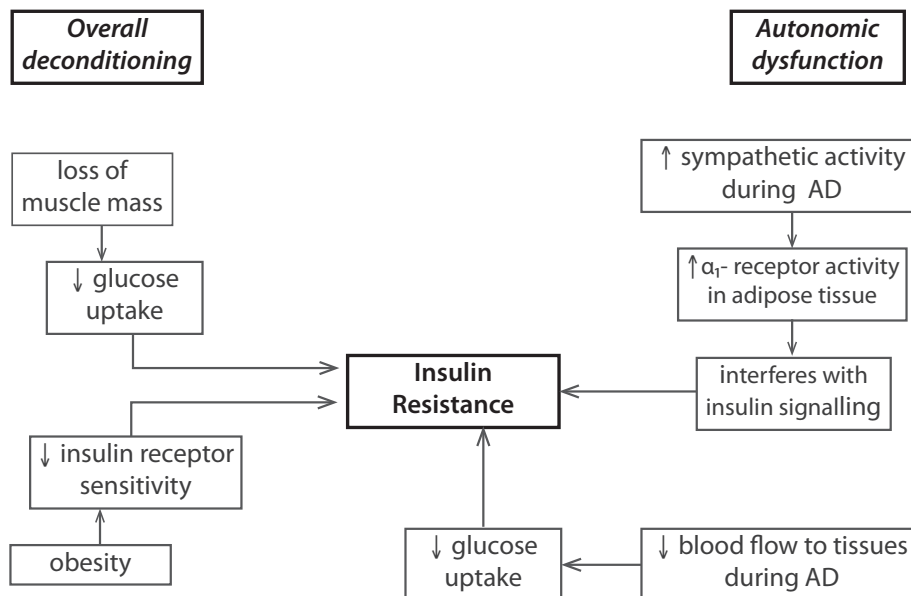


Figure 6.1. Overview of proposed mechanisms leading to insulin resistance after spinal cord injury

Many different mechanisms have been proposed by which spinal cord injury can impact insulin mediated glucose uptake that can cause insulin resistance. On the left side of the figure, the mechanisms related to general deconditioning are shown. On the right side are those mechanisms shown that are specifically related to autonomic dysfunction after spinal cord injury. Insulin mediated glucose uptake in individuals with autonomic dysfunction after spinal cord injury can thus be affected by the mechanisms listed on both sides.

These complicated relationships between SCI and IR have not been addressed. One study showed increased IR in individuals with tetraplegia¹²⁷. They did not find resting noradrenaline nor resting blood pressure and heart rate in the SCI group to be lower compared to controls, indicating that not all individuals had autonomic impairments¹²⁷. This study measured noradrenaline throughout a 24-hour period and showed episodes of AD as identified by spikes in noradrenaline. These episodes of AD were proposed to be related to IR, as they measured an increase in insulin during induced mild AD. The complexity of the effects of SCI on IR, were not addressed by this study.

In this chapter I have shown that many different factors contribute to risk of CVD after SCI. It is therefore challenging to determine the level of risk for each individual with SCI. For the general population, tools for predicting CVD risk have been developed and are widely used. However, these tools might not have the same accuracy in determining risk in individuals with SCI, as many factors contributing to CVD have changed after SCI. In Chapters 4 and 5 of this thesis, the goal was to explore options for predictors of CVD specific to SCI.

Obesity indices specific for individuals with spinal cord injury

In Chapter 4, I explored whether any of the proposed indices for obesity is superior in terms of the ability to detect adiposity and CVD risk, and practicality of use, for individuals with SCI. Waist circumference (WC) and waist-to-height ratio (WHtR) both showed strong correlations with abdominal adiposity and the Framingham risk score. WC had the strongest correlation with CVD risk, but most importantly, it is most practical to use for individuals with SCI as it does not require any measurement of height or weight. An optimal cut-off for WC was provided at 94 cm for both men and women.

These findings are supported by previous studies reporting strong correlations between WC and visceral adiposity, both in the general population and in individuals with SCI^{173,174,188}. Visceral adiposity has been shown to be the more important than other measures of adiposity in predicting CVD risk. The proposed cut-off for individuals with SCI is lower than the cut-off for men in the general population (102 cm), but slightly higher than the cut-off for women (88 cm)¹⁷⁰. Analysis of only male participants from

our study indicated the optimal cut-off to remain the same (94 cm), which could be explained by the large percentage of males in our sample, representative of the population of individuals with SCI as a whole.

The lower cut-off for WC is in line with a study that showed an underestimation of adiposity when standard cut-offs for BMI were used in individuals with SCI¹³⁶. It is also supported by a study that showed that individuals with SCI had more visceral adipose tissue compared to able-bodied controls with the same WC, BMI, and weight¹⁷³.

Cardiac arrhythmias and autonomic function

Cardiac arrhythmias have been shown to occur during episodes of AD and are thus related to autonomic impairment after SCI. In addition to an index for obesity, which can be used as a tool to predict adverse CVD risk profiles, a marker to predict the risk for cardiac arrhythmias would be valuable in order to stratify risk management towards those at highest risk for cardiac arrhythmias specifically.

In Chapter 5, abnormal electrocardiogram (ECG) characteristics were observed in individuals with SCI. The increases in $T_{\text{peak}}-T_{\text{end}}$ variability, P-wave variability and QTVI observed occurred only in those with cardiovascular autonomic impairment. The fact that cardiac arrhythmias in individuals with SCI have been shown to occur mostly during AD, a condition specific to those with cardiovascular autonomic dysfunction, provides a strong link between these altered ECG markers and risk for cardiac arrhythmia, even though we did not show a direct correlation with the occurrence of cardiac arrhythmias. In further support, it was shown that the magnitude of impairment to cardiac autonomic pathways was directly correlated with the severity of these ECG abnormalities. I, therefore, propose that these ECG characteristics provide useful measures of impairment of cardiovascular autonomic function, and may prove to be valuable in predicting susceptibility to cardiac arrhythmia after SCI.

Only one recent study has reported on these ECG characteristics in a SCI population. They showed that QTVI was increased in individuals with SCI compared to able-bodied controls, but they did not show a correlation with autonomic impairment (differences were only reported on the basis of lesion level²¹⁸). The two other ECG parameters that we evaluated have not previously been studied in this population.

As this study did not investigate direct correlations with the occurrence of arrhythmias, it is not yet possible to provide a clear recommendation for the use of these ECG characteristics to predict susceptibility to cardiac arrhythmia; a follow-up study is necessary to provide an optimal cut-off for these markers.

Implications and future directions

The study presented in Chapter 2 showed that signs of cardiovascular dysfunction (e.g. hypotension and bradycardia) are not improving with time after injury. Although this represents an important finding on its own, the question arises as to how these signs are related to symptoms of cardiovascular dysfunction, and what the consequences are in relation to activities of daily living. Perhaps individuals with SCI adapt to or learn to cope with these lower resting blood pressure and heart rate values in such a way that the impact on participation in activities of daily might improve with time after injury. It would be interesting to investigate how resting blood pressure and heart rate are related to participation in activities of daily living and whether any changes in this relationship occur with time after injury.

Another important implication demonstrated throughout this thesis is that completeness of injury as assessed by AIS scoring, does not necessarily relate to severity of autonomic impairment of injury. An important step has been made by incorporating a qualitative assessment of autonomic function in the 'Autonomic Standard Assessment Form' of the AIS assessment⁸¹. However, it is important for SCI research and clinical practice to have a standard method to quantify autonomic impairment after injury.

The most complex questions for future studies arise from Chapter 3. As explained, the relationship between changes in body composition, autonomic function after SCI, and altered insulin mediated glucose uptake is extremely complex. A future direction would be to explore these relationships in more depth using more sophisticated tools such as the insulin clamp technique to explore glucose and insulin changes in interaction with each other. It would be interesting to conduct these tests both at rest and during an episode of AD, to investigate the potential short-term effects of elevated sympathetic activity. In addition, the effects of physical fitness and muscle mass on these relationships should also be investigated.

The WC cut-off for predicting adverse CVD risk profiles can be used by individuals living with SCI as a simple and practical aid to maintain a healthy body composition. The main follow up question to this study is to define a cut-off for WC that is specific to females. In the able-bodied population sex-specific cut-offs are used and the cut-off for women is much lower than for men. Even though there are more men living with SCI, it is still important to determine these sex specific cut-offs. Conducting this research project might be challenging, as the recruitment of women with SCI will be more complicated due to the smaller population of women with SCI.

As mentioned previously, in Chapter 5 altered ECG-based markers for arrhythmias were detected in individuals with autonomic dysfunction after SCI compared to controls, but did not provide a correlation with occurrence of arrhythmias directly. A future direction would be to investigate the relationships between these markers and the occurrence of cardiac arrhythmias during an episode of AD, triggered in a controlled laboratory setting. Potentially these research studies can aid the identification of a simple tool to predict the risk for cardiac arrhythmias after SCI.

In broader terms, these insights into the role of autonomic dysfunction after SCI in the development of overall CVD, and perhaps more specifically in relation to abnormal glucose regulation, insulin resistance or even diabetes, can raise awareness for clinicians and individuals living with SCI of the seriousness of autonomic dysfunction after injury and the importance of managing signs of autonomic dysfunction early. In the longer term, this might lead to new strategies to manage signs and symptoms of autonomic dysfunction (e.g. prevention or relief of episodes of AD) and new therapies to improve autonomic function.

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Appendix.

List of publications arising from this thesis

Peer reviewed journal articles

H.J.C. Ravensbergen, S.A. Lear and V.E. Claydon (2013) Waist circumference is the best index for obesity-related cardiovascular disease risk in individuals with spinal cord injury. *Under revision, Journal of Neurotrauma*.

H.J.C. Ravensbergen, S. de Groot, M.W.M. Post, H.J. Sloomman, L.H.V. van der Woude and V.E. Claydon (2013). Cardiovascular function after spinal cord injury: prevalence and progression of dysfunction during inpatient rehabilitation and 5 years following discharge. *Accepted for publication in Neurorehabilitation and Neural Repair*.

H.J.C Ravensbergen, M.L Walsh, A.V Krassioukov, and V.E. Claydon (2012). Electrocardiogram-based predictors for arrhythmia after spinal cord injury. *Clinical Autonomic Research* **22**. 265-273.

Peer reviewed published research abstracts

H.J.C. Ravensbergen, I.S. Sahota, S.A. Lear and V.E. Claydon (2012). The impact of injury to autonomic pathways on cardiovascular disease risk after spinal cord injury. *Clinical Autonomic Research* **22**:244

H.J.C. Ravensbergen, MC Keenleyside, S.A. Lear and V.E. Claydon (2012). What is the best marker for obesity in individuals with spinal cord injury *Clinical Autonomic Research* **22**:245

H.J.C. Ravensbergen, I.S. Sahota, S.A. Lear and V.E. Claydon (2012). The impact of injury to autonomic pathways on cardiovascular disease risk after spinal cord injury. *European Journal of Neurology* **19**: Suppl. 1, 808-826

H.J.C. Ravensbergen, I.S. Sahota, S.A. Lear, A.V. Krassioukov and V.E. Claydon (2011). Does autonomic dysfunction play a role in the development of cardiovascular disease after spinal cord injury? *Clinical Autonomic Research* **21**:185–213

H.J.C. Ravensbergen, I.S. Sahota, S.A. Lear, A.V. Krassioukov and V.E. Claydon (2011). Does autonomic dysfunction play a role in the development of cardiovascular disease after spinal cord injury? *Autonomic Neuroscience* 163:109

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