The validation of heart rate variability in individuals with spinal cord injury

by

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Dedication

I would like to dedicate this thesis to my mom Karyn, my dad Fred and my sisters Alyson, Heather and Christine. I would also like to dedicate this thesis to my supervisor Dr. Dave Ditor. I would like to thank you all for your endless support.
Abstract

The current classification system for spinal cord injury (SCI) considers only somatic information and neglects autonomic damage after injury. Heart rate variability (HRV) has the potential to be a valuable measure of cardiac autonomic control after (SCI). Five individuals with tetraplegia and four able-bodied controls underwent 10 min continuous ECG recordings during rest, after Metoprolol administration (max dose=3x5mg) and after Atropine administration (0.02mg/kg) in both supine and 40° head-up tilt. After Metoprolol administration there was a 61.8% decrease in the LF:HF ratio in the SCI participants suggesting that the LF:HF ratio is a reflection of cardiac sympathetic outflow. After Atropine administration there was a 99.1% decrease in the HF power in the SCI participants suggesting that HF power is highly representative of cardiac parasympathetic outflow. There were no significant differences between the SCI and able-bodied participants. Thus, HRV measures are a valid index of cardiac autonomic control after SCI.
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List of Abbreviations

Ach - Acetylcholine
ANOVA – analysis of variance
ANS – autonomic nervous system
ASIA – American Spinal Injury Association
BWSTT – body weight support treadmill training
ECG - electrocardiogram
HF – high frequency
HR – heart rate
HRV – heart rate variability
HUT – head-up tilt
LF – low frequency
MAP – mean arterial pressure
MSNA – muscle sympathetic nerve activity
NE - Norepinephrine
SA node – sino-atrial node
SCI – spinal cord injury
SD – standard deviation
SNS - sympathetic nervous system
SSR – sympathetic skin response
Chapter 1

1.1 Introduction

Spinal cord injury (SCI) is a devastating and life-altering condition. It includes both psychological and physiological consequences and affects both the somatic (SNS) and autonomic (ANS) nervous systems. The ANS consists of two parts; the sympathetic and parasympathetic systems. The preganglionic sympathetic fibres originate from the first thoracic segment to the second lumbar segment (T1-L2) of the spinal cord and the parasympathetic originates from the vagus nerve in the caudal medulla of the brainstem and between the sacral segments two through four (S2-S4) of the spinal cord (Vander et al., 2001). The heart is innervated by both the sympathetic and parasympathetic nervous systems. The sympathetic innervation of the heart originates from the first to the fourth thoracic segments of the spinal cord, and the vagus nerve provides the parasympathetic innervation of the heart (Vander et al., 2001).

1.2 Literature Review

1.2.1 Incidence and Prevalence of Spinal Cord Injury

In Canada the incidence of spinal cord injury is approximately 35 injuries per million people, which translates to approximately 1050 new injuries every year (Sekhon et al., 2001). In the USA the incidence is 40 per million people, translating to approximately 12,000 new injuries every year (Sekhon et al., 2001). The slightly higher incidence in the USA is attributed to gun and stab wounds (Sekhon et al., 2001). There are approximately 36,000 Canadians and 205,000 Americans living
with SCI today (Sekhon et al., 2001). Of the approximately 12,000 new injuries every year in the USA, roughly 4000 die before reaching the hospital and around 1000 die during hospitalization (Sekhon et al., 2001). Not all of these deaths are attributable to the actual SCI, but instead from other injuries sustained at the accident. Males sustain approximately 75% of all SCI (Ho et al., 2007). In Canada, the most common cause of SCI is car and motorcycle accidents which comprise approximately 55% of all injuries (Ho et al., 2007).

1.2.2 ASIA Examination

The American Spinal Injury Association (ASIA) has provided a neurological examination which is the currently accepted criteria for the classification of the level and severity of a spinal cord injury. This examination however has a major limitation, as it only takes into consideration the somatic motor and sensory information. Absent from all aspects of this examination is any consideration for the autonomic nervous system. A spinal cord injury may have profound effects on the entire body. Along with the obvious paralysis of muscles and loss of sensation below the level of the injury, SCI results in devastating consequences to many other systems of the body such as impairments to bladder and bowel function, sexual function, temperature regulation, and cardiovascular function (Forsythe et al., 2006; Sipski et al., 2006; Lombardi et al., 2007; Lynch et al., 2000). However, it is important to note that, different levels of the spinal cord are responsible for sending autonomic outflow to the various organ systems of the body. For example, autonomic outflow to the bladder originates from T11-L1 (sympathetic) and S2-S4 (parasympathetic) while autonomic outflow to the heart originates from T1-T4 (sympathetic) and the vagal nerve (parasympathetic) (Vander et al., 2001). Thus, like somatic impairments, the
autonomic impairments, that accompany any given SCI depend on the level and severity of injury.

The ASIA classification system for SCI includes a determination of the level of the injury and the severity of the injury. When discussing the level of an injury the terms tetraplegia and paraplegia are used. Tetraplegia refers to damage within the cervical segments of the spinal cord. It causes impairment or loss of motor and/or sensory function in the upper and lower extremities, trunk and pelvic regions. Paraplegia results from damage to the thoracic, lumbar or sacral segments of the spinal cord. Motor and sensory function is normal in the upper extremities in this case. Complete and incomplete injuries are used to describe the severity of SCI. An injury is considered to be complete when both sensory and motor function are absent in the lowest sacral segments of the spinal cord (S4 and S5). An incomplete injury is when there is some sensory and/or motor function preserved below the level of the lesion, including sensory and/or motor function in the lowest segments of the spinal cord (S4 and S5).

The ASIA impairment scale then further distinguishes the severity of the injury with a graded letter system. Complete injuries are designated ASIA A, whereas incomplete injuries are separated into ASIA B, C, D and E (Lin et al., 2000). An ASIA B injury indicates sensory but not motor function below the neurological level including the sacral segments S4 and S5. An ASIA C injury denotes motor and sensory function below the neurological level such that more than half of the key muscles below the neurological level have a muscle grade of less than 3 out of 5, with 3 denoting anti-gravity strength against no resistance. ASIA D represents motor and sensory function preservation below the neurological level, such that at least half of
the key muscles below the neurological level have a muscle grade of 3 or more out of 5, with 4 and 5 denoting active movement with full range of motion, against moderate and full resistance, respectively. Finally, an ASIA E individual has normal motor and sensory function. This classification system is sufficient for the somatic motor and sensory systems, but does not consider the ANS at all (Lin et al., 2000). As stated above, there are many autonomic complications that accompany SCI and therefore methods to measure autonomic function after SCI are greatly needed. Such methods would not only help to identify the amount of autonomic impairment that an individual has, but will also help to gauge improvements in autonomic function with subsequent exercise, drug or even neuroregenerative therapies. See Appendix A for an example of the ASIA neurological exam.

1.2.3 Cardiovascular Dysfunctions associated with Spinal Cord Injury

As mentioned, cardiovascular dysfunction is one of the many autonomic complications following SCI. Two common cardiovascular dysfunctions following SCI are autonomic dysreflexia and orthostatic intolerance. The severity of any given episode of these conditions may range from a mild change in blood pressure to a drastic one that should be considered a medical emergency. This highlights the great need for a valid and reliable measure of autonomic function after SCI which is both easily administered and non-invasive.

Autonomic dysreflexia is an abnormal sympathetic reflex response triggered by a noxious stimulus below the level of the injury (Krassioukov et al., 2006). This condition most commonly occurs in individuals with SCI at levels T5 and above but, may also occur in individuals with SCI between T6 and T10 (Krassioukov et al., 2006). It is estimated that 50-75% of individuals with SCI at T6 or higher experience
autonomic dysreflexia. However, this is a rough estimate as this is one of the most under diagnosed conditions in SCI with many doctors not even being aware that it exists. It is more severe in those with T5 or above because of the vasoconstriction of the large splanchnic vascular bed in the abdomen which receives sympathetic input from T6-T12. A very common cause of autonomic dysreflexia is bladder distension. Afferent information to the spinal cord causes a reflex of sympathetic outflow below the injury. This leads to intense vasoconstriction and therefore increased blood pressure, which may lead to strokes, seizures and even death. Systolic blood pressures can reach as high as 300 mmHg (Krassioukov et al., 2006). Damage to the pathways in the spinal cord, that carry inhibitory input, from the lower brainstem to the sympathetic preganglionic neurons, may play a role in autonomic dysreflexia (Krassioukov et al., 2006). This damage allows for over-activity in the spinal reflexes below the level of the lesion connecting spinal afferent projections to preganglionic neurons, causing moderate to extreme vasoconstriction and therefore increased blood pressure (Krassioukov et al., 2006).

In contrast, orthostatic intolerance is the inability to withstand an upright posture without experiencing moderate to large decreases in blood pressure and possibly syncope. The symptoms of this condition include fatigue or weakness, light-headedness, dizziness, blurred vision, dyspnea and restlessness (Frisbie et al., 1997; Sclater et al., 2004). This is a problem for individuals with acute or chronic high-level spinal cord injuries (Mathias et al., 2002; Mathias et al., 1995). Individuals with SCI often suffer from this condition due to a low level of functioning or complete loss of sympathetic drive to the abdominal vessels (splanchnic vascular bed) and the large vessels of the legs, thus, being unable to properly vasoconstrict below the injury level and maintain blood pressure while upright. The issue is made even more problematic
by the absence of sympathetic drive to the heart which would otherwise increase cardiac output and to a lesser extent the absence of somatic motor function in the lower extremities as these lower limb muscles would otherwise act as a muscle pump. Resulting from this is a greatly decreased blood pressure in the large veins emptying into the atria of the heart (Jacobsen et al., 1992; Faghri et al., 2001). This leads to moderate to extreme decreases in overall blood pressure and the aforementioned symptoms. Both of these cardiovascular dysfunctions have a large effect on the quality of life of individuals with SCI. The ability to measure cardiovascular autonomic damage after SCI may allow practitioners to predict the severity of these cardiovascular dysfunctions.

1.2.4 Heart Rate Variability

Power spectral analysis of heart rate variability (HRV) is a well-established non-invasive measure of autonomic cardiac control in the able-bodied population that allows for the estimation of the relative sympathetic and parasympathetic outflow to the heart (Pomeranz et al., 1985), and if validated, this technique would be valuable for the assessment of autonomic regulation after SCI. The theory behind HRV is that the heart does not beat with the regularity of a metronome. Instead there are subtle variations in the time intervals between successive heart beats. It is important to realize that it is the intervals between successive R spikes on an electrocardiogram (ECG) that are being analyzed rather than the heart rate itself. After mathematical analysis of the R-R intervals, a frequency spectrum describing the relative predominance of sympathetic and parasympathetic outflow to the heart over a given time period is created. There are two methods of evaluating the variability among R-R intervals. Other than the frequency domain analysis mentioned above, R-R intervals
can also be analyzed in the time domain. The disadvantage of time domain analysis, however, is that this method is primarily only an estimate of vagal outflow to the heart (Task Force, 1996). Power spectral analyses, which is a frequency domain measure estimates both sympathetic and parasympathetic regulation (Task Force, 1996). For the purposes of this study, the frequency domain will be the only method of R-R interval analysis discussed.

1.2.4.1 Analysis of Heart Rate Variability

For power spectral analysis of HRV, the intervals between successive R-R intervals are first measured and plotted against beat number. When this is done, a complex signal called a tachogram is produced. In general, healthy, able-bodied people have a uniform and predictable tachogram signature. The individual spikes of the tachogram are the high frequency oscillations and the smooth waves along a tachogram are the low frequency oscillations. Mathematical analysis using most commonly a Fast Fourier Transformation is then completed to separate the various frequencies and determine the relative predominance of each. Fast Fourier transformation includes a mathematical algorithm which is relatively simple to use and has a very high processing speed (Task Force, 1996). The relative predominance or power of the high frequency (HF) and low frequency (LF) oscillations are then quantified and represented graphically in a power spectrum. See Appendix B for an example of the stages of the analysis. In healthy, able-bodied individuals, there are two particularly prominent frequencies embedded in the tachogram; 0.1Hz which is denoted as the low frequency (LF) and 0.25 Hz, which is denoted as the high frequency (HF). Thus, with respect to the power spectrum, LF power refers to the area under the curve between 0.04 and 0.15 Hz (centred around 0.1 Hz) and HF power
refers to the area under the curve between 0.15 and 0.4 Hz (centred around 0.25 Hz) (Pomeranz et al, 1985).

1.2.4.2 Validation and Reliability of Heart Rate Variability

In a study conducted by Pomeranz et al., (1985), Atropine and Propranolol were administered to observe the effects on the HF and LF powers in the HRV power spectrum. In this study Atropine, a parasympathetic blocker, blocking acetylcholine (Ach) and Propranolol, a beta-sympathetic blocker, blocking norepinephrine (NE) were administered to eight healthy able-bodied participants. ECG data was then collected from each participant and HRV was determined via power spectral analysis. The results showed that Atropine alone caused an almost complete loss of the HF power in the spectrum in both supine and standing postures. They interpreted this as HF corresponding to cardiac parasympathetic activity. Administration of Propranolol alone showed approximately a 75% reduction in LF power in standing posture. The addition of Atropine with the Propranolol caused the remainder of the LF power to disappear. This was also the case with the reverse order of the drugs. This was interpreted as the LF corresponding to both sympathetic and parasympathetic cardiac activity. Thus, Pomeranz and colleagues (1985) concluded that power spectral analysis of HRV is a valid measure of autonomic cardiac control in the able-bodied population, and further the ratio of LF:HF power may be used as an estimate of cardiac sympathovagal balance (Pomeranz et al., 1985); a lower ratio corresponding to parasympathetic predominance.

A study by Mukai and Hayano (1995) showed that the LF:HF ratio of the HRV spectrum analyzed by autoregressive transformation, increased progressively as angles increased during head-up tilt in healthy able-bodied participants. This
coincides with the findings from the Pomeranz study connecting the LF:HF ratio of the spectrum to sympathovagal balance. These studies also point out the importance of posture when describing the relationship between cardiac autonomic input and the HRV power spectrum. In healthy able-bodied people the LF power seems to correspond primarily to cardiac sympathetic outflow, however, this relation may only be detectable during a cardiovascular challenge such as upright posture. In contrast, parasympathetic activity influences the low and high frequencies of the power spectrum in both supine and standing postures (Pomeranz et al., 1985 & Mukai et al., 1995).

Numerous studies have shown the reproducibility of spectral analysis of HRV in able-bodied populations. In a study by Dimier-David et al., (1994), there were no significant differences seen in HRV measures taken with a month separating the measures. Marks et al., (1999), showed that although only moderate there is reliability in resting heart rate variability in the frequency domain. LF and HF powers had intraclass correlation values of .78 and .76 respectively between days 1 and 2, however the sympathovagal ratio had an R value of .96 between days, showing a much higher reproducibility. Pitzalis et al., (1996), showed that under conditions of rest, controlled respiration and passive head-up tilt, frequency domain measures of HRV are reproducible in healthy individuals. In particular, the HF and total powers were shown to be reproducible under conditions of controlled breathing and rest, respectively, while the LF power was reproducible under all three test conditions.

1.2.4.3 Heart Rate Variability in the Spinal Cord Injured population

To date a few studies have begun to show promise for power spectral analysis of HRV in the SCI population. First, Ditor et al. (2005b) observed that day-to-day
measures of resting HRV are reproducible after SCI. Measures of heart rate, LF, and LF:HF were found to be highly reproducible, however the reproducibly of HF was not as strong for all participants (Ditor et al., 2005b). Inoue et al. (1990) analyzed HRV among a population of six quadriplegic males using autoregressive spectral analysis. They found that only the HF component was observed in the power spectrum and suggested that the disappearance of the LF component was caused by the interruption of the spinal pathways linking supraspinal cardiovascular centres with the peripheral sympathetic outflow. They also suggested that the cervical spinal sympathetic pathways may be key in the production of the LF component in humans (Inoue et al., 1990).

In a later study by Inoue et al., nine of fifteen individuals with quadriplegia showed no LF component in the power spectrum, in agreement with their previous work. However, in the remaining six participants, the LF component was observed. It was suggested that the physiological mechanism of low frequency in these six quadriplegics may be different than the controls, for example there may be reflexive contribution from the spinal sympathetic nervous system in the absence of supraspinal input. On the other hand, the individuals with paraplegia, involved in this study, (with intact 1st-4th thoracic spinal cord segments), showed both LF and HF components. However total power, the power of the LF component and that of the HF component were smaller than those in nine healthy, sedentary age-matched controls. The authors suggested that the low HRV may be explained by the dysfunction of the sympathetic nerves to the vessels below the level of the lesion and/or the compensatory vagal suppression (Inoue et al., 1995).
In contrast to the earlier study by Inoue et al. (1990) a study by Grimm et al. (1997) showed that although reduced, high frequency and low frequency oscillations both exist on the power spectrum after SCI. They also showed that these oscillations centre around the same frequencies of 0.1 Hz and 0.25 Hz as seen in the able-bodied population (Grimm et al., 1997). Finally, they showed that there is maintenance of the LF:HF ratio (Grimm et al., 1997). Koh et al. (1994) provide strong evidence that the parasympathetic nervous system contributes in an important way to low-frequency and high frequency R-R interval spectral power (Koh et al., 1994). Similar to Pomeranz et al. (1985) however in the SCI population, they also used Atropine to block the cardiac parasympathetic outflow, while in supine, and saw that it nearly abolished the LF and HF fluctuation in the spectrum (Koh et al., 1994). However, the actual decrease in the HF power was not quantified and no able-bodied controls were included in that study.

Finally, there have also been some studies investigating interventions to improve cardiovascular function after spinal cord injury which have used spectral analysis of HRV to measure the improvements. Exercise training in the able-bodied population leads to a reduction in the LF:HF ratio, corresponding to vagal predominance (De Meersman et al., 1993). Similarly, Ditor et al. (2005a) had an incomplete SCI population perform six months of aerobic exercise training with body weight support treadmill training (BWSTT) and observed that HRV changes with exercise training after SCI in a similar way as has been shown in the able-bodied population, in particular, a lowering of the LF:HF ratio (Ditor et al., 2005a). These findings were also shown in those with complete SCI following four months of BWSTT (Ditor et al. 2005c). Autonomic function and LF HRV after spinal cord injury may be influenced by pharmacological interventions in positive ways also.
Segal et al. (2002) showed that 4-Aminopyridine, a potassium channel blocker, increased the LF activity of those with spinal cord injury to a level indistinguishable from the LF activity of the able-bodied controls.

In summary, the majority of studies show both LF and HF powers exist in the power spectrum after SCI. It has also been shown that these powers still centre around 0.1 Hz for LF and 0.25 Hz for HF. It has been shown that these measures are reproducible in both able-bodied and SCI populations. Studies have shown that these values change with exercise in a way that suggests that they correspond to sympathetic and vagal outflow, again in both able-bodied as well as SCI populations. However, this has never been truly validated as a measure of cardiac autonomic outflow after SCI as in the able-bodied population.

1.2.4.4 Limitations of Heart Rate Variability Measures

Despite the fact that heart rate variability has the potential to be a very useful tool in measuring cardiac autonomic outflow and integrity it does have a major limitation. There is a common misconception when using HRV measures regarding what is actually being measured. The misconception exists as it is often thought that the HF component of HRV is a direct index of vagal outflow, when it is actually a measure of the heart’s response to the vagal outflow to the heart. The limitation exists in that the autonomic receptors at the heart do not necessarily respond in the same way as has been shown in the able-bodied population. Wecht et al. (2006) suggest that after spinal cord injury, particularly an injury resulting in tetraplegia, the heart’s response to autonomic outflow may be altered. They suggest that there is a desensitization of the sino-atrial (SA) node to vagal outflow in individuals with tetraplegia. A possible mechanism for this reduced SA node sensitivity to vagal
influences may be a chronic upregulation of the renin-angiotensin system in these individuals (Wecht et al., 2006). There is evidence to suggest that individuals with tetraplegia rely on the renin-angiotension system for blood pressure maintenance during up-right postures (Mathias et al. 1975 & 1980 & Wecht et al., 2005). Studies have shown that in patients with heart failure a significant negative relationship is observed between plasma renin activity and cardiac vagal tone (Osterziel et al., 1994 &1996). Chronically high plasma renin concentrations in individuals with tetraplegia may have a large impact on cardiovascular autonomic modulation as well. However, this is an area requiring further investigation.

To measure the actual autonomic tone to the heart one would have to measure the activity of the nerves of the heart, specifically at the SA node. To do this, muscle sympathetic nerve activity (MSNA) measures would have to be studied. This type of research has been done for various areas of the body (Macefield et al., 1999). To measure the autonomic outflow to the nerves at the heart would be a very invasive and unrealistic procedure. The next best option to obtain similar information would be to use HRV, with the understanding of what is actually being measured.

1.2.5 Sympathetic Skin Response

Another already validated measure of autonomic control in the SCI population is the sympathetic skin response (SSR), which measures the integrity of the descending sympathetic cholinergic pathways to the sweat glands (Cariga et al., 2002). This measure is commonly used to investigate possible autonomic complications following SCI, and it is measured in the palmar and plantar surfaces of the hands and feet respectively as they are rich in eccrine glands (Cariga et al., 2002). The sweat glands of hairless skin on the body are innervated by the sympathetic
sudomotor system which has both preganglionic and postganglionic neurons synaptically connected in the sympathetic paravertebral ganglia (Reitz et al., 2002). The sudomotor pathways are thought to be controlled by supraspinal descending pathways originating in the brainstem, hypothalamus and other higher structures (Reitz, et al., 2002).

In this technique changes in skin conductance are recorded after activation of sweat glands under the neural control of sympathetic cholinergic fibers (Uncini et al., 1988). Data is inconclusive regarding the pathways of the SSR in the upper and lower limbs. In some studies it appears as though the conducting pathway of the SSR in the spinal cord for the upper limb descends to the upper thoracic cord (T4-T6) and the conducting pathway in the lower limbs travels to the lower thoracic cord between T9 and T10 (Ogura et al., 2004). Other clinical findings indicate that sudomotor neurons may generally pass through the segments T1-T6 to the upper limb and through the segments T8-T12 to the lower limb, and even more investigations have shown the pathways to the upper limb to pass through T2-T9 and the lower limb, T10-L3 (Reitz, et al. 2002). Potentials generated by the SSR can be recorded in response to various stimuli; including gasps for breath, acoustic stimuli, magnetic stimulation of nerves and electrical peripheral nerve stimulation (Cariga et al., 2002). For the purpose of this research project, electrical peripheral nerve stimulation will be the only method discussed in this paper. Mild electrical stimulation is given at the wrists and ankles while electrodes are placed on the hands and feet to record the responses. There are various methods of measuring sympathetic skin responses. Some researchers measure the amplitude and latency of each of the responses, whereas others count the number of responses present from ten stimulations (Shahani et al., 1984; Knezevic et al.,
1985; Uncini et al., 1988; Hoeldtke et al., 1992; Levy et al., 1992; Claydon et al., 2006a, 2006b). See Appendix C for an example of an SSR recording.

1.2.5.1 Validation and Reliability of Sympathetic Skin Response

SSR is a valid technique used to assess the activity of sympathetic sudomotor pathways (Shahani et al., 1984). There is evidence to suggest reproducibility in this technique in various populations (Hoeldtke et al., 1992; Shahani et al., 1984).

Although mean amplitudes were significantly lower on Day 2 than Day 1 (0.706 ± 0.10 vs. 0.85 ± 0.1 mV, respectively), there were no significant differences found in the mean latencies of SSR responses between two consecutive days of testing in a healthy able-bodied population of 24 individuals (Hoeldtke et al., 1992). Claydon (2006a, 2006b) and Krassioukov (2006) also demonstrate validity and reproducibility in the able-bodied population as their controls consistently show perfect responses to all ten stimuli presented to them.

1.2.5.2 Limitations of the Use of Sympathetic Skin Response

There are, however limitations to the sympathetic skin response technique. Habituation, although easily overcome is one of these limitations (Cariga et al., 2001). Habituation leads to a progressive loss of amplitude to repeated stimuli (Cariga et al., 2001). Various approaches have been used to overcome habituation. Included in this are waiting for longer periods of time between consecutive stimuli (Yokota et al., 1991; Curt et al., 1996), progressively increasing the intensity of the stimulation (Hoeldtke, et al., 1992) and averaging several single traces (Knezevic et al., 1985). The time course of habituation varies from person to person (Cariga et al., 2001).

SSR also requires strict regulations of skin and room temperature (Deltombe et al.,
Skin temperature has a large effect on the latency and amplitude of SSR (Deltombe et al., 1998). At low temperatures the latency is prolonged and the amplitude is decreased (Deltombe et al., 1998). There is a linear correlation between skin temperature and latency and amplitude of SSR (Deltombe et al., 1998). It is therefore vital for SSR to be conducted in controlled conditions to ensure reliable findings.

1.2.5.3 Sympathetic Skin Response in the Spinal Cord Injured Population

Motor and sensory completeness as assessed by the ASIA scale and autonomic completeness as assessed by SSR do not always correlate (Claydon et al., 2006b). This suggests that somatic and autonomic damage may differ with respect to level and severity after SCI. As such, there is a strong argument for the need for valid and reliable measures of autonomic control after SCI. SSR may assist in the identification of individuals at the greatest risk of temperature dysregulation and possibly other impaired cardiovascular controls such as orthostatic intolerance (Claydon et al. 2006b). These conclusions again only further advocate the need for autonomic assessments for the classification of SCI.

The latency of the SSR in individuals with SCI is approximately 5 seconds after the stimulus (Claydon et al., 2006a, 2006b). It is slightly more delayed in the plantar surface of the foot if the wrist was stimulated and vice versa. In general, individuals with complete tetraplegia or high paraplegia show no SSRs from either the hands or the feet (Curt et al., 1996). In individuals with complete paraplegia SSRs are generally seen in the hands but not the feet and in individuals with complete paraplegia below T8, SSRs are usually seen in both hands and feet (Curt et al., 1996). On the other hand, approximately 50% of individuals with incomplete tetraplegia or
high paraplegia show normal SSR potentials in both the hands and the feet (Curt et al., 1996). In some cases potentials are observed in the hands and not the feet of these individuals (Curt et al., 1996).

1.3 Objectives

The purpose of the proposed research is to validate power spectral analysis of heart rate variability in individuals with tetraplegia. As a secondary purpose, this study will also determine if sympathetic impairments as determined by HRV are consistent with sympathetic impairments as determined by the SSR technique. The final purpose of this study is to investigate if autonomic impairment after SCI as determined by HRV is related to the actual clinical cardiovascular impairments of orthostatic intolerance and autonomic dysreflexia.

1.4 Hypotheses

The primary hypothesis for this study is that HRV will indeed be a valid measure of autonomic cardiac control following SCI. It is hypothesized that the HF power will correspond to parasympathetic outflow and that the LF:HF ratio will correspond to sympathovagal balance. The studies that have used this measure in the SCI population lend support to the thought that the HRV in the SCI-population will be similar to that in the able-bodied population (Grimm et al., 1997, Ditor et al., 2005a, 2005b, 2005c). It is expected that the LF:HF ratio will correlate positively to the SSR. If the first hypothesis proves to be correct than both HRV and SSR will be valid, and since they both measure sympathetic control they may prove to therefore be related to each other. It is also expected that impairments seen in autonomic outflow will be
associated with autonomic cardiovascular dysfunctions such as orthostatic intolerance and self-reported autonomic dysreflexia.
1.5 References


Chapter 2 – The Validation of Power Spectral Analysis of Heart Rate Variability after Spinal Cord Injury
2.1 Introduction

Spinal cord injury (SCI) is a devastating and life-altering condition which affects both the somatic and autonomic nervous systems. The American Spinal Injury Association (ASIA) has provided a neurological examination which is the currently accepted criteria for the classification of the level and severity of a spinal cord injury. However, this examination only takes into account the somatic motor and sensory information and fails to assess the autonomic damage after a spinal cord injury. There is a great need for an easy non-invasive measure of autonomic integrity following spinal cord injury.

A spinal cord injury may have profound affects on the entire body. Along with the obvious loss of sensation and paralysis of muscles below the level of the injury, SCI results in devastating consequences to many other systems of the body. Among these are deficits to cardiovascular autonomic control leading to an increased risk of cardiovascular disease and dysfunction. Cardiovascular disease has become the number one killer after SCI (DeVivo et al., 1993). Cardiovascular dysfunction is also a major problem after SCI. Two common cardiovascular dysfunctions include autonomic dysreflexia and orthostatic intolerance. Autonomic dysreflexia is an abnormal reflex response caused by a noxious stimulus below the level of the injury. This stimulus causes a reflex autonomic outflow to the blood vessels causing them to constrict and therefore increasing blood pressure. Blood pressures may rise to as high as 250-300mmHg systolic, resulting in seizures, stroke and even death. Orthostatic intolerance is the inability to withstand movement to an upright posture without experiencing large decreases in blood pressure potentially leading to syncope. Despite these autonomic cardiovascular deficits, there is no validated measure of cardiovascular autonomic control following spinal cord injury.
Heart rate variability (HRV) has the potential to be a valuable measure of cardiovascular autonomic control after SCI. It is a well-established, non-invasive measure of autonomic cardiac control in the able-bodied population that allows for the estimation of the relative parasympathetic and sympathetic outflow to the heart (Pomeranz et al., 1985). Heart rate variability measures the time intervals between successive heart beats, which are known to have subtle differences. A frequency spectrum, describing the relative predominance of sympathetic and parasympathetic outflow to the heart, is created after mathematical analysis of the R-R interval time intervals. This technique has been validated in the able-bodied population, but has yet to be validated in the SCI population. Using pharmacological blockade, Pomeranz and colleagues (1985) were able to determine that the high frequency (HF) oscillation present in the power spectrum corresponds to parasympathetic activity and that the low frequency (LF) corresponds to both sympathetic and parasympathetic outflow in the able-bodied population. Thus, the ratio of LF:HF is considered a more accurate measure of sympathetic outflow than LF on its own. This ratio is called the sympathovagal ratio.

To date there has been some promising literature regarding the use of HRV in the SCI population. Grimm and colleagues (1997) showed that although reduced, high and low frequency oscillations both exist on the power spectrum following spinal cord injury. They also showed that these oscillations center around similar frequencies as seen in the able-bodied population (0.1Hz – low frequency & 0.25Hz – high frequency). Finally, they showed that there is maintenance of the LF:HF ratio after SCI. Ditor et al. (2005b) observed that day-to-day measures of resting HRV are reproducible after SCI. This group (2005a, 2005c) was also able to show that HRV changes with exercise training after SCI in similar ways as has been shown in the
able-bodied population, in particular a lowering of the LF:HF ratio. Koh et al. (1994) provide strong evidence that the parasympathetic nervous system contributes in an important way to low frequency and high frequency R-R interval spectral power. Similar to Pomeranz et al. (1985), however in the SCI population, they also used Atropine to block the cardiac parasympathetic outflow, while in supine, and saw that it nearly abolished the LF and HF fluctuation in the spectrum. Despite the many studies using HRV after SCI, it has never truly been validated using pharmacological blockade of both the parasympathetic and sympathetic systems, following SCI.

Sympathetic skin response (SSR) is another already validated measure of autonomic outflow commonly used to investigate possible autonomic complications following SCI. SSR measures the sympathetic outflow to the sweat glands by measuring changes in skin conductance after activation of sweat glands under the neural control of sympathetic cholinergic fibres. Claydon and colleagues (2006b) showed that motor and sensory completeness as assessed by the ASIA scale and autonomic completeness as assessed by SSR do not always correlate, suggesting that somatic and autonomic damage may differ with respect to level and severity after SCI. This further advocates the need for a reliable and valid measure of autonomic control following SCI. The question exists as to whether HRV and SSR are redundant and interchangeable or whether they provide different information and therefore would both be necessary during the measurement of autonomic damage post-injury.

The primary purpose of the present study was to pharmacologically validate power spectral analysis of heart rate variability in the spinal cord injured population. Secondarily, autonomic measures as determined by HRV were compared to autonomic measures as determined by SSR to determine whether these measures are interchangeable or whether they must co-exist for autonomic damage assessment. The
final purpose was to observe the relationship between measures of HRV and SSR with measures of orthostatic intolerance and self-determined autonomic dysreflexia. It is hypothesized that power spectral analysis of heart rate variability will indeed be a valid measure of autonomic outflow after spinal cord injury. It is also hypothesized that measures of autonomic outflow as determined by heart rate variability will correlate positively to measures of autonomic outflow as determined by sympathetic skin response. It is also expected that impairments seen in autonomic outflow will be associated with autonomic cardiovascular dysfunctions such as orthostatic intolerance and self-reported autonomic dysreflexia.
2.2 Methods

2.2.1 Participants and Sample Size

The current cross-sectional investigation measured indices of HRV (LF, HF, and LF:HF) both before and after pharmacological autonomic blockade. The relative change in the measures of HRV upon blockade was compared between individuals with SCI and age-matched able-bodied controls. Autonomic blockade was accomplished with the administration of Atropine and Metoprolol. Atropine is a muscarinic cholinergic antagonist which blocks the parasympathetic transmission to the sinoatrial (SA) node, and Metoprolol is an adrenergic antagonist that blocks the sympathetic transmission to the SA node. The sample for this study consisted of five individuals with cervical SCI and four healthy able-bodied individuals. Males and females between the ages of 25 and 64, with incomplete (ASIA B-D) tetraplegia, with a minimum of one year post-injury were included in this study.

As this was the first attempt to validate measures of HRV in the SCI population, there were no recognized expectations of the amplitude of the drug effect, or its variability, on the measures obtained from the analysis of HRV. Therefore, no power calculations were conducted to estimate the sample size chosen to participate. Nevertheless, in Pomeranz et al., (1985) eight subjects were included and the authors were able to show large and statistically significant percentage changes in the HF and LF powers after administration of Atropine and Propranolol. With the anticipation that measures of HRV would be valid after SCI five participants with SCI and four able-bodied controls were included in this study. Participants for the SCI group were recruited by telephone from an approved list of patients of Dr. Richard McMillan, who also screened each participant for suitability into the proposed study.
Appendix D for the telephone script used to recruit participants. The able-bodied subjects were recruited by word of mouth, and consisted mainly of Brock University graduate students, faculty and staff. All procedures were approved by the Brock University Ethics Review Board and the Hotel Dieu Shaver Ethics Review Board.

2.2.2 General Protocol

The participants were tested on two separate occasions. Day 1 included a visit with Dr. Richard McMillan for medical clearance to participate in the study. This included screening for any history of cardiovascular disease, asthma, glaucoma, or smoking, as well as recording weight, nature of the injury, level of the injury and ASIA classification. Upon completion of this visit the participants went to the Spinal Cord Injury Laboratory at Brock University where they were given an information and consent session. The participants with spinal cord injury filled out a questionnaire on self-reported autonomic dysreflexia (Appendix C for the Autonomic Dysreflexia questionnaire). This questionnaire included questions about the frequency of autonomic dysreflexia experienced in both the acute period post injury (first six months of injury) and the chronic state (in the six months prior to inclusion in the study). The questionnaire also included questions about the number of symptoms and severity of symptoms. Finally, SSR testing was completed on this day as well. Day 2 included the HRV testing. During this visit the drug administration protocol occurred. (See below)
2.2.3 Measurements

2.2.3.1 Anthropometric Measurements

To ensure that the participants were a similar population except for SCI vs. able-bodied, basic baseline anthropometric measures were taken. The age, height and weight of each subject were recorded to make certain that there were no significant differences between the SCI group and the able-bodied group. Each participant was asked about their medical history. Information about any history of cardiovascular disease, any history of smoking, any past or present symptoms of asthma or glaucoma, the neurological classification of injury as well as the years post-injury for the SCI group was obtained. Questions were asked regarding asthma and glaucoma because of the effects of the drugs used in this study on those conditions. Atropine has been shown to have a negative effect on glaucoma patients as it causes pupil dilation. Dilation of the pupil crowds the iris into the angle of the anterior chamber, where it impedes the drainage of aqueous. In a normal eye this may be uncomfortable but has no real negative effects. In an eye which has drainage problems due to glaucoma this issue can cause blinding. Metoprolol worsens symptoms of asthma by aggravating breathing problems and contra-indicates Ventolin and other drugs used for asthma.

This personal health information was used for inclusion and exclusion criteria as healthy individuals were required for both the SCI and control groups. Each participant was required to be clear of any history of cardiovascular disease, asthma or glaucoma. In addition, a list of current medications was obtained to ensure that nothing contraindicated the Atropine and Metoprolol administrations. No medications were ceased during the protocol to ensure that the results were based on all factors...
which are a part of the participants’ lives. See Table 6 in the Results section for participant characteristics (pg.45)

2.2.3.2 Heart Rate Variability

The protocol for the HRV testing was as follows. The participants came into the lab after emptying their bladder and were transferred to a tilt table. A catheter was inserted into the anticubital vein for drug administration. The participants had two different ECGs attached to them. One of these was a twelve lead ECG and was connected to the cardiac monitor for rhythm observation for safety throughout the entire protocol. The other ECG was three lead and was used to collect the data to measure heart rate variability. Sampling was done at 1000 Hz to ensure an accurate R spike was recorded. The three lead ECG used for all of the data collection was recorded using the Powerlab program. Any episodes of autonomic dysreflexia or spasticity were recorded and excluded from analysis.

Each participant then lay supine on a tilt table in a dark quiet room for approximately 10 minutes. This was to ensure that the subject was in a resting state. After these 10 minutes of rest, 10 minutes of baseline ECG was recorded in the supine position followed by another 10 minutes of ECG recording with the participant in 40° head-up tilt (HUT). During the 10 minutes of HUT participants also submerged their right hand in 10° water and clenched their jaw. All of these were used as sympathetic stimuli to ensure the sympathetic nervous system was activated. Grimm et al. (1995) used these three provocative maneuvers and found that only when used together did the LF:HF ratio change significantly from supine baseline. Houtman and colleagues (2000) determined that tilt alone is not sufficient to increase sympathetic activity in a tetraplegic population.
The subjects were then returned to supine. Once returned to supine, a maximum dose of 15 mg of Metoprolol was administered intravenously in three 5 mg doses. Each 5 mg dose was given over a 5 minute period with 5 minutes of rest between doses. The heart rate (HR) was monitored as it began to decrease upon administration of Metoprolol. Metoprolol administration was terminated once the heart rate reached a nadir or if it was anticipated that another dose would decrease heart rate to less than 40 beats/min. Ten minutes of ECG readings in the supine position were then recorded and another 10 minutes in the 40° head-up tilt position with the hand submerged in 10° water and the jaw clenched.

The participant was then returned to supine and Atropine at a dosage of 0.02mg/kg was administered intravenously. It was felt that Atropine at a dose of 0.02mg/kg was appropriate for parasympathetic blockade, as it was shown that there was no difference in the drug effect using a range from 0.02-0.04mg/kg of Atropine in the SCI population in a study by Koh et al (1994). Ten minutes of ECG was then recorded in supine and another 10 minutes in the sympathetically stimulated tilt conditions. Heart rate and blood pressure were monitored during all drug infusions. With respect to the 40° head-up tilt, if participants were unable to maintain the upright position for the entire 10 minutes, they were returned to the supine position upon their request due to feeling uncomfortably lightheaded. Mean arterial pressures were also determined by brachial auscultation during all measures and position changes to measure orthostatic intolerance.

ECG tracings were analyzed with custom software developed by the Clinical Cardiovascular Physiology Lab of Dr. John S. Floras at the University Health Network. Heart rate variability indices were then determined using the TSAS software.
package developed by Dr. Yoshiharu Yamamoto. This program allowed for editing of the ECG recordings so as to remove any ectopics beats from analysis. Low frequency power was defined as the area under the curve for frequencies between 0.04 and 0.15 Hz and high frequency was defined as the area under the curve for frequencies between 0.15 and 0.4 Hz.

2.2.3.3 Sympathetic Skin Response

In addition to the ECG recordings and drug infusions, sympathetic skin response testing was conducted. This testing involved electrical stimulation of the median and peroneal nerves, at the wrist and ankle, respectively, while recording the evoked potentials at the palms of the hands and the plantar surfaces of the feet. Each participant underwent ten stimulations at the median nerve of both the right and left wrists and ten stimulations of the peroneal nerve of both the right and left ankles. Each stimulus lasted between 0.2 and 1.5 msec at an intensity of 1.5 times the motor threshold of the stimulated nerve (approximately 8-10mA). The motor threshold is defined as the minimum amplitude at which a twitch is observed at the site being stimulated. There was 1-2 minutes of rest between each stimulus. This stimulation protocol has been used extensively by others (Cariga et al., 2001; Cariga et al., 2002; Nicotra et al., 2005; Claydon et al., 2006a, 2006b) and is not associated with pain, even in able-bodied individuals with intact sensation.

Appropriate precautions were made to ensure that habituation to the stimulus did not occur. This phenomenon has been shown in both able-bodied and SCI populations (Donadio et al., 2005; Aramaki et al., 1997). Stimuli had random time intervals ranging from 1-2 minutes between each other and the participant laid on a plinth table with his or her eyes closed, so that the time of the stimulus was
unexpected. The individuals' hands and feet were cleaned with soap and water, before the procedure began, as any debris or bacteria may affect the response. In addition the skin was examined where the electrodes were placed to avoid placement over an area of poor skin integrity. Rough skin with blisters and sores will create inaccurate responses and therefore the electrodes must be adjusted accordingly.

Specifically, self-adhesive electrodes were placed on the palmar and dorsal surfaces of the hands and the plantar and dorsal surfaces of the feet. The electrodes on the palmar and plantar surfaces served as recording electrodes and the electrodes placed on the dorsal surfaces served as reference electrodes. A ground electrode was placed on each wrist and ankle as well. A stimulator was taped to one ankle and another to one wrist. The participant was then left alone to lie silently in the dark quiet room for ten minutes. Room temperature and skin temperature were closely monitored at this point. Studies have shown that both of these have effects on SSRs (Deltombe et al., 1998). Low skin temperatures may lead to decreased amplitudes of the SSR, leading to errors in reading the responses by the investigators (Deltombe et al., 1998). At a low skin temperature, the SSR amplitude decreases anywhere from 0.065 mV/ °C to 0.302 mV/ °C (Deltombe et al., 1998). For individuals with SCI, who already have very low SSR amplitudes, this may lead to errors in the determination of positive and negative results. Skin temperatures were maintained at 30-33°C as measured by a thermistor. Participants' hands and feet were washed with warm water before testing to achieve the desired range of skin temperature.

After ten minutes, the investigator re-entered and started to manually apply random stimulation to the ankle and the wrist with various time intervals between each stimulus. The electrodes recorded from all four sites; two palms and two plantar
foot surfaces for each of the stimuli. Refer to Appendix C for an example. An eight second trace was recorded after each stimulus. The first response that is seen, immediately following the stimulus artifact, is the motor response. Approximately 3-5 seconds after the stimulus, the SSR if present is seen. Regardless of the amplitude, if there is a response at all, it is considered a positive finding. For the purposes of this study, amplitude and latency were not measured, but instead just the presence or lack of response to the stimuli (Claydon et al., 2006a, 2006b). Data was continuously recorded using an analog-to-digital converter interfaced with a computer (Claydon et al., 2006a, 2006b).

2.2.4 Statistical Analyses

All of the statistics were calculated using Statistica, Excel and Prism. The HRV values used in the statistical analysis included HF power and LF:HF ratio. LF power alone was not used, as LF:HF ratio is thought to be a better representation of sympathetic predominance. Student’s t-tests were used to determine any differences in anthropometric measurements between the SCI and able-bodied groups. T-tests were also used to compare LF:HF ratios between the able-bodied and the SCI groups to determine whether there is a maintenance of this ratio after spinal cord injury, and to compare the percentage change in measures of HRV with drug administration and cardiovascular stress between the SCI and able-bodied groups.

The percent changes in HF power and LF:HF ratio after drug administration were analyzed by descriptive statistics for both the able-bodied and SCI groups. Changes in the HF power and LF:HF with drug administration were also compared within the SCI group using a one-way repeated measures ANOVA. Tukey’s honestly
significant difference post-hoc analyses were used to determine specific differences between means when required.

Pearson correlation comparisons were used to measure the strength of relationships between sympathetic impairments as measured by HRV and sympathetic integrity as measured by SSR. Specifically, this included comparisons between baseline LF:HF vs. total SSR, SSR after wrist stimulation and SSR after ankle stimulation in both the SCI and able-bodied groups. These comparisons were also made between the Δ in LF:HF ratio from supine to cardiovascular stress in the natural state vs. total SSR, SSR after wrist stimulation and SSR after ankle stimulation in both the SCI and able-bodied groups.

Pearson correlations were used to measure sympathetic impairments as measured by HRV and orthostatic intolerance. Specifically, this included comparisons between baseline LF:HF vs. baseline MAP and the Δ in MAP from supine to cardiovascular stress in the natural state. These comparisons were also made between the Δ in LF:HF ratio from supine to cardiovascular stress in the natural state vs. baseline MAP and the Δ in MAP from supine to cardiovascular stress in the natural state. These comparisons were made in both the SCI and able-bodied groups.

Finally, Pearson correlations were also used to compare sympathetic impairments as measured by HRV and measures of autonomic dysreflexia. Specifically, this included comparisons between baseline LF:HF vs. questionnaire scores for acute (within the first six months of their injury) frequency of AD, acute number of symptoms during episodes of autonomic dysreflexia and the severity of these symptoms in the acute phase of injury. These comparisons were also made between baseline LF:HF vs. chronic (within the six months before this study began)
frequency, chronic number of symptoms and the severity of symptoms during episodes of autonomic dysreflexia in the chronic state. Correlations were determined between the Δ in LF:HF ratio from supine to cardiovascular stress in the natural state vs. acute frequency of AD, acute number of symptoms during episodes of autonomic dysreflexia and the severity of these symptoms in the acute phase of injury. Finally, correlations were determined between the Δ in LF:HF ratio from supine to cardiovascular stress in the natural state vs. chronic frequency, chronic number of symptoms and the severity of symptoms during episodes of autonomic dysreflexia in the chronic state. The comparisons between measures of HRV and measures of autonomic dysreflexia were done exclusively in the SCI group. Statistical significance was set at $P \leq 0.05$ and values are expressed as means ± SD.
2.3 Results

2.3.1 Validation of Power Spectral Analysis of Heart Rate Variability after Spinal Cord Injury

2.3.1.1 Case Study Reports

Participant One – TZ

TZ is a twenty-five year old male with a C5 incomplete ASIA B spinal cord injury. TZ sustained his injury while playing hockey in 2000 and is therefore 8 years post-injury.

Changes in High Frequency Power

In the supine position without drug administration the HF power was 535.3 (beats/min)$^2$. Regarding the effects of drug administration, there was a 24.9% increase in supine HF power with Metoprolol administration, and a 99.9% decrease in supine HF power with Atropine administration. Thus, compared to the supine state with no drug administration, there was a modest increase in HF power with Metoprolol and a near abolishment of HF power with Atropine. (Table 1)

Changes in the LF:HF ratio

In the supine position with no drug administration the LF:HF ratio was 3.06. This value rose to 9.10 during cardiovascular stress. Regarding the effects of drug administration there was a 72.3% reduction in the LF:HF ratio during cardiovascular stress after Metoprolol administration and a further reduction of 12.4% after Atropine administration. Thus, compared to the stressed state with no drug administration, there was a large reduction in the LF:HF ratio with Metoprolol and a further modest reduction with Atropine. (Table 1)
Table 1-Absolute Values of Power Spectral Analysis of Heart Rate Variability for TZ

<table>
<thead>
<tr>
<th></th>
<th>HF (bt/min)$^2$</th>
<th>LF (bt/min)$^2$</th>
<th>LF:HF</th>
<th>HR (bt/min)</th>
<th>MAP (mmHg)</th>
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<tr>
<td>Supine</td>
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Participant Two – JM

JM is a forty-one year old male with a C5 incomplete ASIA D spinal cord injury. JM sustained his injury after falling from a tree in 2001 and is therefore 7 years post-injury. JM experienced an episode of autonomic dysreflexia during this portion of the data collection. His data is being reported in this case study section but was not included in the group analysis in the following section as his data may have been compromised from the autonomic dysreflexia.

Changes in High Frequency Power

In the supine position with no drug administration the HF power was 101.4 (beats/min)$^2$. Regarding the effects of drug administration, there was a 38.1% decrease in supine HF power with Metoprolol administration, and a 99.3% decrease in supine HF power with Atropine administration. Thus, compared to the supine state with no drug administration, there was a modest decrease in HF power with Metoprolol and a further decrease to near abolition with Atropine. (Table 2)

Changes in the LF:HF ratio

In the supine position with no drug administration the LF:HF ratio was 1.04. This value rose to 2.16 during cardiovascular stress. Regarding the effects of drug administration, there was a 181.5% increase in the LF:HF ratio during cardiovascular
stress after Metoprolol administration and there was a 59.7% reduction after Atropine administration when compared to the stress natural condition. Thus, compared to the stressed state with no drug administration, there was a large increase in the LF:HF ratio after Metoprolol most likely caused by the onset of an episode of autonomic dysreflexia and a modest reduction in the LF:HF ratio after Atropine administration.

(Table 2)

Table 2-Absolute Values of Power Spectral Analysis of Heart Rate Variability for JM

<table>
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<tr>
<th></th>
<th>HF (bt/min)^2</th>
<th>LF (bt/min)^2</th>
<th>LF:HF</th>
<th>HR (bt/min)</th>
<th>MAP (mniHg)</th>
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<td>105.91</td>
<td>1.04</td>
<td>53.20</td>
<td>90.00</td>
</tr>
<tr>
<td>Stress</td>
<td>16.18</td>
<td>35.01</td>
<td>2.16</td>
<td>66.35</td>
<td>79.30</td>
</tr>
<tr>
<td>Metoprolol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>62.77</td>
<td>372.86</td>
<td>5.94</td>
<td>52.86</td>
<td>93.33</td>
</tr>
<tr>
<td>Stress</td>
<td>41.17</td>
<td>250.37</td>
<td>6.08</td>
<td>60.61</td>
<td>82.00</td>
</tr>
<tr>
<td>Metoprolol + Atropine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>0.69</td>
<td>0.61</td>
<td>0.88</td>
<td>97.25</td>
<td>85.33</td>
</tr>
<tr>
<td>Stress</td>
<td>0.80</td>
<td>0.70</td>
<td>0.87</td>
<td>96.11</td>
<td>85.33</td>
</tr>
</tbody>
</table>

Participant Three – BA

BA is a forty-six year old male with a C5 incomplete ASIA C spinal cord injury. BA sustained his injury while playing hockey in 1988 and is therefore 20 years post-injury.

Changes in High Frequency Power

In the supine position with no drug administration the HF power was 185.82 (beats/min)^2. Regarding the effects of drug administration, there was a 59.6% decrease in supine HF power with Metoprolol administration, and a 98.2% decrease in supine HF power with Atropine administration. Thus, compared to the supine state with no drug administration, there was a modest reduction in HF power with Metoprolol and a near abolishment of HF power with Atropine. (Table 3)
Changes in the LF:HF ratio

In the supine position with no drug administration the LF:HF ratio was 0.54. This value rose to 2.64 during cardiovascular stress. Regarding the effects of drug administration, Metoprolol caused a 74.2% reduction in the LF:HF ratio during cardiovascular stress and with the addition of Atropine there was a further reduction of 10.6%. Thus, compared to the stressed state with no drug administration, there was a large reduction in the LF:HF ratio with Metoprolol and a further modest reduction after Atropine. (Table 3).

Table 3-Absolute Values of Power Spectral Analysis of Heart Rate Variability for BA

<table>
<thead>
<tr>
<th></th>
<th>HF (bt/min)²</th>
<th>LF (bt/min)²</th>
<th>LF:HF</th>
<th>HR (bt/min)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>185.82</td>
<td>99.63</td>
<td>0.54</td>
<td>51.07</td>
<td>75.33</td>
</tr>
<tr>
<td>Stress</td>
<td>42.39</td>
<td>111.72</td>
<td>2.64</td>
<td>55.50</td>
<td>74.67</td>
</tr>
<tr>
<td>Metoprolol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>75.05</td>
<td>306.95</td>
<td>4.09</td>
<td>46.45</td>
<td>87.33</td>
</tr>
<tr>
<td>Stress</td>
<td>76.43</td>
<td>52.35</td>
<td>0.68</td>
<td>52.20</td>
<td>92.67</td>
</tr>
<tr>
<td>Metoprolol + Atropine</td>
<td>3.29</td>
<td>4.84</td>
<td>1.47</td>
<td>61.39</td>
<td>84.67</td>
</tr>
<tr>
<td>Stress</td>
<td>3.71</td>
<td>1.49</td>
<td>0.40</td>
<td>62.28</td>
<td>87.33</td>
</tr>
</tbody>
</table>

Participant Four - GC

GC is a sixty-one year old male with a C7 incomplete ASIA B spinal cord injury. GC sustained his injury after a brick fell on his head in 1987 and is therefore 21 years post-injury.

Changes in High Frequency Power

In the supine position with no drug administration the HF power was 50.4 (beats/min)². Regarding the effects of drug administration, there was a 212.2% increase in supine HF power with Metoprolol administration, and a 99.1% decrease in supine HF power with Atropine administration. Thus, compared to the supine state
with no drug administration, there was a large increase in HF power after Metoprolol and a near abolition of HF power with Atropine. (Table 4)

Changes in the LF:HF ratio

In the supine position with no drug administration the LF:HF ratio was 6.51. This value fell to 4.82 during cardiovascular stress. Regarding the effects of drug administration, Metoprolol caused a 38.9% reduction in the LF:HF ratio during cardiovascular stress and the addition of Atropine caused a further reduction of 35.8%. Thus, compared to the stressed state with no drug administration, there was a large reduction in the LF:HF ratio after Metoprolol and a further large reduction with Atropine. (Table 4)

Table 4 - Absolute Values of Power Spectral Analysis of Heart Rate Variability for GC

<table>
<thead>
<tr>
<th></th>
<th>HF (bt/min$^2$)</th>
<th>LF (bt/min$^2$)</th>
<th>LF:HF</th>
<th>HR (bt/min)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>50.45</td>
<td>328.27</td>
<td>6.51</td>
<td>64.00</td>
<td>96.00</td>
</tr>
<tr>
<td>Stress</td>
<td>56.00</td>
<td>269.79</td>
<td>4.82</td>
<td>64.69</td>
<td>94.00</td>
</tr>
<tr>
<td>Metoprolol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>157.51</td>
<td>812.36</td>
<td>5.16</td>
<td>55.49</td>
<td>86.67</td>
</tr>
<tr>
<td>Stress</td>
<td>83.79</td>
<td>246.54</td>
<td>2.94</td>
<td>58.98</td>
<td>92.67</td>
</tr>
<tr>
<td>Metoprolol + Atropine</td>
<td>0.44</td>
<td>0.45</td>
<td>1.03</td>
<td>81.49</td>
<td>92.67</td>
</tr>
</tbody>
</table>

Participant Five – JL

JL is a thirty-five year old male with a C4 incomplete ASIA C spinal cord injury. JL sustained his injury in 1997 after falling off of a roof and is therefore 11 years post-injury. JL experienced an episode of autonomic dysreflexia during this portion of the data collection. His data is being reported in this case study section but was not included in the group analysis in the following section as his data may have been compromised from the autonomic dysreflexia.
Changes in High Frequency Power

In the supine position with no drug administration the HF power was 1096.95 (beats/min)². Regarding the effects of drug administration, there was a 44.7% decrease in supine HF power with Metoprolol administration, and a 99.9% decrease in supine HF power with Atropine administration. Thus, compared to the supine state with no drug administration, there was a modest decrease in HF power with Metoprolol and a near abolition of HF power with Atropine. (Table 5)

Changes in the LF:HF ratio

In the supine position with no drug administration the LF:HF ratio was 1.43. This value rose to 3.11 during cardiovascular stress. Regarding the effects of drug administration, Metoprolol caused a 30.2% increase in the LF:HF ratio during cardiovascular stress and the addition of Atropine caused a reduction of 69.3%. Thus, compared to the stressed state with no drug administration, there was a modest increase in the LF:HF ratio with Metoprolol and a large reduction with Atropine.

(Table 5)

Table 5 - Absolute Values of Power Spectral Analysis of Heart Rate Variability for JL

<table>
<thead>
<tr>
<th></th>
<th>HF (bt/min)²</th>
<th>LF (bt/min)²</th>
<th>LF:HF</th>
<th>HR (bt/min)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natural</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>1096.95</td>
<td>1571.20</td>
<td>1.43</td>
<td>64.36</td>
<td>86.67</td>
</tr>
<tr>
<td>Stress</td>
<td>42.10</td>
<td>130.83</td>
<td>3.11</td>
<td>81.68</td>
<td>78.67</td>
</tr>
<tr>
<td><strong>Metoprolol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>606.28</td>
<td>1288.01</td>
<td>2.12</td>
<td>61.65</td>
<td>82.67</td>
</tr>
<tr>
<td>Stress</td>
<td>17.87</td>
<td>72.37</td>
<td>4.05</td>
<td>80.84</td>
<td>84.00</td>
</tr>
<tr>
<td><strong>Metoprolol + Atropine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>0.69</td>
<td>5.05</td>
<td>7.31</td>
<td>92.26</td>
<td>99.33</td>
</tr>
<tr>
<td>Stress</td>
<td>0.39</td>
<td>0.37</td>
<td>0.95</td>
<td>100.85</td>
<td>77.33</td>
</tr>
</tbody>
</table>
2.3.1.2 Spinal Cord Injury VS. Able-bodied

The data obtained from participants #2 and #5 (JM and JL respectively) was not included in this portion of the analysis as they both experienced episodes of autonomic dysreflexia during the recording and therefore their data was likely confounded by the large sympathetic outflow associated with this condition. JL’s blood pressure rose to 135/90 during his episode and JM’s blood pressure rose to 145/100 during his episode.

Participant Characteristics

There were no significant differences observed between the SCI and able-bodied groups for any of the anthropometric measures obtained.

Table 6 – Participant Characteristics for SCI and Able-Bodied Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SCI</th>
<th>Able-Bodied</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.6 ± 13.4 years</td>
<td>33.0 ± 7.8 years</td>
<td>0.372</td>
</tr>
<tr>
<td>Sex</td>
<td>5 male</td>
<td>3 males, 1 female</td>
<td>n/a</td>
</tr>
<tr>
<td>Weight</td>
<td>70.8 ± 15.2 kg</td>
<td>83.0 ± 18.9 kg</td>
<td>0.266</td>
</tr>
<tr>
<td>Height</td>
<td>174.6 ± 10.8 cm</td>
<td>176.3 ± 9.8 cm</td>
<td>0.500</td>
</tr>
<tr>
<td>Years Post Injury</td>
<td>13.4 ±13.4 years</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>ASIA score</td>
<td>2 ASIA B, 2 ASIA C &amp; 1 ASIA D</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Level of Injury</td>
<td>1 C4, 3 C5 &amp; 1 C7</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Heart Rate Variability Results

Changes in High Frequency Power

The HF powers were normalized to the values obtained during the supine condition in the absence of drug administration. Regarding the effects of drug administration, Metoprolol caused a 61.9 ± 138.2 % increase in supine HF power, and Atropine caused a 99.1 ± 0.8% decrease in supine HF power. There was no main effect for condition on HF power (p=0.15; Figure 2). However, a t-test showed that the 99.1% decrease in supine HF power with Atropine was statistically significant (p=0.00002).
It is also important to note that all 3 participants experienced decreases in supine HF power with Atropine administration, compared to the supine natural state.

**Changes in LF:HF ratio**

The LF:HF ratios were normalized to the values obtained during cardiovascular stress in the absence of drug administration. The administration of Metoprolol caused an average reduction of 61.8 ± 19.8% during cardiovascular stress (stress without Metoprolol compared to stress with Metoprolol). Subsequently, the administration of Atropine caused a further decrease of 18.6 ± 5.8%. There was a main effect for condition on the LF:HF ratio (p=0.004; Figure 3 & Appendix 8). A Tukey post-hoc test revealed that there was a significant difference between the stress natural and stress Metoprolol conditions (p=0.004) and between the stress natural and stress with Metoprolol and Atropine (p=0.002). It also revealed that there was no significant difference between the stress with Metoprolol condition and the stress with Metoprolol and Atropine condition (p=0.16) (Figure 3). It is also important to note that all 3 participants experienced decreases in LF:HF ratio with Metoprolol administration, compared to the cardiovascular stress natural state.

Tables 8 & 9 and Figures 4 & 5 compare the percentage change in measures of HRV with drug administration between the able-bodied and SCI participants. There was no significant difference between the changes in HF power from the supine natural condition to supine after Atropine administration between the SCI group and the able-bodied group. There was also no significant difference between the change in LF:HF ratio from cardiovascular stress without drug administration to cardiovascular stress after Metoprolol administration between the SCI group and the able-bodied group.
Table 7 – Mean Heart Rate Variability Results for the Able-Bodied Group

<table>
<thead>
<tr>
<th>Natural</th>
<th>Supine</th>
<th>Stress</th>
<th>( \text{HF (bt/min)}^2 )</th>
<th>( \text{LF (bt/min)}^2 )</th>
<th>( \text{LF:HF} )</th>
<th>HR (bt/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supine</td>
<td>14031.7</td>
<td>56801.3</td>
<td>2.11</td>
<td>41.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress</td>
<td>694.1</td>
<td>593.9</td>
<td>1.80</td>
<td>51.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metoprolol +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>8.0</td>
<td>7.8</td>
<td>2.57</td>
<td>83.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress</td>
<td>5.7</td>
<td>24.7</td>
<td>6.90</td>
<td>85.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8 – Change in High Frequency Power from Supine Natural to Supine after Atropine Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Supine</th>
<th>Supine Metoprolol and Atropine (beats/min²)</th>
<th>Percentage Change</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI (3 individuals)</td>
<td>100 ± 0</td>
<td>0.908 ± 0.8</td>
<td>-99.1 ± 0.8%</td>
<td>0.49</td>
</tr>
<tr>
<td>Able-Bodied (4 individuals)</td>
<td>100 ± 0</td>
<td>3.982 ± 7.0</td>
<td>-96.0 ± 7.0%</td>
<td></td>
</tr>
</tbody>
</table>

Table 9 – Change in LF:HF Ratio from Cardiovascular Stress in the Natural State to Stress after Metoprolol Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Stress</th>
<th>Stress with Metoprolol</th>
<th>Percentage Change</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI (3 individuals)</td>
<td>100 ± 0</td>
<td>38.24 ± 19.8</td>
<td>-61.8 ± 19.8%</td>
<td>0.10</td>
</tr>
<tr>
<td>Able-Bodied (4 individuals)</td>
<td>100 ± 0</td>
<td>70.4 ± 59.8</td>
<td>-29.6 ± 21.5%</td>
<td></td>
</tr>
</tbody>
</table>

LF:HF ratios were compared between the SCI group and the able-bodied group during the supine natural state to determine if there was maintenance in the LF:HF ratio after spinal cord injury. There was no significant difference between the values (p=0.66).

2.3.2 Autonomic Measures as determined by Heart Rate Variability vs. Autonomic Measures as determined by Sympathetic Skin Response

There were no significant correlations observed between sympathetic predominance as determined by HRV and sympathetic function as determined by SSR. In other words, there were no significant correlations between baseline LF:HF and SSR scores (total SSR, total SSR after wrist stimulation or total SSR after ankle stimulation;
There was also no significant correlations between the percentage change in LF:HF from supine to stress in the natural state and SSR scores (total SSR, total SSR after wrist stimulation or total SSR after ankle stimulation; p>0.05).

**Table 10 – Sympathetic Skin Response Scores for Participants with Spinal Cord Injury**

<table>
<thead>
<tr>
<th>SCI</th>
<th>Total SSRs</th>
<th>Total SSRs after Wrist Stimulation</th>
<th>Temperature of Hand During Testing (°C)</th>
<th>Total SSRs after Ankle Stimulation</th>
<th>Temperature of Foot During Testing (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZ</td>
<td>0</td>
<td>0</td>
<td>32.8</td>
<td>0</td>
<td>29.9</td>
</tr>
<tr>
<td>JM</td>
<td>69</td>
<td>65</td>
<td>35.0</td>
<td>4</td>
<td>33.0</td>
</tr>
<tr>
<td>BA</td>
<td>0</td>
<td>0</td>
<td>31.5</td>
<td>0</td>
<td>30.5</td>
</tr>
<tr>
<td>GC</td>
<td>0</td>
<td>0</td>
<td>31.3</td>
<td>0</td>
<td>30.5</td>
</tr>
<tr>
<td>JL</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**Table 11 - Sympathetic Skin Response Scores for Able-Bodied Participants**

<table>
<thead>
<tr>
<th>Able-Bodied</th>
<th>Total SSRs</th>
<th>Total SSRs after Wrist Stimulation</th>
<th>Temperature of Hand During Testing (°C)</th>
<th>Total SSRs after Ankle Stimulation</th>
<th>Temperature of Foot During Testing (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>148</td>
<td>75</td>
<td>32.0</td>
<td>73</td>
<td>31.2</td>
</tr>
<tr>
<td>DD</td>
<td>156</td>
<td>79</td>
<td>32.0</td>
<td>77</td>
<td>30.1</td>
</tr>
<tr>
<td>GI</td>
<td>150</td>
<td>76</td>
<td>30.5</td>
<td>74</td>
<td>30.3</td>
</tr>
<tr>
<td>TW</td>
<td>146</td>
<td>80</td>
<td>32.9</td>
<td>66</td>
<td>30.1</td>
</tr>
</tbody>
</table>

2.3.3 Autonomic Measures as determined by Heart Rate Variability vs. Autonomic Dysfunctions

2.3.3.1 Orthostatic Intolerance

There were no significant correlations observed between orthostatic intolerance and sympathetic predominance as determined by HRV. In other words, there were no significant correlations between baseline LF:HF and orthostatic intolerance measures (baseline MAP or % change in MAP from supine to stress; p>0.05). There were also no significant correlations between the percentage change in LF:HF from supine to stress in the natural state and orthostatic intolerance measures (baseline MAP or percentage change in MAP from supine to stress; p>0.05).
Table 12-Mean Arterial Pressure Values for Individuals with Spinal Cord Injury

<table>
<thead>
<tr>
<th>SCI</th>
<th>Baseline MAP (mmHg)</th>
<th>% Change MAP from supine to stress (no drug administration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZ</td>
<td>72.67</td>
<td>-1.84</td>
</tr>
<tr>
<td>JM</td>
<td>90.00</td>
<td>-11.89</td>
</tr>
<tr>
<td>BA</td>
<td>75.33</td>
<td>-0.88</td>
</tr>
<tr>
<td>GC</td>
<td>96.00</td>
<td>-2.08</td>
</tr>
<tr>
<td>JL</td>
<td>86.67</td>
<td>-9.23</td>
</tr>
</tbody>
</table>

2.3.3.2 Autonomic Dysreflexia

There were no significant correlations observed between self-reported autonomic dysreflexia and sympathetic predominance as determined by HRV. In other words, there were no significant correlations between baseline LF:HF and autonomic dysreflexia questionnaire scores (acute frequency, acute number of symptoms, acute severity of symptoms, chronic frequency, chronic number of symptoms or chronic severity of symptoms; p>0.05). There were also no significant correlations between the percentage change in LF:HF from supine to stress in the natural state and autonomic dysreflexia scores (acute frequency, acute number of symptoms, acute severity of symptoms, chronic frequency, chronic number of symptoms or chronic severity of symptoms; p>0.05).

Table 13. Autonomic Dysreflexia Questionnaire Scores for the SCI group

<table>
<thead>
<tr>
<th></th>
<th>Acute Frequency</th>
<th>Acute # of Symptoms</th>
<th>Acute Severity of Symptoms</th>
<th>Chronic Frequency</th>
<th>Chronic # of Symptoms</th>
<th>Chronic Severity of Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZ</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JM</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BA</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GC</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>JL</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
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2.4 Discussion

2.4.1 Validation of Power Spectral Analysis of Heart Rate Variability after Spinal Cord Injury

The mean high frequency power for individuals with spinal cord injury showed a non-significant increase after the administration of a beta sympathetic blocker; Metoprolol (P=0.48). In contrast, upon Atropine administration a Student’s t-test determined there was a very large and significant decrease in mean HF power of 99.1% (p<0.001). This would suggest that when the parasympathetic nervous system is blocked by Atropine that the mean HF power is nearly abolished and is therefore largely representative of parasympathetic outflow to the heart. There was also no significant difference between the percentage decrease in HF power after Atropine administration between the SCI and the able-bodied participants (99.1 ± 0.8% and 96.0 ± 7.0%, respectively)

Regarding the LF:HF ratio, a one-way repeated measures ANOVA revealed a main effect for condition when comparing the mean LF:HF ratio during cardiovascular stress in the natural condition to the mean LF:HF ratio during stress after drug administration. Post-hoc analysis showed a significant difference between the LF:HF ratio during stress in the natural state compared to the LF:HF ratio during stress following Metoprolol administration. Subsequent administration of Atropine did not result in a further decrease in the LF:HF ratio during stress. This strongly suggests that the LF:HF ratio is highly representative of cardiac sympathetic outflow. There was no significant difference observed in the change between the LF:HF ratio from stress to stress with Metoprolol between the SCI and the able-bodied population. Despite the impaired connection between the sympathetic fibers in the thoracic spinal cord and the higher brain centres after a cervical spinal cord injury the LF:HF ratio
may still be used as an index of sympathovagal balance. The large decrease observed in the HF power following Atropine administration suggests that power spectral analysis of heart rate variability is valid in the incomplete tetraplegic population.

This study coincides well with the study by Pomeranz and colleagues (1985) that followed a very similar protocol to the present study except in the able-bodied population. In the Pomeranz study there was a 92% decrease observed in supine HF power after Atropine administration. This is very similar to the 96% decrease observed in the able-bodied group and the 99.1% decrease observed in the SCI group in the current study. There was a 73% decrease in LF power observed in the standing position after Propranolol (another beta-blocker) administration in the Pomeranz study. Although, the LF power alone was not analyzed in the above study, there was a 61.8% decrease in the SCI group and an approximately 30% decrease in the able-bodied group in the LF:HF ratio after Metoprolol administration during cardiovascular stress. The smaller magnitude of effect of the beta blockade on the LF:HF ratio in the able-bodied individuals in this study compared to the Pomeranz study may be attributed to a number of different factors.

Firstly, the two studies measured two different although presumably similar measurements; LF power vs. LF:HF ratio. As was shown in both studies the LF power and the LF:HF ratio are both representative of sympathetic outflow. It is thought however that LF:HF is a more accurate representation of sympathetic outflow. In fact, results from the Pomeranz study showed that LF power corresponds to both sympathetic and parasympathetic outflow, in roughly equal proportions, which is why the LF:HF ratio was used as the measure of sympathovagal balance in the present study. For the sake of comparison LF power (during stress) was reduced by an
average of 49.9% with Metoprolol administration in the current study. Secondly, in the Pomeranz study the beta-blocker was administered according to weight (0.15mg/kg), whereas in the current study each participant was given only enough Metoprolol to lower their heart rate to 40-45 beats/min. This varied between all of the able-bodied participants. For the sake of safety the present protocol gave 5mg doses of Metoprolol until the heart rate decreased to 40-45 beats/min or until participants had received the maximum allowed dosage of 15mg of Metoprolol. It was felt that a larger amount of Metoprolol would have been unsafe. None of the able-bodied participants in the current study were given all three doses of Metoprolol, as their heart rates would have decreased past the pre-determined limit of 40 beats/min. It is possible that the able-bodied participants in this study were therefore not fully blocked by the Metoprolol. This could explain the smaller effect of beta-blockade on the measure of sympathetic predominance in the current study compared to the Pomeranz study in able-bodied participants. In contrast, all of the SCI participants required all three 5mg doses of Metoprolol and therefore were probably almost completely blocked possibly attributing to the greater percent decrease in LF:HF in this group. Pomeranz and colleagues were able to validate power spectral analysis in the able-bodied population and with similar percent changes in the current study it is suggest that this is a valid measure in the SCI population as well.

Grimm et al. (1997), Wang et al. (2000) and Ditor et al. (2005a, 2005c) showed that both the LF and HF powers exist after spinal cord injury and that they center around similar frequencies as has been shown in the able-bodied population (low frequency- 0.1 Hz and high frequency - 0.25 Hz). Grimm et al. also showed that there is a maintenance of the LF:HF ratio after spinal cord injury. The current study showed similar findings in all of these conclusions. A Students t-test revealed there was no
significant difference between resting LF:HF in individuals with SCI and able-bodied individuals. This suggests that similarly to the Grimm et al. (1997) study there was a maintenance of the LF:HF ratio after SCI.

**2.4.2 Autonomic Measures as determined by Heart Rate Variability vs. Autonomic Measures as determined by Sympathetic Skin Response**

There were no significant relationships observed between sympathetic predominance as determined by HRV and sympathetic function as determined by SSR. This would suggest that when assessing an individual's autonomic damage after a spinal cord injury, it would be necessary to use both of these measures, at the least, to get an accurate idea of their complete autonomic damage. It was determined that these two measures offer different information and therefore are not redundant and interchangeable. The sympathetic skin response test measures the sympathetic outflow to sweat glands through the sympathetic cholinergic nervous system (or at least the response of the sweat glands to this outflow), whereas heart rate variability provides an estimate of parasympathetic and sympathetic outflow to the heart and therefore it is reasonable that the two tests should not be redundant or interchangeable. Further, these findings support the need for separate autonomic testing for all of the various systems affected by SCI (bladder, bowel, and sexual function for example).

**2.4.3 Autonomic Measures as determined by Heart Rate Variability vs. Autonomic Dysfunctions**

There were no significant relationships observed between sympathetic predominance as measured by HRV and measures of autonomic dysfunction. This suggests that autonomic outflow to the heart is not directly related to the frequency or severity of autonomic dysreflexia nor to the susceptibility to orthostatic intolerance.
and thus HRV measures may not be appropriate to use as an index of autonomic dysfunctions such as autonomic dysreflexia and orthostatic intolerance.

2.4.4 Relevance to the Current Literature

The findings of this study are extremely important for validating studies using HRV measures in the SCI population to date. Ditor et al. (2005a, 2005c), showed that the LF:HF ratio adapts in a similar way as shown in the able-bodied population after exercise training. It was shown that resting LF:HF was decreased after long-term aerobic exercise training. These authors concluded that the exercise training program resulted in a shift toward vagal predominance in their SCI participants despite their autonomic impairment. With the validation of HRV in the SCI population, this statement is more appropriate to make. Koh et al. (1994) provide strong evidence that the parasympathetic nervous system contributes in an important way to the HF power. The current study confirmed that indeed there is a strong contribution of parasympathetic outflow in the HF power and also quantified this contribution and showed it to be equal to the contribution in the able-bodied population. Mukai and colleagues (1995) measured the LF power during graded head-up tilt in the able-bodied population. The current study coincides with the results of the Mukai study showing that there is an increase in sympathetic activity during tilt in both the able-bodied and the SCI population.

2.4.5 Clinical Relevance

The results of this study are important for the spinal cord injured population for a number of different reasons. Primarily, as a valid measure of autonomic outflow to the heart after spinal cord injury, power spectral analysis of heart rate variability may be used in conjunction with the ASIA impairment scale, as well as other
autonomic outflow measures such as SSR to assess the entire degree of an individual's impairment following spinal cord injury. As a valid measure of cardiac autonomic outflow, HRV could be used to assist in the classification of the autonomic completeness of an injury as well. Based on the location on the spinal cord of sympathetic innervations to the heart (ie. T1-T4), this measure could help to determine a general range of autonomic level and severity of an injury vs. solely the somatic level and severity as is classified using the ASIA impairment scale. This is also helpful since the ASIA scale does not measure motor function in the thoracic myotomes but rather infers it from the corresponding sensory function.

Cardiac disease has become the number one cause of death after spinal cord injury. A tool such as power spectral analysis of heart rate variability may also be useful in the SCI population to determine the potential risk of cardiac disease. Guzzetti and colleagues (1988) reported that individuals with hypertension are characterized by greater LF:HF ratios during supine rest. This may also be true in the SCI population and therefore power spectral analysis of heart rate variability may be a useful tool in predicting hypertension after spinal cord injury. An increase LF:HF ratio has also been linked to an increased risk of cardiac mortality, as Lanza et al. (1997) observed a higher LF:HF ratio in individuals with cardiac disease. This may also be the case in individuals with spinal cord injury and therefore HRV would be a valuable tool to predict cardiac disease and risk of mortality. Power spectral analysis of heart rate variability would also be a very useful tool to determine improvements made to the autonomic nervous system following various interventions. If individuals were to determine their autonomic outflow pre- and post-drug, exercise or even neuroregenerative interventions, improvements in autonomic function and outflow could be measured.
It was important to compare results between HRV and SSR to determine if these assessment tools were redundant and therefore interchangeable or whether one must be used in conjunction with the other to fully understand the autonomic integrity of an individual with a spinal cord injury. Comparing autonomic outflow measured by HRV to autonomic dysfunctions such as autonomic dysreflexia and orthostatic intolerance is important because these dysfunctions are very common after spinal cord injury and to predetermine the frequency and severity in advance would be very beneficial. There was however no significant relationship between the HRV measures and the measures of autonomic dysfunctions found in this study.

2.4.6 Limitations

Two of the participants with spinal cord injury were excluded from the group analysis as they both experienced episodes of autonomic dysreflexia during the drug infusion protocol. It is not entirely clear when the episodes began, however, both of these participants showed unexpectedly large values in their LF:HF ratios during stress after Metoprolol administration. Since it was shown that LF:HF is highly representative of sympathetic outflow it would be appropriate to say that during an episode of autonomic dysreflexia, as characterized by a large sympathetic discharge, it would not be unusual to have a larger than expected LF:HF ratio. These individuals both showed other symptoms of autonomic dysreflexia, such as a headache and increased blood pressure during the stress with Metoprolol condition and were therefore thought to have been experiencing autonomic dysreflexia during this condition, if not before.

As mentioned before, a potential limitation to this study was that the able-bodied population was possibly not completely blocked with Metoprolol. However, this study
was looking to validate this measure in the SCI population and each of the individuals with SCI were given all 15mg of Metoprolol and therefore received equal amounts of Metoprolol and each received the maximum dose. Another possible limitation was that the Metoprolol was not administered based on weight and therefore a larger individual would have received a relatively smaller amount of drug compared to their body size. This did not seem to make a difference in the SCI population as each of the participants showed moderate to large decreases in LF:HF ratio after Metoprolol administration.

The Pomeranz et al. (1985) study pharmacologically validated HRV in the able-bodied population. In their study, they had the participants come to the lab twice and reversed the order of the drugs. This was not possible to do in the current study, due to time constraints. Koh and colleagues (1994) showed the pharmacological effects of Atropine on HRV measures in the SCI population, so it was felt that a second day with reversed drug order was not necessary for the current study and would only provide redundant information to that of the Koh study (1994). The current study involved drug administration on one day administering Metoprolol first allowing for new information regarding the effects of a beta-blocker alone on HRV measures.

Autonomic dysreflexia was measured by a self-reported questionnaire. There is always potential for symptoms to be over and under reported in this case. There is however no validated questionnaire for self-reported autonomic dysreflexia currently available. This condition is not something that occurs on a regular schedule and is therefore not medically recorded for the majority of individuals with spinal cord injury. The International Collaboration On Repair Discoveries (ICORD) laboratory in British Columbia induces autonomic dysreflexia during vibrostimulation in males
with spinal cord injury in order to measure the number of symptoms and the severity of autonomic dysreflexia. The actual induction and measurement of autonomic dysreflexia was beyond the scope of the current study and would have entailed a high degree of risk. Moreover, inducing autonomic dysreflexia would have provided information on the severity of autonomic dysreflexia during a specific stimulus, but would not have provided information on the frequency of autonomic dysreflexia in everyday life, which is a more practical consideration. Therefore a self-reported questionnaire was used instead.

Measuring the difference in MAP from supine to stress may not have been an accurate representation of orthostatic intolerance, as the individuals were put into a situation of further cardiovascular stress, which also included a cold pressor response test and isometric jaw contraction. In the able-bodied population the cold pressor test has been associated with increased HR and blood pressure. However, after spinal cord injury it has been shown that there is a blunted cold pressor response and therefore the MAP may not have been increased enough to effect the results (Catz et al. 2008).

2.4.7 Future Directions

There have already been many studies using power spectral analysis of heart rate variability in the spinal cord injured population. The validation of this measurement in the SCI population only further opens the door to future research in the SCI population using this assessment tool. It is important to conduct a larger study than the current one to truly validate this measure and perhaps to develop an actual classification system for cardiac autonomic function similar to the ASIA scale for motor and sensory function. A greater number of participants would only further validate this measure with a larger statistical power. All of the SCI participants in the
current study had ASIA classifications ranging from ASIA B-D. The current study is therefore valid for individuals with somatic incomplete SCI. A future study should attempt to validate this study in individuals with somatic complete spinal cord injuries (ASIA A).

Similar to the studies by Ditor et al. (2005a, 2005c) there is great potential for future studies to use this as measurement of autonomic improvement after exercise interventions, drug therapies or even different neuroregenerative techniques. As mentioned earlier, there are many studies in the able-bodied population regarding cardiac disease being linked to a higher LF:HF ratio. Future studies should determine if this is also true in the spinal cord injured population.

2.5 Conclusion

The current study shows that power spectral analysis of heart rate variability is a valid measure of cardiac autonomic control in the spinal cord injured population, specifically somatic incomplete tetraplegics. Heart rate variability measures and sympathetic skin response measures provide different information regarding the autonomic nervous system and therefore are not redundant and interchangeable. Autonomic measures as determined by HRV do not provide information directly related to autonomic dysfunctions such as autonomic dysreflexia or orthostatic intolerance and therefore cannot be used to predict the severity of these dysfunctions.
2.6 References


The Effect of Drug Administration on HF Power in individuals with SCI

![Graph showing the effect of drug administration on HF power.](image)

**Figure 1.** The Effect of Drug Administration on HF Power in Individuals with SCI. Values are expressed as means ± SD and normalized to the HF power obtained during supine rest in the absence of drug administration. * indicates a significant difference in HF power compared to that in the supine condition without drug administration (P<0.05)
The Effect of Drug Administration on the LF:HF Ratio in individuals with SCI

Figure 2. The Effect of Drug Administration on LF:HF Ratio in Individuals with SCI. Values are expressed as means ± SD and expressed normalized to the cardiovascular stress condition in the absence of drug administration. * indicates a significant difference compared to the LF:HF ratio obtained during the cardiovascular stress condition without drug administration. (P<0.05)
The Effect of Drug Administration on HF power:
SCI vs. Able-bodied

Figure 3. The Effect of Drug Administration on HF Power: SCI vs. Able-bodied. A comparison between SCI and able-bodied participants for the mean percentage change difference in high frequency power from the supine condition without drugs to the supine condition after Atropine administration.
Figure 4. The Effect of Drug Administration on LF:HF ratio: SCI vs. Able-bodied. A comparison between SCI and able-bodied participants for the mean percentage change difference in LF:HF ratio from the stress condition without drugs to the stress condition after Metoprolol administration.
Chapter 3 - Appendices
Appendix A - The ASIA neurological exam to assess motor and sensory impairment.
Appendix B – A) An ECG recording showing RR-intervals, B) A tachogram showing RR-intervals plotted against beats C) A power spectrum showing the low frequency power centered around approximately 0.1Hz, and high frequency power centered around approximately 0.25 Hz.
Appendix C – A sympathetic skin response recording of a 37 year old male with a T5-T6 complete spinal cord injury (Claydon et al., 2006)

37 y.o. man T5-6 ASIA A SCI

R arm

L arm

R foot

L foot

Stimulus Artifact

L Med. N. Stim
Appendix D – Telephone Script

LISA- Hello my name is Lisa Cotic. I am a Masters student at Brock University. I got your name and phone number from (Dr. Richard McMillan and Assunta Berardocce at Hotel Dieu Shaver Hospital or from Dave Ditor) because they felt that you would be a good candidate for a research study that we are conducting through Brock University. The purpose of the study is to understand the effects of SCI on the cardiovascular system. In particular we are interested in the effects of your injury on the control of your heart and heart rate. In general, we need to record your heart rate after giving you two different drugs. One drug will increase your heart rate to about 100-120 bts/min. and the other will decrease it to about 40-50 bts/min. We will also need to record your heart rate while you are lying down and while you are lying upright on a tilt table. Would you be interested in hearing more details about the study?

If NO: Thank you for your time. If you change your mind my contact number is (905)688-5550 ext. 5826.

If YES: There will be two testing sessions in total. The first will be at Brock University and the second will be located at a clinic in St. Catharines. In the first session, at Brock University, we will describe the study again in detail and have you fill out a questionnaire about your experience with autonomic dysreflexia. We will then conduct what is called a sympathetic skin response test. This procedure includes having electrodes placed on your hands, wrists, feet and ankles. The electrodes on your wrists and ankles will stimulate your nerves at these locations with ten mild pain-free electrical pulses. The electrodes on your hands and feet are recording electrodes which will simply record your skin’s response to the mild pain free stimulation. This first visit should last approximately one and half hours. On the second visit you will be asked to come to the Brock Heart Institute in St. Catharines. During this visit a nurse at the clinic will give you two different drugs. One drug, called Atropine, will increase your heart rate to about 100-120 bts/min. and the other, called Metoprolol, will decrease it to about 40-50 bts/min. During and after we give you these drugs we will monitor your heart rate while you are lying down and then while you are lying upright on a tilt table. These visits should last for approximately two and a half hours. Again, this study will help to increase our knowledge of cardiovascular control after spinal cord injury. Do you have any questions? Would you be interested in coming to the first visit for more information?

If NO: Ok well thank-you for your time and if you change your mind please feel free to contact me at (905) 688-5550 ext. 5826.

If YES: Age? Level of injury? Severity of injury? Date of injury? Asthma? Glaucoma? Smoking? Weight? Height? History of cardiovascular disease? When you come to Brock University on (insert date here) you will park in the visitor parking in front of the Arnie Lowenberger Residence. You will come to Welch Hall 143 where the first session will be held. Thank-you very much for your time and I look forward to meeting you on (insert date here).
Appendix E – Autonomic Dysreflexia Questionnaire

Frequency and Severity of Autonomic Dysreflexia

Please answer the following questionnaire to the best of your ability. If you have any questions please feel free to ask the investigator present.

Autonomic Dysreflexia

Definition: Autonomic dysreflexia is a reflex that can be triggered by various stimuli below the level of a spinal cord injury. Most commonly it is a full bladder or bowel that causes this condition, however, other unpleasant stimuli such as skin irritation can also trigger this reflex. In response to the unpleasant stimulus, there is a reflex constriction of the blood vessels below the level of injury, and as a result blood pressure can rise to dangerous levels.

The common symptoms of autonomic dysreflexia include:

- Pounding headache
- Sweating above the level of the injury
- Nasal congestion
- Nausea
- Flushed skin above the injury
- Cool pale skin below the level of the injury
- Elevation of blood pressure
- Goosebumps below the level of the injury
- Blurred vision
- Increased spasticity

Before today, did you know what autonomic dysreflexia was?
Yes or No (please circle one)
Within the first 6 months of your injury, how frequently did you experience autonomic dysreflexia?

1. **NEVER**: I NEVER experienced autonomic dysreflexia during the first 6 months

2. **RARELY**: Only a few times during the first 6 months

3. **OCCASIONALLY**: Approximately 1-3 times per month

4. **OFTEN**: Approximately 1-3 times per week

5. **QUITE FREQUENTLY**: More than 3 times per week

Refer to the list of symptoms associated with autonomic dysreflexia below. Please circle the symptoms that you would typically experience during the episodes of autonomic dysreflexia that you encountered within the first 6 months of your injury.

- Pounding headache
- Sweating above the level of the injury
- Nasal congestion
- Nausea
- Flushed skin above the injury
- Cool pale skin below the level of the injury
- Elevation of blood pressure
- Goosebumps below the level of the injury
- Blurred vision
- Increased spasticity
Did you experience headaches during episodes of autonomic dysreflexia within the first 6 months of your injury?

**YES** or **NO** (circle one)

If you answered YES above, please indicate how severe a typical headache would have been.

1. Very mild
2. Mild to moderate
3. Moderate
4. Moderate to intense
5. Intense

If you did not experience headaches during autonomic dysreflexia, what was the most common other symptom that you would have experienced?

__________________________________________

Please indicate how severe that symptom would have been.

1. Very mild
2. Mild to moderate
3. Moderate
4. Moderate to intense
5. Intense
Within the last 6 months, how frequently did you experience autonomic dysreflexia?

1  NEVer: I NEVER experienced autonomic dysreflexia during the last 6 months

4  RARELY: Only a few times during the first 6 months

5  OccasionAlly: Approximately 1-3 times per month

4  OfTen: Approximately 1-3 times per week

5  quItE FREquently: More than 3 times per week

Refer to the list of symptoms associated with autonomic dysreflexia below. Please circle the symptoms that you typically experience during the episodes of autonomic dysreflexia that you encountered within the last 6 months.

- Pounding headache
- Sweating above the level of the injury
- Nasal congestion
- Nausea
- Flushed skin above the injury
- Cool pale skin below the level of the injury
- Elevation of blood pressure
- Goosebumps below the level of the injury
- Blurred vision
- Increased spasticity
Did you experience headaches during episodes of autonomic dysreflexia within the last 6 months?

**YES** or **NO** (circle one)

If you answered YES above, please indicate how severe a typical headache would have been.

1. Very mild
2. Mild to moderate
3. Moderate
4. Moderate to intense
5. Intense

If you do not experience headaches during autonomic dysreflexia, what is the most common other symptom that you experience?

______________________________.

Please indicate how severe that symptom typically is.

1. Very mild
2. Mild to moderate
3. Moderate
4. Moderate to intense
5. Intense
Appendix F – Letter of Information and Consent Form

LETTER OF INVITATION

The Validation of Power Spectral Analysis of Heart Rate Variability in Individuals with Spinal Cord Injury

You are being invited to voluntarily participate in a research study led by Dr. David Ditor from the Department of Physical Education and Kinesiology at Brock University and Dr. Richard McMillan from the Hotel Dieu Shaver Hospital in St. Catharines, Ontario. The study is described in this Invitation Letter which is yours to keep. This letter contains information to help you decide whether or not to participate in this research study. It is important for you to understand why the study is being conducted and what it will involve. Please take the time to read this carefully and feel free to ask questions if anything is unclear or there are words or phrases you do not understand.

If you have any questions or concerns about this research, you may contact any of the following members of the research team listed below.

Dr. David Ditor       Brock University       (905)688-5550 ext. 5338       deditor@brocku.ca
Miss Lisa Cotie      Brock University       (905)688-5550 ext. 5826       lc06ya@brocku.ca

If you wish to speak to a third party who is not involved in the study please feel free to contact:
Dr. John Luce     Hotel Dieu Shaver Hospital (905)685-1381 ext. 4282
JohnThomasMD.Luce@hoteldieushaver.org

PURPOSE OF THE STUDY

The heart receives neural input that can cause either an increase or decrease in the heart rate. The nerves that increase heart rate are called sympathetic nerves and the nerves that decrease heart rate are called parasympathetic nerves. In the able-bodied population, a technique called power spectral analysis of heart rate variability can be used to determine the balance of sympathetic and parasympathetic outflow to the heart. Spinal cord injury causes an impairment of skeletal muscle control, but it also causes impairments to the neural control of the heart. Therefore, it would be very useful to use power spectral analysis of heart rate variability in individuals with spinal cord injury in order to determine how badly the neural control of the heart is damaged, and if it can improve with drug, exercise or even future nerve regeneration therapies. The purpose of this study is therefore, to investigate if power spectral analysis of heart rate variability is still a valid measure of neural control of the heart in individuals with spinal cord injury.

STUDY PROCEDURES

If you participate in this study you will be required to make 2 separate visits to meet with the investigators. The first visit will be at Brock University in the
Electromyographic Kinesiology Laboratory and the Human Factors Laboratory. The second visit will be at the Brock University Heart Institute.

During the first visit the study will be explained to you and you will have the opportunity to ask as many questions as you like. You will also be asked several questions about your medical history including questions about any previous cardiovascular disease, whether or not you smoke cigarettes, what medications you are currently taking, your current level of physical activity and, if you have ever had episodes of autonomic dysreflexia, you will be asked about the frequency and severity of these episodes. If you are eligible for the study and chose to participate, you will then be asked to sign a consent form. Once the consent form has been signed, the first testing session will begin. In this first session we will conduct a sympathetic skin response test. This test is also designed to test the sympathetic nerves in your body, and we will compare the results from this test to the results that we get from the power spectral analysis of heart rate variability test. In the sympathetic skin response test we will place a small stimulating electrode on your skin at the wrist and at the ankle. These electrodes will emit mild electrical stimulation of the nerves under your skin that you will not find painful. We will also place small recording electrodes on your palms and on the bottom of your feet. These electrodes will not emit any electrical stimulation; they will just record the effects of the electrical stimulation that was provided at the wrist and ankle. After finding the correct intensity of stimulation we will stimulate your nerves 10 times with at least 1 minute of rest between each stimulus.

During the second visit, you will be asked to lie on a table and a needle will be placed into the vein at your elbow. The needle will stay in place for the duration of the visit and it will be used to provide you with medication that will either increase or decrease your heart rate. You will also have a heart rate monitor placed around your chest underneath your clothes. After the needle and heart rate monitor are in place you will lie down and relax for approximately 20 minutes. We will then record your ECG signal for 10 minutes during which time you will continue to lie down and remain as quiet and still as possible. We will then continue to record your ECG signal for another 10 minutes after tilting the table to 40 degrees in the upright position. We will also have you place your hand up to the wrist in 10 degree Celsius water and clench your jaw. This is to provoke cardiovascular stress. You will be strapped onto the table to prevent you from slipping or falling off if necessary and your blood pressure will be taken with a cuff at your upper arm every time you are tilted on the table. You may feel lightheaded while the table is tilted and you will be immediately returned to the lying down position before the 10 minutes if you request. Following the ten minutes of tilt (if possible) the table will then be returned to the lying down position, and you will receive a dose of a drug called Metoprolol (15mg in 3x5mg doses) through the needle in your arm. This drug will cause a decrease in your heart rate, but should not cause any other discomfort and your heart rate and blood pressure will be monitored during this drug administration. After the drug has been given, you will rest for 5-10 minutes. We will then record your ECG signal again for 10 minutes as you are lying down, and then again for 10 minutes after the table is tilted to 40 degrees. You will then be returned to the lying down position and you will be given a dose of a drug called Atropine 0.02 mg/kg which will cause a slight rise in your heart rate, but should not cause any other discomfort. With both drugs your heart rate should be approximately 100-110 beats/minute. We will then record your ECG signal again for
10 minutes as you are lying down, and then again for 10 minutes after the table is tilted to 60 degrees. After this final recording, the table will be returned to the lying down position and the testing will be finished.

Below, is a summary of each visit:

**Day 1: (Brock University)**

Information and signed consent
Medical history, including frequency and severity of autonomic dysreflexia episodes
Sympathetic skin response testing

**Day 2: (Brock University Heart Institute)**

Insert needle, and put on heart rate monitor, then 20 min rest while lying down
10min ECG while lying down
10min ECG at 40° tilt
Return to lying down
Administer Metoprolol, wait 5-10min, 10min ECG lying down, 10min ECG at 40° tilt
Return to lying down
Administer Atropine, wait 5-10min, 10min ECG lying down, 10min ECG at 40° tilt

Please note: The ECG testing described above is not diagnostic and will not be used to determine if you have any cardiac abnormalities.

**ACCESSING STUDY FINDINGS**

At the completion of the study, you will have access to the study’s findings. You will receive a newsletter that summarizes the study findings and also be invited to an education session that discusses the overall study findings. If you choose, you can have your personal results mailed to you or discussed with you upon completion of the study.

**POTENTIAL RISKS AND DISCOMFORTS**

There are only small risks associated with the drug administration. First, there is a very small chance of infection with the insertion of the needle. However, this risk is extremely small as the administration of the drug will be performed by a qualified nurse, in the sterile setting of the Brock University Heart Institute. As mentioned, you will also experience an increase in heart rate when Atropine is administered and a decrease in heart rate when Metoprolol is administered. The changes in heart rate, however, will only be moderate and with both drugs given at the same time your resting heart rate will be approximately 100-110 beats/minute.

Aside from the increase in heart rate the common adverse reactions (1 to 10%) to Atropine are: headache, restlessness, insomnia, dizziness, tachycardia, dry skin, hot skin, light sensitivity. In addition, Atropine should not be used in someone with glaucoma or asthma. Also note that Atropine may cause urinary retention or constipation in some people, so pay special attention to your bladder and bowel routine the day after testing sessions.

Aside from the decrease in heart rate the known side effects of Metoprolol are: dizziness, lightheadedness, tiredness, drowsiness, upset stomach, diarrhea, shortness of breath, swelling of hands, feet ankles or lower legs, and fainting.

In addition, Metoprolol should not be used in people with: known hypersensitivity to Metoprolol and derivatives or other beta-blockers, sinus bradycardia, sick sinus syndrome, second and third degree A-V block, right ventricular failure secondary to pulmonary hypertension, overt heart failure, cardiogenic shock, severe peripheral arterial circulatory disorders, anesthesia with agents that produce myocardial
depression (eg., ether), untreated pheochromocytoma, asthma and other obstructive respiratory diseases.

However, the above side effects of Atropine and Metoprolol are rare and the dosages that we are using have been chosen to minimize these risks.

If you would like more information about Atropine and Metoprolol please ask either Lisa Cotie or Dr. David Ditor for more details.

There is also a risk that you will feel lightheaded and possibly faint during the 60 degree tilt on the tilt table. However, you may return to the lying down position, if you wish, as soon as you feel that you may faint. Any lightheadedness that you experience should quickly resolve after you return to the lying down position. Finally, there is a very small risk of discomfort and skin irritation during the sympathetic skin response test with the electrical stimulation of the nerves at your wrist and ankle. However, the stimulation will be very mild and should not produce any pain, and any skin irritation that you may experience should be limited to temporary redness at the stimulating electrode site.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY
There are no direct benefits for participation in this study other than a modest remuneration described below. However, the results of this study will help to develop power spectral analysis of heart rate variability as a valid measure of neural control of the heart in individuals with spinal cord injury. This would be an extremely helpful clinical measure as it will help to determine the extent of damage to cardiac nerves after SCI, and the improvements with future exercise, drug or even nerve regeneration interventions.

PAYMENT FOR PARTICIPATION
You will receive a remuneration of $50.00 once you have completed both of the testing sessions.

CONFIDENTIALITY
Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law.
All information will be confidential and will be kept in a locked filing cabinet in Dr. Ditor’s research office for 5 years. Access to this information will be granted only to the researchers and their research assistants. Your identity will never be revealed in any reports regarding this study. After 5 years, paper documents will be destroyed.

PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. You indicate your voluntary agreement to participate by signing the consent form that is part of this letter. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind, and your relationship with any of the physicians in the study will not be harmed. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you feel uncomfortable answering and still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so (e.g., if there are any safety concerns). Any data collected prior to a withdrawal will still be used for analysis purposes unless you wish it to be removed.
The Validation of Power Spectral Analysis of Heart Rate Variability in Individuals with Spinal Cord Injury

CONSENT STATEMENT

I have read the “LETTER OF INVITATION”, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

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### Appendix G - Raw Data

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### Appendix H – ANOVA Table and Post-hoc Analysis

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(Numbers indicate p-values for the post-hoc test.)