12-2016

Evaluating cardiovascular dysfunction during increased activity and exercise rehabilitation following incomplete thoracic spinal cord injury in the adult rat.

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EVALUATING CARDIOVASCULAR DYSFUNCTION DURING INCREASED ACTIVITY AND EXERCISE REHABILITATION FOLLOWING INCOMPLETE THORACIC SPINAL CORD INJURY IN THE ADULT RAT

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A Dissertation
Submitted to the Faculty of the
School of Medicine at the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy in Anatomical Sciences and Neurobiology

Department of Anatomical Sciences and Neurobiology
University of Louisville
Louisville, Kentucky

December 2016

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A Dissertation Approved on

November 9, 2016

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Dr. Jeffrey Petruska

DEDICATION

This dissertation is dedicated to my parents,

George and Deborah Harman,

who have always been my biggest supporters and have

provided me with the love, discipline, and opportunities to succeed.

ACKNOWLEDGEMENTS

First, I would like to extend tremendous gratitude to my mentor, Dr. David S. K. Magnuson, for his encouragement and guidance during my graduate studies. His devotion to my personal and educational goals has allowed me the opportunity to pursue many facets of my career, both as an instructor and a scientist. His scientific knowledge and compassionate mentorship has had a dramatic impact on my approach to science, my students, and my future endeavors. Further, I would like to acknowledge my committee members, Drs. James Hoying, Jeffrey Petruska, Alexander Ovechkin, and Richard Benton for their encouragement and scientific expertise throughout my graduate studies.

I would also like to recognize Dr. Ann Swank for allowing me to be a part of her team in the Health and Sports Sciences department. Her guidance and support have made an invaluable impact on my personal and professional growth and have deepened my love as an educator.

Additionally, I express special thanks to Amanda Pocratsky, Kathryn DeVeau, and Anastasia Keller for their unwavering friendship throughout my graduate studies. I truly appreciate the many hours of scholastic discussion and encouragement, all of which have made me a better person and a stronger scientist. In my short time as a graduate student, I have gained lifelong friends… I could not have achieved this goal without you.

I extend great gratitude to my friends, parents, and family for their unfaltering love and reassurance. I thank my family for believing in me, even when I did not believe in myself. I also offer gratitude to Spencer Harman for being someone I can always count on… you are an incredible brother and a noble friend. A special thanks goes to Judy Sawyers, whose devotion to my education started many years ago… I still remember you reading to me as a child. You have all contributed to my successes and the person I am today; I will be forever grateful.

Finally, I would like to thank the Department of Anatomical Sciences and Neurobiology and the Kentucky Spinal Cord Injury Research Center for the opportunity to pursue this degree. I would also like to thank all of faculty members, as each contributed in some way to my growth as a scientist and an educator.

ABSTRACT

EVALUATING CARDIOVASCULAR DYSFUNCTION DURING INCREASED ACTIVITY AND EXERCISE REHABILITATION FOLLOWING INCOMPLETE THORACIC SPINAL CORD INJURY IN THE ADULT RAT

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Spinal cord injury (SCI) affects approximately 17,000 new patients each year in the United States. In addition to the obvious paralysis caused by SCI, patients, especially those with cervical and high thoracic lesions, develop a multitude of complications including deficiencies in bowel, bladder, and sexual function, bouts of neuropathic pain and spasticity, and altered cardiovascular (CV) homeostasis.

While there has been great deal of effort focused on improving locomotor function after injury, few labs have attempted to tackle the burden of developing therapeutic strategies to combat secondary complications of SCI; particularly, dysfunctions of the autonomic nervous system. This is problematic as cardiovascular disease (CVD) continues to be the leading cause of morbidity and mortality in the chronic SCI patient population. Injury-induced denervation of sympathetic pathways and subsequent remodeling of neural circuitry involved in CV control can lead to episodes of orthostatic hypotension and autonomic dysreflexia (AD); both of which make rehabilitation efforts difficult and contribute to poor quality of life for SCI patients.

In addition to the inherent disruption of neural circuits important for CV control, there is a sharp decline in physical activity immediately after injury. Due to denervation-induced immobility and/or the time needed to recover from polytrauma typical of SCI, many patients are truly sedentary for weeks to months after the initial insult. Acute remodeling of CV structures (i.e. the heart and vasculature) further impacts mechanisms of CV control during everyday living and in response to instances of increased cardiopulmonary stress. Specifically, left ventricular atrophy and malfunction along with changes in vascular wall properties have been shown to contribute to disordered CV homeostasis in the SCI patient population. Despite the evident risk of SCI individuals to develop CVD, the body of literature investigating the combined effects of maladaptive CV plasticity post-injury and ensuing immobility on CV remodeling and function is limited. Currently, there is little focus on implementing appropriately timed *acute* rehabilitation techniques aimed to curtail maladaptive remodeling and improve CV control and function. Furthermore, no basic science or clinical studies have investigated the most appropriate time course for exercise implementation or compared the CV effects of different exercise modalities and intensities. While most clinical studies examine the benefits of exercise in the chronic SCI population, a time by which maladaptive plasticity of the ANS may already be negatively impacting control of the CV system, we hypothesize that acute implementation of exercise rehabilitation will protect against maladaptive autonomic remodeling, improve CV control and function, and result in cardio-metabolic protective effects post-SCI

The body of work presented in this dissertation is focused on elucidating the physiological mechanisms responsible for maintaining CV control following both high and

low thoracic SCI. Using a clinically relevant contusive model of incomplete SCI, the temporal progression of CV function was examined using implantable telemetry in rodents at rest, during exercise challenge, and in response to acutely-implemented exercise rehabilitation. Echocardiography and Dobutamine stress testing was also employed to gain insight into the structure and function of the heart following contusive SCI. My studies revealed that incomplete SCI resulted in austere CV dysfunction, even following injuries in which the critical sympathetic outflow to the heart was spared. Further, neither passive hindlimb cycling nor active swimming exercise rehabilitation initiated one week after injury was able to attenuate the lack of CV control during hemodynamic provocation (i.e. pressor responses to AD).

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CHAPTER I

UNDERSTANDING AUTONOMIC AND CARDIOVASCULAR DYSFUNCTION FOLLOWING INCOMPLETE SPINAL CORD INJURY

GENERAL INTRODUCTION

Cardiovascular Demise following Spinal Cord Injury

Individuals living with spinal cord injury (SCI) experience a wide array of autonomic and somatic dysfunction. Cardiovascular (CV) abnormalities following SCI have been implicated in increased morbidity and mortality in the patient population (Whiteneck *et al.* 1992, Warburton *et al.* 2007, Inskip *et al.* 2012). Autonomic changes related to the level and severity of the lesion have been shown to greatly impact the extent of CV dysfunction following injury (Weaver *et al.* 2012). Both central and peripheral alterations in cardiac and vascular structures negatively impact mechanisms of CV control during everyday living and in response to instances of increased cardiopulmonary demand. Bouts of orthostatic hypotension (OH) and autonomic dysreflexia (AD) are very common after cervical and high thoracic lesions and prove to be a large factor hindering rehabilitation and recovery (Harkema *et al.* 2008, Krassioukov *et al.* 2009, Weaver *et al.* 2012). To date, most animal studies have utilized full spinal transection injury models and have focused on instances of exaggerated system breakdown, such as AD. However, most clinical injuries are anatomically incomplete and little is known about the subtle, underlying autonomic dysfunction that occurs both acutely and chronically following

incomplete high thoracic or cervical injuries, which may ultimately lead to this extreme system disarray. Furthermore, physical inactivity following SCI leads to lifestyles that do not support healthy cardiac or vascular function. Risk factors associated with sedentary lifestyles and subsequent cardiovascular disease (CVD), including glucose intolerance and alterations in body composition are higher in SCI patients (Devillard *et al.* 2007, Myers *et al.* 2007, Bauman and Spungen 2008). Abrupt and persistent immobility combined with the anatomical disruption of the autonomic system following injury create a physiological condition that is not conducive to maintaining adequate CV health or function. Many studies have shown that exercise initiated acutely post-injury favorably impacts neuronal circuitry responsible for locomotion and somatosensation. Until recently, it was not known whether or not this type of adaptive plasticity is possible within the autonomic nervous system. Using a model of complete spinal transection, West and colleagues (West *et al.* 2014, West *et al.* 2015) have shown that passive rehabilitation initiated acutely post-injury has the ability to reduce cardiac dysfunction and normalize hemodynamic control during experimentally-induced AD. However, in an effort to better understand CV pathology in the clinical population, investigations using contusive SCI models are warranted. CVD presents earlier and is more aggressive in the SCI patient population compared to their able-bodied counterparts. Because CVD continues to be one of the main causes of death for chronic spinal cord injured patients, inquiry into mechanisms that curtail the temporal deterioration of CV fitness and the subsequent decline in CV control continues to be a main priority within the SCI field. Here, we have completed a series of experiments that examined the CV dysfunction, both subtle and overt, that occurs following incomplete SCI

and implemented clinically-relevant, acute rehabilitation strategies to counteract the consequences of immobility and maladaptive plasticity responsible for CV failure.

Mechanisms of Cardiovascular Control

Maintaining proper cardiovascular output in normal individuals

Normal function of the CV system relies on both efficient cardiac activity and appropriate responses in peripheral vasculature to changes in systemic pressures and tissue demands. The primary goal of the CV system is to maintain mean arterial pressure (MAP) within a narrow range during instances of varied cardiopulmonary demand (Figure 1). This is accomplished through calculated manipulations of cardiac output (CO) and total peripheral resistance (TPR). Cardiac output, defined as the volume of blood pumped per minute by each ventricle, is dictated by the stroke volume (SV; the volume of blood pumped per cardiac contraction) and the cardiac rate (beats per minute). Variables such as end-diastolic volume (EDV), the strength of ventricular contraction, and total peripheral vascular resistance all contribute to the SV of a given cardiac cycle. The rate of cardiac contraction is influenced by many factors including neurochemicals of the autonomic nervous system (ANS) and the adrenal medulla. Total peripheral resistance is the other major determinant of MAP. As such, changes in TPR without compensatory adjustments in CO can drastically alter systemic pressure. There are a number of factors that affect TPR including vessel diameter, vessel compliance, and the fluid dynamics of the blood (viscosity).

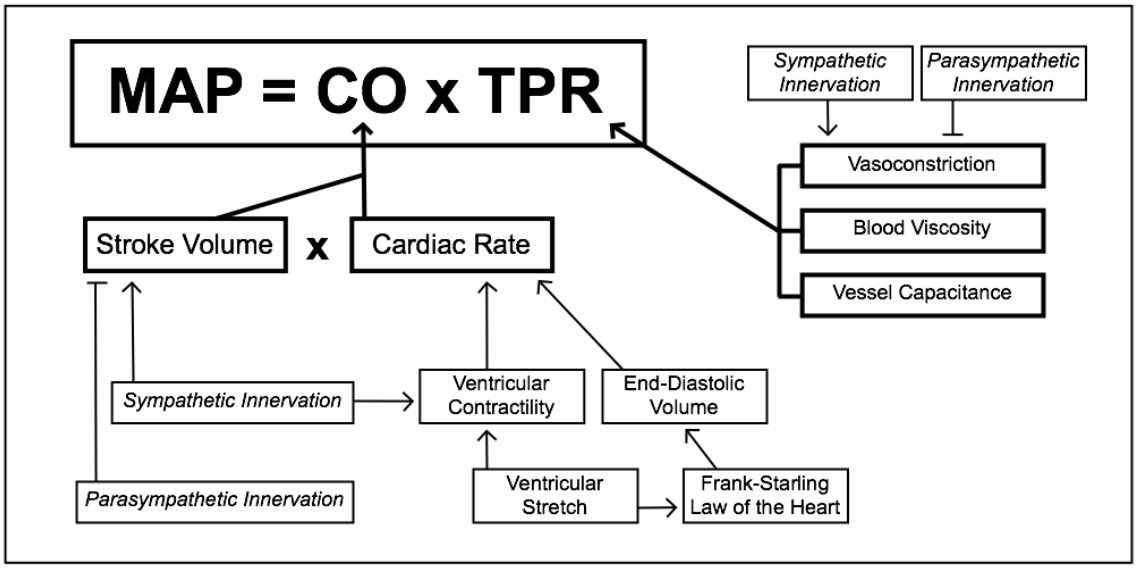

Figure 1: **Maintenance of mean arterial pressure**. Diagrammatic representation of the mechanisms responsible for the maintenance of mean arterial pressure (MAP). Blood pressure control is accomplished through precise manipulations in cardiac output (CO) and total peripheral resistance (TPR).

SCI and the effect of lesion level on cardiovascular control

The heart and vasculature are under both tonic and reflexive autonomic control to ensure adequate blood perfusion during a wide range of physiological conditions (Inskip *et al.* 2009). This control is mediated by the sympathetic and parasympathetic branches of the ANS working in concert (Figure 2). Disruption of either of these circuits results in dramatic changes to the function and homeostasis of the CV system (Krassioukov 2012). Following SCI, CV dysfunction can be partially attributed to the complete or partial loss of supraspinal control over spinally located preganglionic sympathetic neurons that innervate the heart and splanchnic vascular bed (Teasell *et al.* 2000, Furlan *et al.* 2003). The heart and upper body vasculature are innervated by preganglionic fibers emerging from the spinal cord (SC) at the T1-T5 segments. High level lesions isolate portions of the spinal cord circuitry responsible for sympathetic modulation of CV function, forcing them to become "self-regulating" and at least partially independent of supraspinal control. The resulting "decentralization" of the sympathetic nervous system (SNS) leads to changes in the homeostatic maintenance of the CV system due to loss of facilitation and/or lack of inhibition within the SNS (Teasell *et al.* 2000). Furthermore, because cardiac vagal fibers travel outside of the cord and are thus unaffected by the initial mechanical injury, the relative proportions of parasympathetic and sympathetic innervation directing CV function become uneven, setting the stage for serious clinical complications. Following cervical and high thoracic SCI, medullary control centers are unable to effectively maintain efferent control over vasomotor tone and functions of the heart. As a result, vascular mechanics (reflexive arterial and venous vasoconstriction), heart rate, and ventricular contractility are compromised leading to reduced CO and poor venous return (Teasell *et al.* 2000). Severe

high level lesions result in many complications including resting hypotension and bradycardia, diminished circadian rhythms, blunted responses to exercise, OH, and AD (Nitsche *et al.* 1996, Teasell *et al.* 2000, Inskip *et al.* 2009).

Injuries occurring below the T5 spinal level also result in CV dysfunction due to the disruption of sympathetic innervation to lower parts of the mesenteric arterial bed and lower body vasculature. However, there is generally sufficient supraspinal control over the heart and a large portion of sympathetically-innervated vascular beds such that the CV system is able to respond appropriately to baroreceptor mediated reflexes, maintain CV homeostasis, and thus limit clinically significant hemodynamic manifestations of system dysfunction (Teasell *et al.* 2000). Although instances of AD have been reported in individuals with lesions as low as T8 to T10 (Gimovsky *et al.* 1985), this type of profound CV dysfunction is rare in the low thoracic and lumbar clinical populations. Severe low thoracic lesions principally elicit dysfunction related to OH, resting tachycardia, and a diminished ability to appropriately respond to the demands of physical activity (Inskip *et al.* 2009).

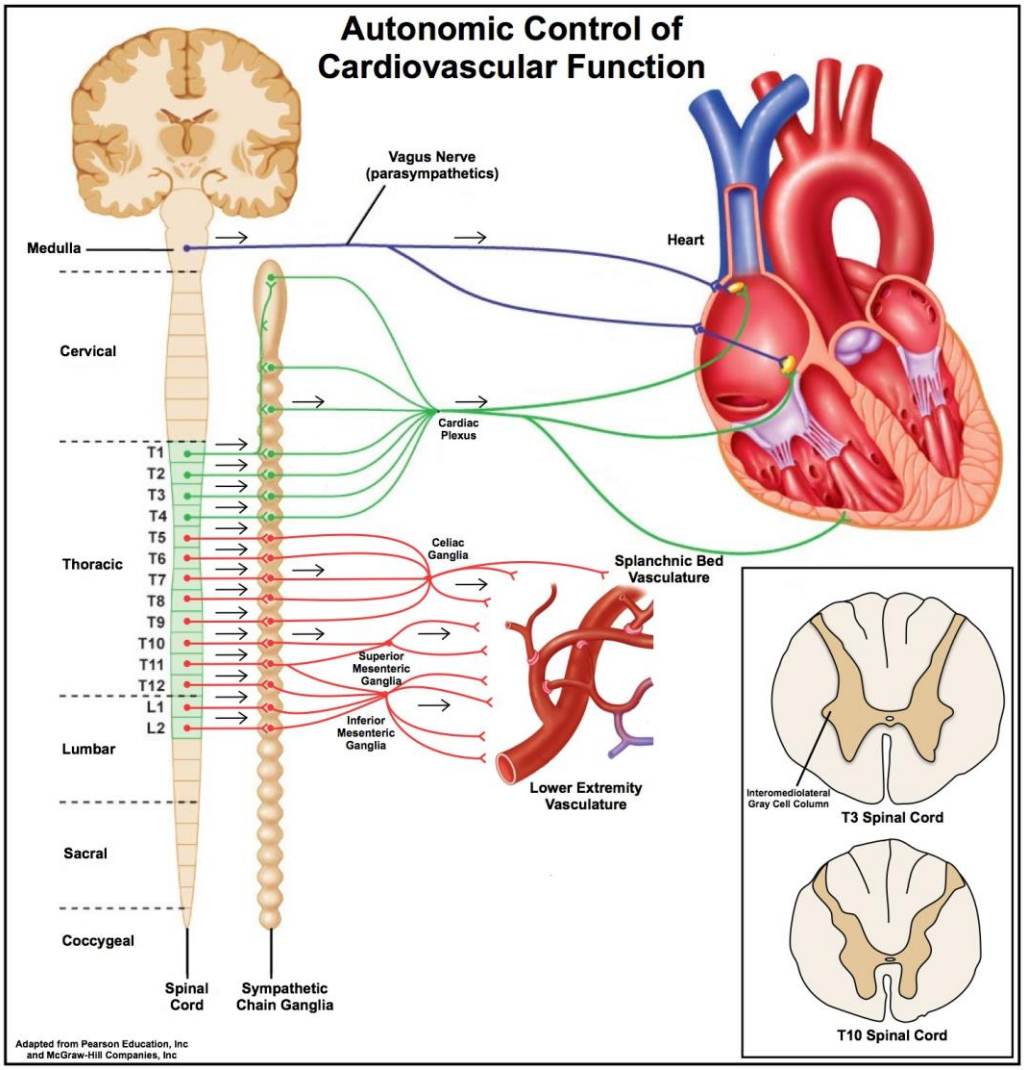

Figure 2. **Autonomic control of cardiovascular function**. Large panel: the control of CV function is accomplished through calculated interactions between the two arms of the ANS, the sympathetic and parasympathetic divisions. Medullary neurons modulating sympathetic function travel within the spinal cord and synapse onto preganglionic sympathetic fibers residing in the interomediolateral (IML) cell column (small box panel). Parasympathetic fibers blue axons are shown in purple. Sympathetic fibers innervating cardiac tissue exit the spinal cord at thoracic levels 1-5 (green axons). Fibers traveling to affect vascular function exit the cord from T5-L2 (gut and lower extremities, red axons).

Temporal Progression of the Clinical Consequences following SCI

Neurogenic Shock

The clinical consequences of SCI on CV control begin immediately following injury and last into the chronic phase (Figure 3). Spinal shock, a condition described by the loss and abnormal return of spinal reflexes (particularly those reflexes modulating somatic activity), begins within hours of the initial insult (Ditunno *et al.* 2004). Neurogenic shock is an extension of spinal shock in which the loss or reduction of sympathetic neuronal activity leads to abnormal autonomic control. The cardiovascular system is particularly affected by this condition given the reduction in vascular tone and peripheral vascular resistance that ensues. Acutely following injury, patients with high level lesions typically experience severe hypotension, bradyarrhythmias, and atrioventricular conduction block (Ditunno *et al.* 2004). Although few studies have examined the etiology and time course of circulatory shock, it appears to evolve alongside the phases of spinal shock. Animal studies utilizing full spinal transection SCI show that sympathetic preganglionic neurons (SPNs) show signs of atrophy acutely following injury, and recover normal morphology over time (Krassioukov and Weaver 1996). The loss of supraspinal inputs onto SPNs and their partial deafferentation is likely responsible for the initial atrophy of SPN dendritic arbor and soma area. Sympathetic atonia and neurogenic shock coincide with the reduction in SPN soma size during this early phase. The literature suggests that following a general dampening of sympathetic activity, autonomic function will begin to emerge within a few days of injury, with hyperreflexia of sympathetic circuits responsible for AD developing in the subsequent weeks to months. Our data suggests that even following mild spinal cord contusion, the autonomic nervous system cannot produce the necessary output to respond

to increased cardiopulmonary demand at one week post-SCI. Swimming exercise challenge at seven days after high *and* low thoracic injury resulted in persistent and progressive bradycardia. Neurogenic shock is possibly responsible for this general blunting of the sympathetic nervous system output and the inability to increase sympathetic tone to the heart myocardium. Upon first glance, it could be theorized that animals with lesions below the critical sympathetic outflow to the heart would respond appropriately to increased cardiopulmonary challenge with increased heart rates. However, in agreement with Summers and colleagues (Summers *et al.* 2013), the level of injury does not solely determine whether or not a patient will develop symptoms of neurogenic shock. Further, the mechanisms responsible for bradycardia (and hypotension) characteristic of neurogenic shock appear to be mixed, with both cardiac and peripheral (vascular resistance and capacitance) origins (Summers *et al.* 2013). This could help explain reasons for why we did not find a clear correlation between the lesion level and the profile of hemodynamic responses during exercise challenge acutely after injury in our animals.

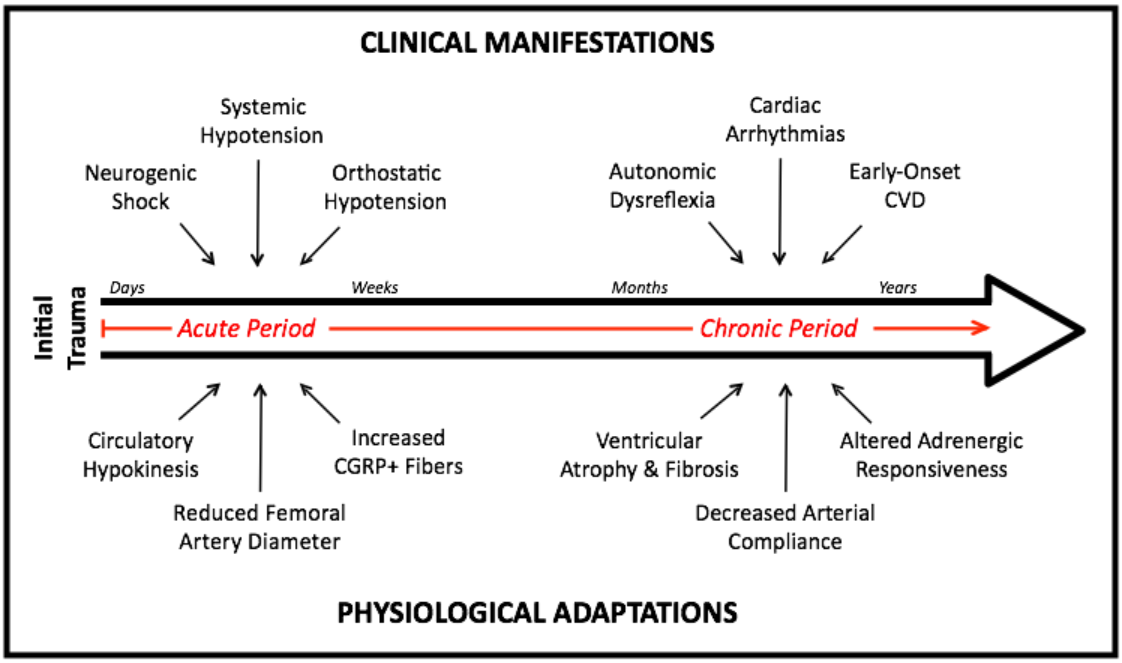

Figure 3. **Temporal profile of physiological adaptations and clinical manifestations of CV demise following SCI**. Schematic timeline illustrating the clinical and physiological consequences of injury with regards to CV structure and function.

Systemic, Orthostatic, Exercise-Induced, and Post-Exertional Hypotension

The maintenance of blood pressure in spinal cord injured patients becomes a life-long battle, even after the period of neurogenic shock has subsided. Abnormal sympathetic cardiovascular control is responsible for periods of hypotension at rest, during postural maneuvers, and following exercise. Orthostatic hypotension (OH) begins acutely following injury and generally lasts into the chronic phase of disease progression. Early literature classified OH as an acute condition. However, recently, it has been shown that OH can last for many years following injury, and will often worsen with time (Frisbie and Steele 1997). Blood pooling in the lower extremities upon standing (or even sitting) results from markedly reduced sympathetic modulation of vasoconstriction below the lesion and causes symptoms of dizziness, blurred vision, and syncope. Hemodynamic instability associated with postural changes greatly affects patients' functional recovery and is likely a contributing factor prolonging the period of bed rest following injury. In a study by Illman et al., it was reported that nearly 60% of both incomplete and complete SCI patients participating in acute care physical therapy involving mobilization experienced symptoms of OH within the first month following injury (Illman *et al.* 2000). Furthermore, patients felt that symptoms of OH significantly limited their ability to fully participate in mobilization procedures and physical therapy in 33% of the treatment sessions. It is likely that in the wake of lower limb paralysis, altered skeletal muscle pump activity is at least partially responsible for reduced venous return, central venous pressure, and end-diastolic volume of the heart, all of which leads to a subsequent drop in blood pressure during postural maneuvers (Rowell *et al.* 1986, Notarius and Magder 1996). The use of compression stockings to reduce venous pooling in the lower extremities and/or

pharmacological agents to increase plasma volume (fludrocortisone) or vasoconstriction (midodrine) are common techniques employed in the clinic to lessen the severity of hypotensive bouts (Groomes and Huang 1991, Barber *et al.* 2000, Freeman 2003, Mathias 2003).

Furthermore, unstable blood pressure control during and following exercise training limits patients' abilities to participate in rehabilitation and community-based sporting activities. Normal responses to exercise require both hemodynamic and neural alterations to effectively meet the metabolic demands of active musculature. Cardiac output (CO), the product of stroke volume (SV) and heart rate (HR), increases in a step-wise fashion as exercise intensity mounts. Contributions of SV and HR to the production of CO vary depending on multiple factors, including the position of the body (Bevegard *et al.* 1966, Vella and Robergs 2005). Oxygenated blood is redistributed away from non-exercising musculature and renal and splanchnic circulatory beds to active muscles via vasoconstriction by sympathetic and adrenal mechanisms (Thomas and Segal 2004). Systemic vascular resistance (total peripheral resistance, TPR) is reduced during exercise due to vasodilation in active limbs. Overall pressor responses to active exercise, therefore, are dependent on the extent of CO and TPR. As exercise intensity grows, systolic BP will increase while diastolic BP is either maintained or decreased slightly, resulting in only modest changes in mean arterial pressures overall (Shepherd 1987). Reflex circuits also contribute to the maintenance of mean arterial pressure at the initiation and continuation of exercise. The exercise pressor response (EPR), in conjunction with central command processes, is responsible for increasing HR at the onset of exercise via muscle afferent signaling (Iwamoto *et al.* 1985, Kaufman and Hayes 2002, Kaufman 2012, Amann *et al.*

2015). Cardiopulmonary and arterial baroreflex arcs monitor mean atrial pressure and volume, making slight adjustments in neural output when necessary. Baroreflex resetting is an important aspect of cardiopulmonary control during exercise initiation, participation, and cessation. During exercise, the EPR and supraspinal centers interact to "shift" the settings of the arterial baroreflex, leading to a larger range of acceptable systemic pressures and thus, ensuring adequate muscle perfusion (Raven *et al.* 2006).

Following SCI, diminished vasomotor tone below the lesion contributes to exertional hypotension. Exertional hypotension is described clinically as a drop in systolic BP of at least 10mmHg (Low *et al.* 2012). Depending on the level of injury, deficits in sympathetically-mediated vasoconstriction and cardiac contractility and rate can lead to inadequate blood redistribution away from the gut, blood pooling in the lower extremities, poor venous return, and diminished cardiac output; all of which may contribute to dampened pressor responses during exercise. Furthermore, previous studies have also highlighted changes in cardiac structure and function that may reduce the heart's ability to perform appropriately during bouts of exercise challenge (Kessler *et al.* 1986). In recent years, a phenomenon known as post-exertional hypotension has gained the attention of SCI researchers. Under normal circumstances, centrally-mediated decreases in sympathetic activity and local vasodilation contribute to the fall in BP during the recovery period (Halliwill *et al.* 2013). The arterial baroreflex also resets to control this new lower pressure (Halliwill *et al.* 1996). In individuals with cardiovascular disorders, not unlike those with SCI, the magnitude and duration of post-exertional hypotension has a tendency to be pathologic, lasting in some patients for up to twelve hours after exercise cessation (Forjaz *et al.* 2000). Although clinical studies examining the incidence of exertional hypotension

after SCI is scarce, one study has shown that patients with cervical lesions, representing injuries with the greatest amount of autonomic dysfunction, do suffer from this debilitating condition (Claydon *et al.* 2006). It is well documented that the sedentary lifestyles adopted by most SCI individuals leads to physical deconditioning and a greater risk of CVD. Therefore, factors that discourage patients from participating in regular exercise further exacerbate their overall physical decline.

Autonomic Dysreflexia

Patients with high level lesions, particularly those above the T6 spinal segment, are prone to a devastating condition known as autonomic dysreflexia, already mentioned above. Characterized by extreme hypertension, episodes of AD often lead to symptoms of upper body flushing and sweating, piloerection, headache, and reflex-mediated bradycardia. Although somewhat uncommon, untreated bouts of AD can become life-threatening and cause seizures, retinal detachment, cerebral hemorrhage, and cardiac arrest (Yarkony *et al.* 1986, Pine *et al.* 1991, Eltorai *et al.* 1992, Karlsson 1999). Studies have estimated that regular bouts of AD can affect nearly 90% of cervical and high thoracic SCI patients (Lindan *et al.* 1980, Elliott and Krassioukov 2006), and is three times more prevalent in patients with complete tetraplegia compared to incompletely lesioned individuals (Curt *et al.* 1997). Symptoms of AD can be elicited as early as one (rodent) to four (human) days following severe SCI of the cervical cord (Osborn *et al.* 1990, Krassioukov *et al.* 2003). However, episodes in this acute time period are often under-recognized, possibly because they present with less severe pressor responses and symptomology (Krassioukov and Weaver 1995). In the chronic stages of disease

progression (4-6 weeks in animal studies), the hypertensive crises in animal and human subjects can reach dangerously high levels. Likewise, we have shown that one-minute bouts of colorectal distension in rodents with incomplete contusion injuries can cause AD with systolic pressures in excess of 200mmHg five weeks post-injury.

The mechanisms responsible for AD are likely multifactorial; however the loss of bulbospinal regulation on sympathetic preganglionic fibers and aberrant plasticity within the spinal cord and peripheral nervous system are contributing factors. Normal autonomic control of CV function (blood pressure and heart rate) depends on complex interactions between descending supraspinal fibers and sympathetic neurons within the spinal cord. Following SCI, the modulatory projections from medullary centers are damaged or lost, leaving SPNs within the interomediolateral (IML) gray column self-regulating. Furthermore, injury-induced increases in spinal levels of nerve growth factor (NGF) leads to sprouting of non-peptidergic and calcitonin gene-related peptide ($CGRP^{+}$) afferent fibers within the thoracolumbar spinal cord. Spinal neurons that have lost their original connections form new synapses with different afferent sources, many of which may be inappropriate (Beattie *et al.* 1993, Murray 1993, Teasell *et al.* 2000). While both noxious and normally non-noxious stimuli have been known to elicit symptoms of AD, most often bouts of AD are triggered by noxious stimulation stemming from the bladder or colon (Figure 4). During a typical bout of AD, afferent input travelling in fibers from the viscera enters the lumbosacral spinal cord igniting a cascade of massive sympathetic discharge below the level of the lesion. Numerous studies have illustrated the significant role of maladaptive propriospinal plasticity on this widespread sympathetic discharge (Hou *et al.* 2008). It has been proposed that propriospinal neurons serve as a substrate for the transfer

of incoming afferent signals to SPNs in the thoracic cord. Vasoconstriction of the lower extremity muscular, cutaneous, and splanchnic vascular beds results in systemic hypertension. In some instances, baroreceptor-mediated reflexes result in bradycardia.

Most animal and human studies to date have focused on instances of CV dysfunction during states of OH and AD (Maiorov *et al.* 1997, Maiorov *et al.* 1998, Mayorov *et al.* 2001, Harkema *et al.* 2008, Inskip *et al.* 2012). However, the underlying autonomic and CV dysfunction that can be seen over time post-SCI in the resting state and during instances of CV challenge is not well characterized. Our data suggests that CV control mechanisms are altered even following injuries in which exaggerated system dysfunction (OH or AD) is not seen. It is likely that the mechanisms responsible for this dysfunction are numerous; however, maladaptive plasticity within autonomic circuitry and general deconditioning within the CV system are both likely to be major contributors to CV decline and altered responses to CV challenge after injury.

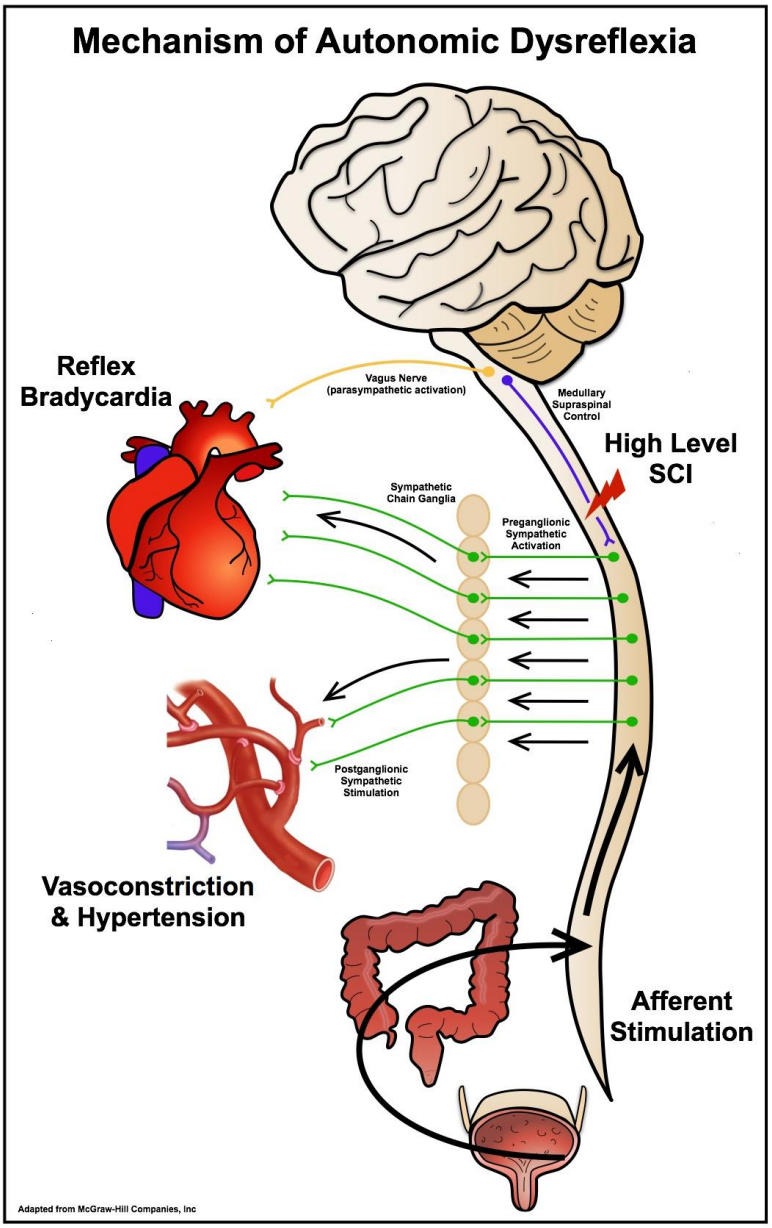

Figure 4. **Mechanism of autonomic dysreflexia.** Simplified schematic of the physiological mechanism leading to AD. Afferent stimulation, most often from the bowel or bladder, triggers excessive, reflex-mediated sympathetic discharge from preganglionic neurons below the level of the lesion (green arrows). Reflex-bradycardia often ensues in an attempt to remedy the widespread vasoconstriction and hypertension.

Implementation of Cardiovascular Rehabilitation after SCI

Deconditioning within the CV system

The sedentary lifestyle adopted by, or forced on most SCI patients results in substantial physical deconditioning which contributes to multisystem dysfunction and physical decline (Jacobs and Nash 2004, Warburton *et al.* 2007). Coupled with the decrease in functional muscle mass below the level of injury, the reduced sympathetic input to the heart, skeletal musculature and peripheral vasculature make rehabilitation very difficult for this population (Teasell *et al.* 2000, Devillard *et al.* 2007). However, implementing physical activity paradigms to counteract deconditioning after SCI is an important component of patient care.

As such, many groups have highlighted the benefits of physical activity and training programs following chronic injury (for review see Devillard et al., 2007)(Devillard *et al.* 2007). Positive changes associated with various types of exercise-related rehabilitation include increased oxygen uptake, favorable muscle fiber type distribution, and improved resistance to fatigue (Mohr *et al.* 1997, Le Foll-de Moro *et al.* 2005, Jacobs 2009). While these adaptations are important for overall patient health and quality of life, CV recovery resulting from exercise training is a crucial consideration for improved life expectancy following injury. In the able-bodied (AB) population, traditional risk factors that contribute to the development of CVD include diabetes, elevated systolic blood pressure, dyslipidemia, age and sex (Jackson *et al.* 2005, D'Agostino *et al.* 2008). Interestingly, following SCI, elements that contribute to CVD are not as clear and do not fully explain the elevated risk for development of CVD in patients. For instance, individuals with high thoracic and cervical lesions have reduced resting blood pressure and a preponderance of

parasympathetic modulation of CV function (Claydon and Krassioukov 2008, West *et al.* 2012). In the AB community, these characteristics would be considered cardioprotective; yet this is not the case for SCI individuals. While many studies have highlighted central cardiac improvements following exercise intervention and their implications for reduced CVD in SCI individuals (Washburn *et al.* 1986, Nash *et al.* 1991, West *et al.* 2014), less is known about how exercise training and the improvement in *vascular function* leads to reduced incidence of CVD (Green *et al.* 2008). It is likely that the deconditioning of the vascular system, particularly endothelial cells, following high-level injury is partially responsible for the increased risk of CVD in the SCI population, apart from traditional risk factors.

Vascular adaptations to systemic deconditioning & exercise training following SCI

The extreme immobility imposed on SCI patients results in rapid and persistent arterial modifications and deconditioning of the vascular system (de Groot *et al.* 2006). Remodeling of the vascular architecture below the lesion has been noted many times following SCI in patients (Olive *et al.* 2003, de Groot *et al.* 2006, Thijssen *et al.* 2012). For instance, there is a 25% reduction in the diameter the femoral artery, which is largely completed by three weeks post-injury (de Groot *et al.* 2006). These findings are similar to those studies examining the consequences of one week of lower-limb casting and four weeks of lower-limb suspension, in which there was a 6% and 12% decrease in femoral artery diameter, respectively (Sugawara *et al.* 2004, Bleeker *et al.* 2005). Changes in femoral artery diameter appear to be a localized consequence of deconditioning in the lower extremities due to paralysis-induced inactivity and muscle atrophy (de Groot *et al.*

2006, de Groot *et al.* 2006), as the structural characteristics of conduit arteries above the lesion are comparable to AB controls (de Groot *et al.* 2004). The rapid and localized inward remodeling of vascular walls is likely an adaptation to alterations in peak shear stress levels and peak oxygen consumption following reduced blood volume and flow in paralyzed limbs (Langille and O'Donnell 1986). Indeed, when femoral artery diameter is normalized to limb volume, no differences are noted in structural parameters suggesting a strong link between vascular remodeling and atrophy of paralyzed musculature (de Groot *et al.* 2006). Additionally, it has been demonstrated that patients with chronic SCI develop thickened arterial walls and increased stiffness (decreased compliance). Enhanced wall thickness appears to be a systemic effect of injury and whole-body inactivity, and does not follow the same pattern of adaptation as inward remodeling. The gradual increase in arterial wall thickness affects vasculature above (carotid) and below (femoral) the lesion level, and does so over many months (Matos-Souza *et al.* 2009, Thijssen *et al.* 2012). It is likely that the increased vascular tone in SCI patients, mediated through angiotensin-II and endothelin-I, contributes to changes in vascular wall thickness after SCI (Thijssen *et al.* 2007, Groothuis *et al.* 2010). These findings suggest that changes in conduit wall thickness and diameter do not follow similar patterns of remodeling, and are likely mediated via distinct mechanisms (Thijssen *et al.* 2012).

The negative effects of inactivity and denervation following SCI can be reversed by regular physical activity, and such changes have been shown to occur within weeks of training initiation. Unfortunately, most of these studies employed functional electrical stimulation (FES) modulation of the lower extremities, which may not be readily accessible to the general population (Gerrits *et al.* 2001, de Groot *et al.* 2005, Thijssen *et al.* 2006).

There is only one study to date that has examined the effects of passive lower extremity cycling exercise on vascular function following SCI (Ballaz *et al.* 2008). While there were no changes in vascular characteristics at rest, paraplegic patients experienced increased mean femoral artery blood flow velocity and reduced peripheral resistance during cycling exercise six weeks after rehabilitation initiation. The improved hemodynamic/vascular response during exercise in these patients is promising, given SCI individuals are known to have blunted responses to instances of increased cardiopulmonary demand. Body-weight supported treadmill training (BWSTT) is a common locomotor rehabilitation technique used in the clinic for individuals with chronic SCI. In a study by Ditor and colleagues, it was found that four months of training improved femoral arterial compliance in both paraplegic and tetraplegic patients (Ditor *et al.* 2005). Unfortunately, most studies examining vascular adaptation to exercise training programs enlist chronically injured patients. It has been shown that the modifications occurring in the vascular architecture functionally correlate with ongoing muscle atrophy in the lower limbs following injury, and reach detrimental levels within a few months of the injury date (de Groot *et al.* 2006). This supports the notion that acute implementation of exercise paradigms to counteract peripheral deconditioning due to immobility and/or denervation could lessen the burden of maladaptive vascular remodeling and improve CV health.

Cardiac decline and subsequent recovery following exercise training in SCI patients

Spinal cord injury induces particular autonomic changes that directly contribute to the rapid decline in cardiac structure and function. Following mid-thoracic (T5 complete) SCI, an injury that spares descending control of preganglionic sympathetic fibers

modulating cardiac function, excessive sympathetic activity above the level of the lesion contributes to abnormal heart rates and contractility. The upregulation of sympathetic modulation of cardiac function can be explained by a general increase in myocardial NGF content and subsequent sympathetic hyper-innervation (Lujan *et al.* 2009, Lujan *et al.* 2010, Lujan *et al.* 2012). It has been demonstrated that calcium overload in the heart leads to myocardial damage and cardiac dyssynchrony, a potent stimulus for the production of NGF, and begins as early as 15 minutes after SCI in both humans and animals (Sharov and Galakhin 1984). Importantly, NGF-mediated elevation of sympathetic tonus following mid-thoracic SCI increases patient susceptibility to ischemia/reperfusion-induced and ischemia-induced sustained ventricular tachycardia (Lujan and DiCarlo 2007, Lujan *et al.* 2009). Higher level lesions, those in which the supraspinal regulation of cardiac sympathetic fibers is disrupted to a greater degree, have not been analyzed in this respect.

Peripheral changes related to the sedentary lifestyles of SCI patients also leads to maladaptive cardiac changes. These include modifications in left ventricular (LV) structure, ventricular atrophy and altered pumping ability, depressed resting SV due to reduced venous return, and circulatory hypokinesis (Hjeltnes and Vokac 1979, Cooper and Tomanek 1982, Nash *et al.* 1991, Nash *et al.* 1996). Deconditioning of cardiac structure following SCI is level-dependent, with tetraplegic patients exhibiting the most severe cardiac atrophy and impairments in systolic cardiac performance (Kessler *et al.* 1986). Cardiac consequences of SCI can be linked to changes in peripheral circulatory volume and systemic pressures (Jacobs and Nash 2004). As such, the inability to maintain blood pressure within a narrow range (between periods of prolonged hypotension and episodic hypertension) in conjunction with volume unloading of the heart likely contributes to the

concentric cardiac remodeling present in SCI patients (West *et al.* 2014). Studies have shown this cardiac remodeling typical of SCI patients is associated with an increase in collagen deposition and myocardial fibrosis (Kehat and Molkentin 2010, West *et al.* 2014), both of which contribute to the decline in cardiac function following SCI. In the nondisabled community, exercise programs, especially those involving endurance training, increase the hemodynamic load placed on the heart leading to LV enlargement and enhanced cardiac performance (Gates *et al.* 2002). Changes in myocardial aerobic requirements, nervous system adaptation including attenuated cardiac sympathetic modulation, and adaptations in trained musculature increase overall CV health and lead to decreased heart rates at rest and during submaximal exercise workloads (Devillard *et al.* 2007). Additionally, endurance training enhances cardiac output, the result of cardiac hypertrophy and improved contraction ability. In turn, this allows for enhanced ventricular filling capacities and the subsequent increase in stroke volume potential (Hellsten and Nyberg 2015). Due to denervation-induced paralysis of the lower extremities, SCI patients are often limited to upper extremity exercise training only. Although positive cardiac benefits of upper body training have been reported following SCI (Huonker *et al.* 1998), exercise in the form of arm ergometry is generally insufficient to maximally load the heart and fails to produce the desired improvements in cardiac structure and function (Devillard *et al.* 2007). However, in a study by Gates et al., (Gates *et al.* 2002) when compared to sedentary control SCI patients, trends for greater wall thickness (power-trained athletes) and LV chamber dimensions (endurance athletes) were noted in wheelchair athletes. This data, while not statistically significant given the heterogeneity of the study subjects, suggests that regular upper extremity exercise programs could be beneficial in reducing

cardiac atrophy in the SCI community. Furthermore, studies evaluating the effects of passive cycle training of the lower extremities report that this type of rehabilitation indeed elicits conditioning effects and have thus been shown to improve vascular and cardiac indices (Ballaz *et al.* 2008, West *et al.* 2014).

Acute exercise training to harvest central plasticity & remedy peripheral deconditioning

Spinal cord injury disrupts all normal physiological processes within the body. Direct damage to spinal cord circuitry and plastic changes within the central nervous system contribute to abnormal cardiovascular control and centrally-mediated reflexes. Additionally, denervation-induced paralysis leads to extended periods of bedrest and immobility. The length of time that a patient remains immobile post-SCI and the degree to which their lifestyles become sedentary have great ramifications on CV outcomes, specifically due to the deconditioning of peripheral CV structures. The decline of physical fitness following SCI is progressive; muscle mass decline, changes in vascular reactivity and structure, and alterations in the body's metabolism increase with time post injury.

Most clinical rehabilitation studies examine only peripheral (heart and vasculature) benefits of exercise in the chronic SCI population, a time in which the physical fitness of the patient may already be at detrimentally low levels and the opportunity for positive adaptive plasticity in central circuitry has declined. Implementing exercise paradigms early after injury may work to not only curtail the patient's decline in physical fitness, but also to take advantage of the inherent changes occurring within autonomic circuitry, ultimately leading to improved cardiovascular control and function.

Acutely following injury, there is a high degree of central nervous system and spinal cord circuitry reorganization and plasticity. Many studies have shown that acute rehabilitation training has the capacity to favorably impact the spinal circuitry responsible for locomotion. For instance, it has been demonstrated that after complete thoracic transection, the lumbar cord regains the capacity to generate weight-supported stepping following treadmill training (Barbeau and Rossignol 1987, Lovely *et al.* 1990, de Leon *et al.* 1998). It is believed that central pattern generators within the lumbar circuitry remain capable of eliciting neural activity in response to sensory input, and are thus responsible for the re-training of stepping following SCI (Roy *et al.* 2012). Furthermore, there is also a large body of experimental evidence for plasticity within the respiratory system following high cervical hemisection. Animal studies have shown that inspiratory drive can be re-established to the ipsilateral phrenic nerve and hemi-diaphragm via spontaneous and contralateral phrenicotomy-induced activation of normally silent commissural and/or interneuronal pathways (Lane *et al.* 2009).

Conversely, previous studies have shown that the spinal cord also experiences maladaptive plasticity after SCI in response to peripheral injury and inflammation. Our lab has shown that activities such as acute wheelchair immobilization and stretching physical therapy negatively impact this reorganization within the spinal cord and produce maladaptive consequences that last into the chronic phase (Caudle *et al.* 2011). Likewise, numerous studies have shown that plastic changes occurring in the lumbar cord after high thoracic transection negatively impact CV function. SCI-induced increases in the density of lumbar $CGRP^{+}$ fibers correlate with the development and severity of AD in rodent models of complete transection (Hou *et al.* 2008, Laird *et al.* 2009, West *et al.* 2015).

Studies of acute exercise training and the effects of rehabilitation on CV outcomes are few in number. To date, it is still too premature to determine whether or not early intervention has the capacity to alter the trajectory of CV dysfunction typical of SCI patients. If the ANS undergoes plasticity similar to locomotor and somatosensory systems, acute exercise rehabilitation may induce adaptive changes in ANS circuitry and lead to more efficient functioning within the CV system. One of the only animal studies to date that has examined the influence of early exercise rehabilitation on CV function comes from Laird et al. (Laird *et al.* 2009). In this study, they evaluated the rat's response to colorectal distension following six weeks of treadmill training. Results suggest that the exercise paradigm that they implemented exaggerated AD responses and resulted in increased afferent fiber density in the lumbar cord. However, they began training only three days after spinal cord transection (SCT), a time in which the animal was still in spinal shock and vascular permeability in the spinal cord remained high. Therefore, the timing of initial exercise bouts in conjunction with the physical attributes of treadmill training (harness application, hindlimb dragging across the treadmill, etc.) may have been nocuous to CV outcomes and recovery. Conversely, in a recent study by West et al. (West *et al.* 2015), it was demonstrated that acute rehabilitation in the form of passive hindlimb cycling decreased the severity of AD in spinal rats and this improvement correlated with the reduction in $CGRP^{+}$ fiber density in the lumbar spinal cord. Because the peripheral and central mechanisms responsible for changes in CV function following SCI are temporally mediated, it is reasonable to believe that acute exercise rehabilitation has the potential to correct maladaptive changes that occur due to both the injury parameters and the inherent immobility typical of SCI. CV challenge initiated in the acute phase of injury will impact

neuronal circuitry and plasticity within the ANS; much like extensive step training positively influences locomotor recovery. Acute training will not only prevent the effects of deconditioning, it will positively influence autonomic reorganization, leading to more permanent changes in CV function. More insight regarding the effects of acute physical activity on CV function and ANS plasticity needs to be established in order to properly treat SCI patients in the clinic.

Dissertation Overview

Overall Goal

Dysfunction of the autonomic nervous system following SCI leads to multisystem decline and diminished quality of life for SCI patients. Coupled with the inherent deconditioning of peripheral CV end organs, injury to the neural circuitry responsible for CV control can lead to episodes of systemic and orthostatic hypotension, cardiac arrhythmias, and autonomic dysreflexia, all of which make activities of daily living and rehabilitation difficult. Despite the elevated risk of SCI patients to develop CVD, the body of literature investigating the combined effects of maladaptive plasticity post-injury and ensuing immobility on CV remodeling and dysfunction is limited. Studies implementing appropriately-timed acute rehabilitation techniques to curtail maladaptive remodeling and improve CV control has received little focus. While chronic exercise rehabilitation has been used in the clinic to improve many facets of patient health, including vascular and bladder function, application of exercise paradigms early after injury is ideal, given that the many pathways within the nervous system is most malleable to circuitry reorganization acutely after injury. Thus, the work presented in this dissertation is focused on the impact

of acute exercise challenge and acute exercise rehabilitation on CV structure and function following a clinically relevant model of contusive thoracic SCI. Using implantable telemetry devices to measure blood pressure (BP) and electrocardiogram (ECG) signaling in freely moving animals, the CV responses to active swimming exercise were evaluated to assess the ability of the CV system to respond to an exercise challenge both acutely and chronically after SCI. Further, various modalities of exercise training beginning acutely after high thoracic SCI were evaluated for their effects on CV control and the development of AD. Discoveries made during these endeavors has allowed us to better understand the underlying causes of CV system demise by examining the effects of not only the primary injury on neurological systems, but also on the impact of deconditioning and immobility following contusive SCI. We have shown that even following mild injuries and injuries that spare the critical sympathetic outflow to the heart, CV control is altered especially during instances of increased cardiopulmonary demand. We have shown that the effects of acute exercise training following incomplete contusion are varied, and it appears as though the modality of training is critical to CV outcomes. Understanding the temporal progression of CV decline and the impact that various rehabilitation paradigms have on hemodynamic control following contusive SCI will allow us to more effectively treat patients in the clinic, and perhaps lessen the burden of CVD in chronic SCI individuals.

Research Aims and Hypothesis

Aim Ia: To develop a model of exercise challenge involving different levels of intensity and duration, to be used before and after contusive SCI as a cardiopulmonary stressor.

Few studies have examined the hemodynamic responses to exercise challenge following SCI. Using various rehabilitation modalities commonly employed in our lab, Aim 1a explored the blood pressure and heart rate responses to exercise challenge of various intensities and duration. Shallow water walking, lap swimming (for varied lengths of time), and continuous swimming against a current were utilized to challenge the cardiovascular system. Blood pressure and ECG were recorded before, during, and after exercise in normal rats and at various time points post T3 or T10 contusion. We hypothesized that the maintenance of CV control would rely heavily on the level of the injury and the intensity of the exercise being performed. Higher lesions lead to global dysfunction (cardiac and peripheral vasculature deficits) and will result in the inability to maintain appropriate cardiac output and control during exercise.

Aim Ib: To characterize the temporal progression of cardiovascular dysfunction during rest, exercise challenge, and exercise recovery following incomplete T3 or T10 contusion SCI in the adult rat.

Hemodynamic data acquired from telemetric blood pressure and ECG devices were used to describe the physiological mechanisms responsible for CV demise following incomplete SCI. Cardiovascular responses (blood pressure and heart rate) were characterized at rest and during instances of increased cardiopulmonary demand in rats

before and for ten weeks after T3 or T10 contusion. To determine how dynamic CV control varied over time post-injury and differed from normal animals, cross-correlation analysis of BP and HR was evaluated during recordings of in-cage rest, exercise challenge, and exercise recovery. Sympathetic and parasympathetic autonomic modulation of CV function was measured using power spectral analysis of heart rate variability (HRV). Additionally, in a small subset of animals, high resolution ultrasound was used to derive structural and functional indices of vascular and cardiac tissues. Further, dobutamine stress testing was employed pre-injury and following T3 or T10 contusion for ten weeks to examine cardiac function directly, without the involvement of potentially damaged cardiac sympathetic pathways. We hypothesized that SCI occurring at the T3 level would produce profound cardiovascular dysfunction that is both more severe and longer lasting than injuries at the T10 level, and that dysfunction will be most evident during the increased cardiopulmonary demand of exercise.

Aim II: To determine how rehabilitation-associated exercise, initiated acutely after incomplete SCI, can influence CV dysfunction and the time course and extent of recovery of dynamic hemodynamic control.

In an effort to harness inherent nervous system plasticity and prevent the effects of deconditioning, we evaluated whether acute exercise rehabilitation could improve CV control following incomplete contusion of the upper thoracic cord. Initially, our goal was to employ lap swimming as an exercise rehabilitation technique to examine the effects of acute training on cross correlation of BP and HR and spectral analysis of HRV. However, given that instances of autonomic dysreflexia are very common in the SCI patient

population, we shifted our focus to investigate the effects of acute exercise rehabilitation on the development and severity of AD using pre-clinical models. Both dynamic swimming exercise and passive hindlimb cycling rehabilitation were evaluated for their effects on CV control and the degree of pressor responses to experimentally-induced AD. I hypothesize that implementation of exercise training at an acute time point following injury would protect against maladaptive autonomic remodeling, improve CV control and function, and result in cardio-metabolic protective effects post-SCI. Further, I believed that active exercise training (swimming) would be most effective in producing the desired CV benefits.

Summary

I hypothesized that following incomplete contusion of the thoracic cord, rodents would present with altered CV control that was most severe during instances of cardiopulmonary challenge and that dysfunction could be remedied with acute exercise rehabilitation. In Aim I, I discovered that animals with both high (T3) and low (T10) SCI were unable to maintain tight control over CV parameters during periods of exercise challenge, although they appeared to function normally at rest. In Aim II, I showed that despite acute rehabilitation therapy, rodents with severe T2 contusion continued to experience episodes of AD.

CHAPTER II

TEMPORAL ANALYSIS OF CARDIOVASCULAR CONTROL AND FUNCTION AT REST AND DURING EXERCISE CHALLENGE FOLLOWING INCOMPLETE T3 SPINAL CORD INJURY IN RATS

Introduction

Autonomic regulation of the cardiovascular (CV) system is disrupted following spinal cord injury (SCI) (Krassioukov and Claydon 2006). Abnormal control of arterial blood pressure and heart rate (HR) leads to instances of extreme system breakdown, such as autonomic dysreflexia (AD) and orthostatic hypotension (OH). As a result, patients suffering from SCI, especially those with cervical and high thoracic lesions, experience increased morbidity and mortality from cardiovascular disease (CVD) compared to their able-bodied counterparts (Whiteneck *et al.* 1992). In addition to maladaptive nervous system reorganization and plasticity following injury (Krenz and Weaver 1998, Hou *et al.* 2008, Hou *et al.* 2009), peripheral alterations in cardiac and vascular structures contribute to aberrant CV regulation and unstable hemodynamics (Laird *et al.* 2008, Lujan *et al.* 2012, Thijssen *et al.* 2012). Due to the anatomical organization of sympathetic outflow to the CV system, the segmental level and severity of the lesion greatly impacts the degree of dysfunction experienced following injury, with lesions above T5 resulting in the most severe CV consequences. Given this information, the majority of studies investigating CV dysfunction use injury models involving complete transections of the upper thoracic cord.

These endeavors have traditionally been preferred over contusion models because complete transections reduce the variability between animals and result in severe and persistent CV dysfunction. However, these models may not be ideal given that the majority of clinical injuries are incomplete, leading to a wide array of locomotor and CV phenotypes in the SCI community.

Additionally, the degree of locomotor recovery and, thus the level of physical activity after injury greatly affects the extent of CV dysfunction in chronic SCI patients. Many studies have shown that exercise training following SCI, both in the acute and chronic injury period, can favorably influence hemodynamic, cardiac, and vascular function. For instance, just four weeks of acutely-implemented passive hind limb cycling (PHLC) in rats has been shown to prevent myocardial fibrosis and cardiac decline, improve blood lipid profiles, and attenuate the severity of experimentally-induced AD (West *et al.* 2014, West *et al.* 2015). Likewise, various forms of exercise training in chronic SCI patients stabilizes hemodynamic parameters at rest and during orthostatic provocation, improves vascular function, and reduces arterial stiffness (Thijssen *et al.* 2005, Thijssen *et al.* 2006, Harkema *et al.* 2008). It is generally assumed that initiating exercise rehabilitation acutely, or even sub-acutely, following injury is beneficial by reducing chronic CV dysfunction, impeding the progression of cardiac and vascular decline, and taking advantage of the inherent central nervous system plasticity within spinal cord circuitry.

However, it is critical that the exercise intensity and timing initiation is optimal to avoid exacerbating CV dysfunction. For example, a study performed by Laird and colleagues (2009) found that treadmill training initiated three days post-SCI resulted in exaggerated pressor responses to colorectal distension, enhanced vasoconstrictor responses

to phenylephrine in renal vasculature, and increased CGRP immunoreactivity in the lumbar cord at six weeks post-injury (Laird *et al.* 2009). Likewise, studies in our lab have shown that swimming rehabilitation initiated within days of a thoracic contusion resulted in increased extravasation of a blood borne markers within the spinal cord and did not lead to improved over-ground locomotion chronically (Smith *et al.* 2009). Conversely, studies by West et al. (West *et al.* 2014, West *et al.* 2015) show that when exercise training is delayed to six days post-injury, a time when spinal shock has largely subsided and hemodynamics have begun to stabilize (Tsai *et al.* 1980, Krassioukov and Weaver 1995, Maiorov *et al.* 1997, Ditunno *et al.* 2004, Guly *et al.* 2008), animals develop chronic CV function that is not different from uninjured controls. However, the exercise employed in these studies was passive in nature, thus mechanisms to control CV dynamics are likely different than during active exercise (treadmill training, swimming, etc.). However, not much is known about how the system responds during active exercise at early time points post-injury, or whether the necessary adjustments in cardiac and vascular output can be appropriately accomplished by a newly injured system. Further, little is known about how incomplete contusion of the thoracic spinal cord and the propensity for increased in-cage activity leads to spontaneous recovery of CV control both at rest and during exercise challenge.

The primary purpose of this study was to evaluate hemodynamic function at rest and during an active exercise challenge following incomplete T3 contusive spinal cord injuries. Using implantable telemetry devices, the blood pressure and heart rate responses to a bout of active swimming exercise challenge were analyzed pre-injury and for ten weeks following contusion. Changes in cardiac structure and function were assessed over time using high resolution ultrasound (echocardiography). In an effort to explore possible

mechanisms for abnormal CV responses during exercise, Dobutamine infusion was employed to test cardiac performance in a dose-dependent manner during increased sympathetic activation, irrespective of sympathetic support from damaged spinal autonomic pathways. We hypothesized that incomplete contusion injury to the upper thoracic cord would result in abnormal cardiovascular control during instances of increased cardiopulmonary demand acutely after injury that is partially due to cardiac decline and altered autonomic modulation of cardiac function. Further, as hindlimb function recovers, increased in-cage activity would lead to enhanced volume loading of the heart and improved CV mechanics at rest and during exercise challenge.

Methods

Ethical Approval

All animal care and surgical procedures were performed in accordance with the NIH Guidelines and with the approval of the University of Louisville Institutional Animal Care and Use Committee.

Experimental Design

Experiments were conducted on adult female SD rats (250-300g; Harlan Laboratories, Indianapolis, IN, USA). Prior to injury, animals were implanted with telemetry devices to deliver measurements of arterial pressure and ECG (Data Sciences International®, St. Paul, MN; C50-PXT or HD-S11 transmitters). Experiments were completed in three phases in order to maximize the number of animals with telemetry devices. Phase I and II animals (n=3 and 4, respectively) were assessed weekly for ten

weeks after injury to look at acute and chronic responses to an exercise challenge. Phase III animals (n=4) were also followed for 10 weeks post-SCI; however, swimming assessments were not assessed at one week post-contusion. Pre-injury (baseline) recordings were collected for three weeks prior to SCI. Rats were then subjected to either a 12.5 g/cm mild (MILD, Phase I, n=3) or a 25 g/cm moderate injury (MOD, Phases II and III, n=4 each) contusion at the T3 spinal level using the NYU Impactor (Mascis, Rutgers University). An additional set of age-matched animals were used as non-injured controls (CON, n=8) for cardiac histology.

Telemetry Implantation

All animals were instrumented with C50-PXT or HD-S11 transmitters (Data Sciences® International, St. Paul, MN) for *in vivo* measurement of arterial pressure and ECG as previously described previously (Brockway *et al.* 1991). Briefly, under isoflurane anesthesia (2% in oxygen), a ventral midline incision was made in the skin and abdominal wall. The body of the transmitter was placed within the peritoneal cavity and sutured to the abdominal wall musculature. The MBP sensing cannula was inserted into the abdominal aorta slightly above the bifurcation of the iliac arteries and advanced rostrally to the point where the left renal vein courses over the aorta. The MBP catheter was fixed in place using a small amount of VetBond tissue adhesive (3 M™ Vetbond™ Tissue Adhesive, St. Paul, MN). The two biopotential leads were subcutaneously sutured in place under the 12th left rib and over the right pectoralis major muscle for ECG signal recordings in a Modified Lead II configuration (Data Sciences® International *public technical notes in webpage*). The abdominal wall musculature and skin were closed in layers using 4-0 nylon and 4-0

silk sutures, respectively. Post-operative care included daily injections of gentamicin sulfate for 7 days (20 mg/kg, SC), twice-daily injections of buprenorphine for 3 days (0.03 mg/kg, SC; and as needed for pain management thereafter), and twice-daily 5ml boluses of lactated ringers for three days (and as needed for hydration thereafter). Animals were allowed to recover for 7-10 days following device placement, after which pre-injury recordings of arterial pressure and ECG were collected at rest and during exercise challenge. In the event that a rat showed signs of peritonitis due to the transmitter implantation, daily doses of the non-steroidal anti-inflammatory ketoprofen (5 mg/kg SC) and additional gentamicin sulfate were administered until symptoms resolved.

Exercise Challenge Recording Protocol

Following recovery from implantation, rats were re-introduced to the swimming pool and testing conditions. Swimming has been used as both rehabilitation exercise and as an assessment for locomotor recovery following SCI in rodents (Smith *et al.* 2006, Gonzenbach *et al.* 2012). For the purposes of this study, swimming was used as a form of exercise to challenge the cardiopulmonary system and assess cardiovascular control after SCI. Briefly, swim assessments consisted of a four-minute session in which the rat is repeatedly placed at one end of a 5-ft long plexiglass pool and encouraged to swim to the opposite end where they exited via a padded ramp. Pool temperatures are maintained at 33-35 degrees C to give rats incentive to exit the pool but also to avoid problems associated with drastic drops in core body temperature and spasticity after injury. Uninjured rats can easily swim upwards of 45 laps in a typical exercise session. Beat-by-beat arterial pressure was collected at rest and in response to the exercise challenge at either 500 Hz (MILD,

Phase 1) or 1000 Hz (MOD, Phase 1 and 2). In-cage recordings of MBP and ECG were acquired before swimming (four minutes) and during exercise recovery (6-10 minutes). Baseline measurements were made 3 times per week for 3-4 weeks.

Spinal Cord Injury

Approximately five weeks after device placement, rats were given T3 contusion injuries using the NYU Impactor. Each animal was anesthetized with a Ketamine (50 mg/kg)/Xylazine (0.024 mg/kg)/ Acepromazine (0.005 mg/kg) cocktail (IP) and given glycopyrrolate (0.08 mg/kg, IM) prior to the contusion procedure. A dorsal midline incision was made in the superficial muscle overlying the T1 – T4 vertebrae. A single level laminectomy was made at the T2 vertebral level. Using clamps applied to the T1 and T4 spinous processes, the spine was immobilized and positioned for impact. The NYU impactor was then used to deliver either mild (MILD, Phase 1; 12.5 g/cm) or moderate (MOD, Phase II and III; 25 g/cm) weight drop contusion injuries. The muscle and skin overlying the injury was sutured in layers and antibiotic ointment was applied to the incision. Injured animals were monitored on heating pads until they recovered from the anesthesia. Rats were then doubly housed in standard cages for the remainder of the study. Post-operative care consisted of daily injections of gentamicin sulfate for 7 days (20 mg/kg, SC), twice-daily injections of buprenorphine for 3 days (0.03 mg/kg, SC; and as needed for pain management thereafter), and twice-daily 5ml boluses of lactated ringers for three days (and as needed for hydration thereafter). Manual bladder expression was conducted three times a day until reflexive voiding was re-established.

CV Analysis and Behavioral Assessments

CV data was collected using the PONEMAH® 5.0 software package and DataQuest Acquisition hardware (Data Sciences® International, St. Paul, MN). Initial arterial pressure and pressure-derived HR data analyses were performed in LabChart version 8.0 (ADInstruments, Colorado Springs, CO). Mean blood pressure (MBP) measurements were calculated for analysis during in cage rest, exercise, and exercise recovery. Mean blood pressure Excursion and HR Drop during swimming was assessed using a custom Excel macro. Briefly, HR Drop was determined by calculating the difference in the mean HR from the first to the last 15 seconds of the entire swim session. Mean blood pressure Excursion was assessed on a lap-by-lap basis and calculated as the average difference between the peak and trough values for each lap. Weekly Louisville Swim Scale (LSS) and BBB assessments were performed to track locomotor recovery.

In Vivo Cardiac Echocardiography and Dobutamine Stress Testing

In a subset of moderately contused animals (Phase II, n=4), echocardiographic assessments (pre-injury and weeks 1, 5, and 10 post-SCI) were performed using a high resolution ultrasound machine (VisualSonics Vevo® 3100) and transducer (MX250S, 24 MHz) designed for preclinical studies. Rats were anesthetized with isoflurane inhalation (1.75% in oxygen), the thorax was shaved, and the animal was placed in dorsal recumbency. Body temperature, heart rate, and arterial blood pressure were monitored using the telemetry system as previously described. Prior to Dobutamine infusion, standard measures of left ventricular structure and function were obtained using M-mode echocardiography in the parasternal short-axis view (SAX) at the midventricular level.

Once an acceptable view of the heart was obtained, the transducer was secured in a stereotaxic stand to minimize variation in image capture (VisualSonics) during the Dobutamine infusion protocol. Thereafter, the tail vein was cannulated using a 25-gauge butterfly needle and secured using surgical tape for Dobutamine infusion. Dobutamine was intravenously infused at progressively increasing doses (5, 10, 20, and 30 µg/kg/min) using an automated perfusion pump (KD Scientific, Holliston, MA). Each dose was infused for four minutes, after which M-mode imaging was performed before advancing to the next dose. Results for five cardiac cycles during expiration were averaged for comparison using the VEVO® LAB software. Blood pressure (mean, systolic and diastolic) and heart rate measures for the final minute of each infusion dose were analyzed using LabChart version 8.0 (ADInstruments, Colorado Springs, CO).

Study Termination and Histological Analysis

Upon completion of the study, rats were euthanized with an overdose of sodium pentobarbital (50 mg/kg IP), transcardially perfused with phosphate buffer and fresh dissected to remove the heart and spinal cord. The heart and SC were post-fixed with 4% paraformaldehyde and cryopreserved in 30% sucrose. Spinal cord tissue was sectioned at 30 µm in six sets and assessed for white matter sparing in and around the epicenter (Magnuson *et al.* 2005, Smith *et al.* 2006). Mid-ventricular heart tissue was sectioned at 10 µm and processed for collagen deposition with Masson's trichrome stain. Images of the left ventricular free wall were captured at 20X magnification. Analysis was completed from five separate sections at least 70µ apart using consistent camera settings. Collagen

deposition was calculated as a percent of the total area of the image and percentages from each section were averaged to deliver one value per animal (Radovits *et al.* 2013).

Statistical Analysis

Data from the phase II and III moderately-contused animals were combined for statistical analysis as there were no differences between these groups. Behavioral assessments (BBB and LSS) were analyzed using repeated measures analysis of variance (RM ANOVA) for time with the group factor (MILD and MOD). Cardiovascular parameters during in-cage rest and swimming were analyzed as raw data with mixed model (fixed effects) ANOVA. Post hoc t-tests were completed with Bonferroni correction. Following normalization to femur length, echocardiography data was analyzed using repeated measures analysis of variance (RM ANOVA) for comparisons in dose responses across time. Post hoc t-tests were completed with Tukey HSD. Terminal histological analyses were analyzed with Independent t-tests between means with equal or unequal variance, as appropriate, followed by Bonferroni correction for multiple comparisons. Statistical analyses were performed with SPSS (v22, Chicago, IL). Telemetry blood pressure data is shown as mean ± standard deviation (SD) and significance was set at $p \leq .05$. Ultrasound data is shown as mean ± standard deviation (SD) in the text and illustrated as mean ± standard error of the mean (SEM) in figures. Ultrasound data significance was set at $p \leq .05$.

Results

T3 contusion results in substantial tissue damage in and around the injury epicenter.

The percent of spared white matter (SWM) assessed as darkly stained compact white matter ranged from 3.40 to 9.52 percent overall (group average 6.325 ± 2.3 percent) for MOD animals (Figure 5A). There was a great deal of variability in the amount of SWM for mildly injured animals (Figure 5A; group average 16.47 ± 8.6 percent).

Locomotor assessments post-injury were executed at the beginning of each week prior to swimming exercise challenge (Figure 5B). Group comparisons revealed that MILD animals performed better on the BBB scale, with a significant difference in mean group score at weeks 1, 3, 4, 5, 6, 8, 9, and 10 (all time points, $p \leq .05$). Timewise comparisons showed that in both groups, BBB scores measured during week 1 were significantly lower than those measured at sub-acute and chronic time points (data not shown; MILD: week 1 vs. weeks 3, 4, 5, 6, 8, 9, and 10 $p \leq .05$; MOD: week 1 vs. all other weeks $p \leq .05$). MILD rodents consistently achieved weight-supported plantar stepping with consistent forelimb-hindlimb coordination at the terminal time point (week 10). Conversely, only a small proportion of the MOD rodents regained forelimb-hindlimb coordination and toe clearance remained poor in most of these animals.

Locomotor ability during swimming varied greatly across animals in both groups. Group comparisons revealed significant differences at two and three weeks post-SCI only, with MILD animals performing better on the LSS than MOD (Figure 5C; $p \leq .05$). Swimming ability did not improve over time post-injury in either group.

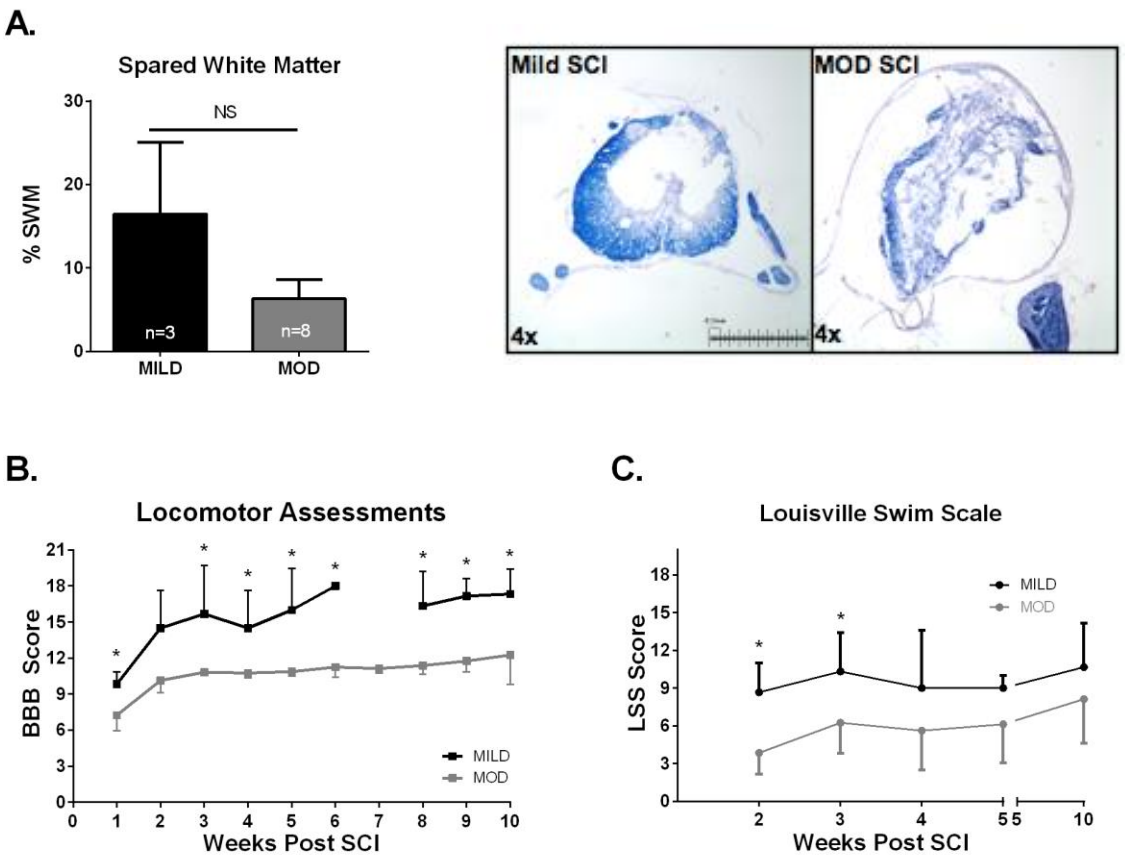

Figure 5**. Graded T3 contusion results in severity dependent tissue damage and locomotor deficits. (A)** Percent spared white matter at the injury epicenter following mild and moderate T3 contusion. **(B)** Group comparisons of weekly BBB scores over time in MILD and MOD animals. Significant group differences were noted at nearly all time points assessed. **(C)** Group comparisons of weekly performance during swimming assessments. Significant group differences are noted at two and three weeks post-SCI. Statistical significance was assessed using Mixed Model ANOVA with Bonferroni *post hoc* t-test. Data is displayed as mean ± SD and statistical significance was set as *p≤ .05.

CV function at rest is not compromised by incomplete high thoracic SCI

Weekly assessments of resting MBP and HR were completed in MILD and MOD. No differences were noted between groups prior to contusion injury (p= 1.0). Group differences in resting CV parameters following injury are highlighted in Table 1.

Incomplete contusion of the upper thoracic cord did not produce altered resting CV hemodynamics in MILD animals over time post-SCI. Conversely, animals that received moderate T3 contusions experienced resting tachycardia one week following incomplete SCI, that quickly resolved by two weeks post-contusion (Table 1; Week 1 vs. Pre-injury, p=.017; Week 1 vs. Week 2, p=.02). Mean arterial pressure in MOD animals was within normal ranges for all time points assessed post-injury (Table 1).

Hemodynamics during In Cage Rest

	Pre-Injury	Week 1	Week 2	Week 3	Week 4	Week 5	Week 10
Mean Blood Pressure (mmHg)							
Mild	120.31 ± 5.54	119.82 ± 6.77	116.82 ± 6.84	115.14 ± 1.13 †	118.59 ± 2.88	116.84 ± 7.19	115.11 ± 5.93
Moderate	120.92 ± 4.62	119.37 ± 5.75	122.69 ± 5.91	123.20 ± 5.13	122.93 ± 5.39	125.65 ± 5.48	122.10 ± 2.70
Heart Rate (bpm)							
Mild	413.50 ± 21.35	418.85 ± 14.23 †	415.59 ± 39.75	379.43 ± 56.25	394.34 ± 31.22 †	392.47 ± 30.02	419.13 ± 11.15
Moderate	422.84 ± 20.16	465.74 ± 22.50	410.12 ± 32.95	429.58 ± 40.30	443.98 ± 21.34	434.51 ± 17.51	442.58 ± 15.03

Hemodynamics during Swimming Exercise Challenge

	Pre-Injury	Week 1	Week 2	Week 3	Week 4	Week 5	Week 10
Mean Blood Pressure (mmHg)							
Mild	133.12 ± 2.65	119.88 ± 2.91	132.88 ± 2.91	135.07 ± 2.98	137.98 ± 2.59	134.17 ± 3.36	131.20 ± 3.13
Moderate	129.45 ± 1.62	114.25 ± 3.03	126.75 ± 1.78	130.24 ± 1.82	133.57 ± 1.58	134.77 ± 2.06	135.13 ± 1.92
Heart Rate (bpm)							
Mild	482.81 ± 8.41	378.45 ± 12.69 †	456.93 ± 11.48	451.50 ± 12.90	474.65 ± 10.60	463.02 ± 12.71	469.53 ± 8.12
Moderate	478.97 ± 5.15	475.02 ± 10.99	469.43 ± 7.03	475.28 ± 7.90	486.90 ± 6.49	487.39 ± 7.78	475.64 ± 4.97

Hemodynamics during Exercise Recovery

	Pre-Injury	Week 1	Week 2	Week 3	Week 4	Week 5	Week 10
Mean Blood Pressure (mmHg)							
Mild	127.02 ± 3.52	126.10 ± 2.37 ††	122.57 ± 4.08	126.33 ± 5.53	124.39 ± 8.45	122.88 ± 6.76	123.84 ± 6.11
Moderate	127.23 ± 2.94	112.96 ± 3.17	115.36 ± 7.89	118.67 ± 7.08	120.73 ± 5.25	121.95 ± 3.75	123.48 ± 4.68
Heart Rate (bpm)							
Mild	464.88 ± 11.06	333.54 ± 30.14 ††	415.95 ± 40.09	422.01 ± 21.88 ††	445.49 ± 8.32 †	439.30 ± 20.37 †	453.14 ± 7.04 †
Moderate	480.75 ± 15.47	460.65 ± 11.95	463.56 ± 25.23	474.61 ± 18.47	487.93 ± 24.32	488.48 ± 16.79	477.92 ± 20.99

Statistical Key: Group Differences †$p \leq .05$; ††$p \leq .01$

Table 1. **Group comparisons of average blood pressure and heart rate over time in MILD and MOD animals.** Statistical significance was assessed using Mixed Model ANOVA with Bonferroni *post hoc* t-test. Data is displayed as mean ± SD and statistical significance was set as $^{\dagger}p \leq .05$ and $^{\dagger\dagger}p \leq .01$.

Responses to acute exercise challenge are altered following high thoracic contusion

CV responses to an exercise challenge (swimming) were evaluated weekly before and after upper thoracic SCI. During pre-injury assessments, there was a modest pressor response at the initiation of swimming that was maintained throughout the four-minute session. Heart rate was also increased and remained stable for the duration of the exercise challenge. One week following mild and moderate contusion, animals were still able to mount a modest pressor response during swimming, although it was somewhat blunted. Conversely, the HR dropped considerably as the swimming session progressed and remained low for at least several minutes after the exercise challenge had ceased. Figure 6 illustrates a representative recordings of MBP (A) and HR (C) during in-cage rest, swimming exercise, and exercise recovery in an individual rodent at baseline (shown in black) and acutely post-T3 moderate contusion (shown in red). The bradycardic response to swimming exercise, measured as HR Excursion, largely recovered by two weeks post-injury in both MILD (data not shown, Week 1 vs. Pre-injury and Weeks 3, 4, and 5, $p \leq .01$) and MOD animals (Figure 7D, Week 1 vs. Pre-injury and Weeks 5 and 10, $p \leq .05$). Also note that following the exercise challenge, animals experienced short periods of exertional hypotension while recovering in their cages acutely after injury (Figure 6A, arrows). While this hypotensive response to exercise was transient in MILD animals, rodents with moderate contusive SCI experienced sustained post-exertional hypotension at one and two weeks post-injury that was significantly greater in magnitude than baseline measurements (Figure 7C, $p \leq .05$).

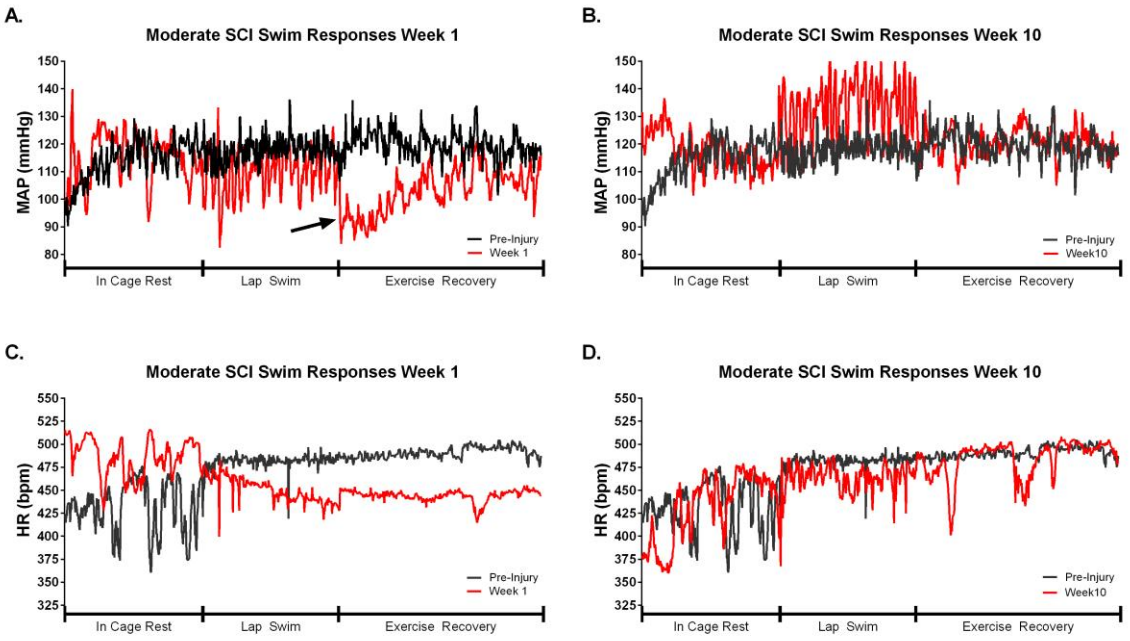

Figure 6. **Rodents with high thoracic contusion are unable to maintain cardiovascular control during swimming exercise challenge.** Representative MBP **(A)** and HR **(C)** responses to swimming exercise challenge before (black lines) and one week post-T3 moderate contusion (red lines). Note the post-exertional hypotension during the Exercise Recovery period acutely after injury (arrow). Representative MBP **(B)** and HR **(D)** responses to swimming exercise challenge before (black lines) and 10 weeks post-T3 moderate contusion (red lines). Note the elevated pressor response to swim challenge at ten weeks post-SCI. Data has been down sampled from 1000 Hz to display one data point per second. Individual recordings of In Cage Rest, Lap Swim, and Exercise Recovery are displayed as one continuous MBP or HR trace.

Lack of CV control persists for many weeks following moderate high thoracic SCI

Deficits in CV function during exercise persisted for many weeks following moderate T3 contusion injuries. While the average MBP over the four-minute exercise session was not different from pre-injury baseline measurements after week 1 (Figure 7B), the range in MBP values measured on a lap-by-lap basis increased substantially over time post-injury. Visually, this is shown as large saw-tooth patterns during chronic swim challenge in representative hemodynamic traces (Figure 7B) and is quantified as MBP Excursion (Figure 7A, MBP Excursion, Weeks 3, 4, 5, and 10 vs. Pre-injury, $p \leq .01$).

Mildly contused animals did not experience increased MBP excursion during exercise challenge over time (data not shown) and absolute changes in HR from the beginning to the end of the four-minute exercise session quickly returned to pre-injury baseline values after week one (data not shown). Average HR during swimming exercise and exercise recovery were also not different past week 1 (data not shown).

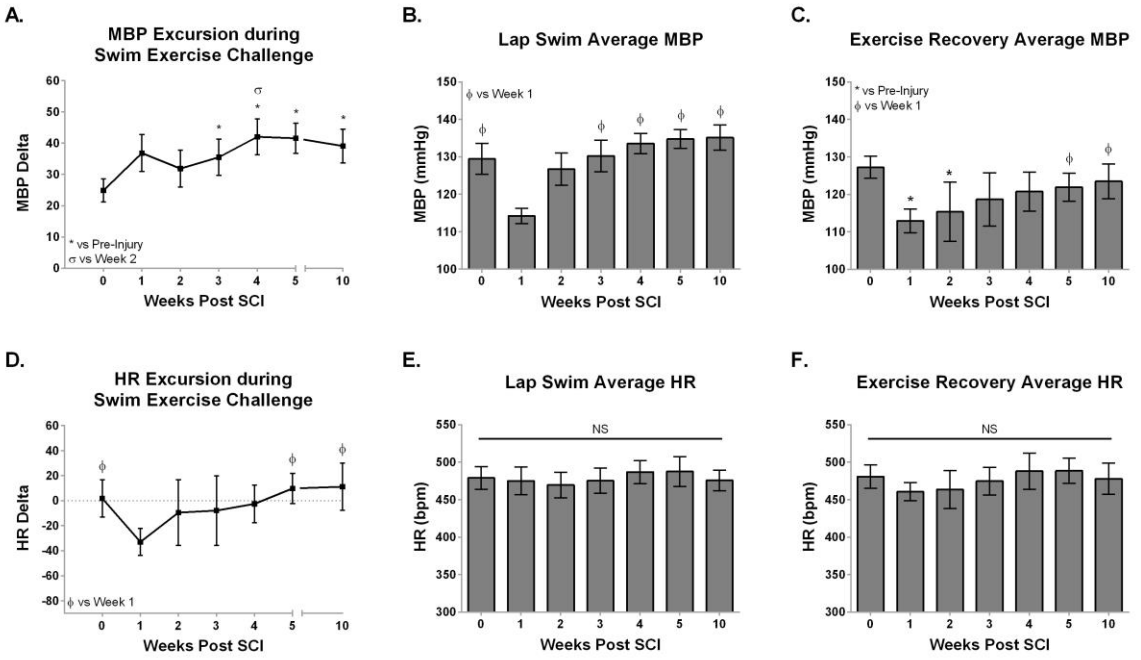

Figure 7. **Lack of cardiovascular control during exercise challenge persists for many weeks following moderate T3 contusion. (A)** Average MBP Excursion measured prior to SCI and each week following injury. Oscillatory changes in MBP during each swim lap are averaged for each time point. The inability to maintain MBP during exercise challenge increased with time post-injury. **(B)** Average MBP during the four-minute swim session. Note the exertional hypotension one week after injury. **(C)** Average MBP during the Exercise Recovery period. **(D)** HR Excursion during the four-minute swim session. Note the drastic drop in HR from the beginning to the end of swimming acutely after injury. **(E)** Average HR during the four-minute swim session. **(F)** Average HR during the Exercise Recovery period. Statistical significance was assessed using Mixed Model ANOVA with Bonferroni *post hoc* t-test. Data is displayed as mean ± SD and statistical significance was set as *$p \leq .05$ vs. pre-injury, $^{\phi}p \leq .05$ vs. week 1, and $^{\sigma}p \leq .05$ vs. week 2.

Echocardiography

Blood pressure measurements (systolic, diastolic, and mean blood pressure) during echocardiography were significantly reduced at all time points assessed after injury (Figure 8A-C; Pre-injury vs. Weeks 1, 5, and 10, $p \leq .001$), indicating blunted pressor responses in the presence of isoflurane anesthesia. Echocardiography revealed that moderately contused female rats had reduced left ventricular internal diameter during diastole (LVIDd) at five and ten weeks post-SCI compared to pre-injury (Table 2, Pre-injury vs. Week 5, $p \leq .001$; Pre-injury vs. Week 10, $p = .046$). End-diastolic volume (EDV) was also reduced at these time points (Figure 8H; Pre-injury vs. Week 5, $p \leq .001$; Pre-injury vs. Week 10, $p = .042$), while end-systolic volume (ESV) remained unchanged (Figure 8I). Further, cardiac output (CO) was decreased at five weeks post-SCI (Figure 8F; Pre-injury vs. Week 5, $p = .024$), most likely due to a lower stroke volume (SV), although this only approached significance (Figure 8D, E; SV $p = .06$).

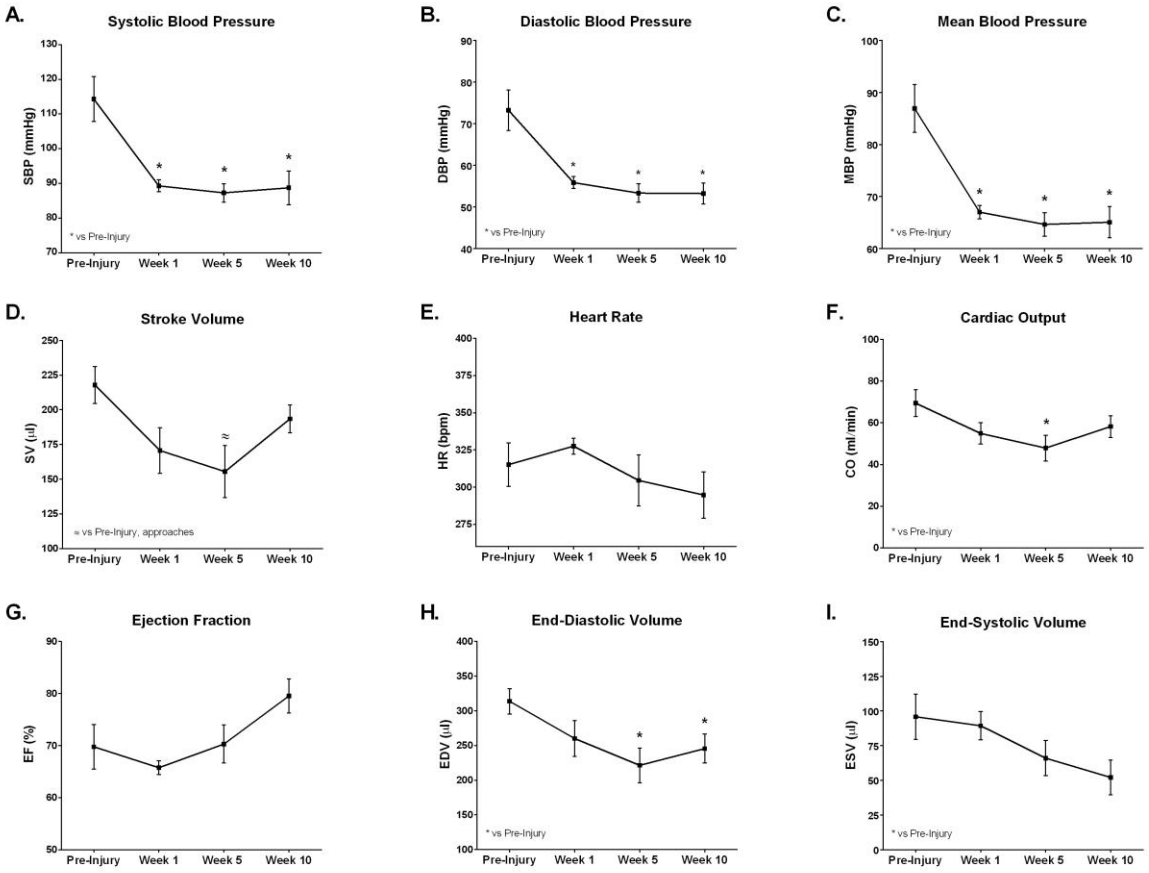

Figure 8. **Assessment of cardiac structure and function following T3 moderate contusion using echocardiography**. Compared to pre-injury, pressor responses in the presence of isoflurane anesthesia were blunted at all time points assessed (SBP **(A)**, DBP **(B)**, and MBP **(C)**). Measures of systolic function (SV **(D)**, CO **(F)**, and EF **(G)**) over time indicate a recovery of function at 10 weeks post-SCI. End-diastolic volume **(H)** was significantly lower at chronic time points; ESV **(I)** and HR **(E)** were not different from pre-injury. Data is displayed as mean ± SEM and significance is set at *p≤ .05 vs. pre-injury.

Influence of Dobutamine on Cardiac Structure and Function

Prior to SCI, Dobutamine infusion resulted in a dose-dependent decrease in EDV and ESV, with a concurrent increase in ejection fraction (Table 2). Flow indices (SV, HR, and CO) did not change significantly with increased Dobutamine concentrations (Table 2).

One and five weeks after moderate SCI, Dobutamine infusion resulted in a decrease in ESV with no change in EDV measurements (Table 2). Similar to pre-injury assessments, moderately contused animals also experienced an increase in EF with increasing concentration of Dobutamine (Table 2). One week following contusion, Dobutamine administration elicited a dose-dependent increase in HR, SV, and CO (Table 2). The dose-dependent increase in stroke volume was still present at five weeks post-SCI.

Much like pre-injury measurements, Dobutamine induced dose-dependent decreases in ESV and increases in EF following injury (Table 2). Unlike pre-injury, however, at ten weeks post-SCI moderately contused rodents responded to increasing concentrations of Dobutamine with increases in HR (Table 2). Stroke volume and CO were unaffected at this later time point. Hemodynamic responses to Dobutamine infusion are shown in Table 2. Systolic and mean blood pressure were not significantly affected by increasing concentrations of Dobutamine infusion. Diastolic blood pressure, however was significantly lower at higher concentrations of Dobutamine during pre-injury assessments and again at ten weeks post contusion. No differences were seen in body weight, heart mass, or collagen deposition verses uninjured, age-matched controls (Table 2); however, there was a significant difference in the body mass to heart mass ratio ($p = .042$).

Anatomical Data

	T3 MOD SCI		Uninjured Control
Body Mass	290.13 ± 13.01		298.88 ± 18.06
Heart Mass	1.0837 ± 0.1097		1.0022 ± 0.0963
Heart/Body Mass Ratio	0.0037 ± 0.0003	ᵀᵀ	0.0034 ± 0.0004
Area Collagen	0.0031 ± 0.0015		0.0031 ± 0.0006
Percent Collagen	0.8400 ± 0.4000		0.8264 ± 0.1572

KEY
* Dose vs. Dose 0ug, p ≤ .05
† Dose vs. Dose 5ug, p ≤ .05
ǂ Dose vs. Dose 10ug, p ≤ .05
ᵀᵀ Group Difference, p ≤ .001

Echocardiographic Data

Dimensions	***Dose***	**Pre-Injury**		**Week 1**		**Week 5**		**Week 10**	
LVIDd (mm)	0 ug	7.48 ± 0.19		6.86 ± 0.66		6.58 ± 0.82		6.87 ± 0.63	
	5 ug	7.60 ± 0.33		7.30 ± 0.52		6.88 ± 0.69		6.53 ± 1.04	
	10 ug	7.26 ± 0.26		7.16 ± 0.68		6.63 ± 0.58		6.69 ± 0.60	
	20 ug	7.06 ± 0.18	†	7.16 ± 0.55		6.61 ± 0.39		6.74 ± 0.46	
	30 ug	7.00 ± 0.26	*†	6.94 ± 0.53		6.51 ± 0.44		6.61 ± 0.56	
LVIDs (mm)	0 ug	4.28 ± 0.57		4.34 ± 0.52		3.87 ± 0.73		3.42 ± 0.84	
	5 ug	4.34 ± 0.72		3.79 ± 0.37		3.41 ± 1.29		2.68 ± 0.57	
	10 ug	2.88 ± 0.68	*†	2.87 ± 0.54	*	2.68 ± 0.87	*	2.21 ± 0.54	*
	20 ug	2.18 ± 0.50	*†	2.55 ± 0.80	*†	2.51 ± 1.86	*	2.01 ± 0.56	*
	30 ug	2.17 ± 0.65	*†	2.13 ± 0.70	*†	1.75 ± 0.81	*†	1.61 ± 0.48	*†
EDV (µl)	0 ug	297.04 ± 17.44		246.10 ± 53.67		225.01 ± 60.60		246.40 ± 51.05	
	5 ug	307.35 ± 29.81		282.22 ± 44.57		247.86 ± 54.61		223.28 ± 76.00	
	10 ug	277.89 ± 22.55		270.61 ± 57.04		227.44 ± 43.72		232.17 ± 46.20	
	20 ug	260.84 ± 15.36	†	269.92 ± 46.41		225.32 ± 30.09		235.36 ± 35.84	
	30 ug	256.02 ± 21.41	*†	251.59 ± 41.86		217.28 ± 32.62		225.64 ± 42.46	
ESV (µl)	0 ug	83.52 ± 26.21		85.91 ± 23.55		67.05 ± 30.93		51.27 ± 30.76	
	5 ug	86.98 ± 31.12		62.17 ± 13.93		54.92 ± 49.72		27.98 ± 12.92	
	10 ug	33.57 ± 17.97	*†	32.68 ± 13.47	*	29.57 ± 21.82		17.59 ± 9.15	
	20 ug	16.82 ± 8.73	*†	26.12 ± 16.93	*	36.90 ± 54.53		14.10 ± 8.70	
	30 ug	17.38 ± 10.84	*†	16.81 ± 11.21	*†	11.56 ± 12.85	*†	8.19 ± 6.35	*

Systolic Function	***Dose***	**Pre-Injury**		**Week 1**		**Week 5**		**Week 10**	
SV (µl)	0 ug	213.52 ± 30.51		160.18 ± 31.14		157.97 ± 45.71		195.14 ± 24.15	
	5 ug	220.37 ± 7.16	*	220.05 ± 35.29		192.94 ± 35.19		195.30 ± 77.31	
	10 ug	244.32 ± 15.78	*	237.94 ± 46.22		197.88 ± 39.36		214.58 ± 47.15	
	20 ug	244.02 ± 8.50	*	243.80 ± 38.39		188.42 ± 27.71		221.26 ± 33.75	
	30 ug	238.63 ± 10.74	*	234.78 ± 32.61		205.72 ± 24.90	*	217.45 ± 38.45	
EF (%)	0 ug	71.85 ± 9.09		65.37 ± 3.14		70.32 ± 8.92		80.19 ± 7.90	
	5 ug	72.19 ± 7.97		78.03 ± 3.36		79.42 ± 15.17		85.97 ± 9.08	
	10 ug	88.13 ± 5.98	*†	88.22 ± 3.51	*	87.15 ± 8.04	*	92.15 ± 4.57	
	20 ug	93.65 ± 3.02	*†	90.64 ± 5.91	*	85.38 ± 20.81	*	94.02 ± 3.36	*
	30 ug	93.42 ± 3.79	*†	93.67 ± 3.87	*†	95.02 ± 4.99	*†	96.51 ± 2.18	*
Q (ml min-1)	0 ug	69.26 ± 15.91		50.53 ± 6.25		46.55 ± 14.79		58.77 ± 12.77	
	5 ug	67.94 ± 4.86		66.40 ± 6.12		55.08 ± 16.02		59.83 ± 28.61	
	10 ug	82.90 ± 5.40		76.68 ± 7.61		57.38 ± 18.33		68.32 ± 21.60	
	20 ug	87.28 ± 1.58		84.50 ± 10.33	*	60.79 ± 9.28		76.15 ± 11.87	
	30 ug	86.09 ± 5.05		84.94 ± 8.27	*	69.01 ± 9.56		78.32 ± 10.81	

Diastolic Function	***Dose***	**Pre-Injury**	**Week 1**	**Week 5**	**Week 10**
E (cm s-1)	0 ug	723.19 ± 131.09	627.70 ± 116.52	690.76 ± 68.30	739.94 ± 67.51

Hemodynamics	***Dose***	**Pre-Injury**		**Week 1**		**Week 5**		**Week 10**	
SBP (mmHg)	0 ug	114.32 ± 12.91		89.29 ± 3.43		87.28 ± 5.31		88.75 ± 9.71	
	5 ug	112.79 ± 8.78		105.60 ± 7.80		92.64 ± 11.74		95.83 ± 10.52	
	10 ug	113.52 ± 8.64		105.65 ± 4.70		95.29 ± 11.27		104.85 ± 14.58	
	20 ug	111.49 ± 8.70		103.27 ± 5.14		97.51 ± 5.61		102.50 ± 14.70	
	30 ug	106.13 ± 8.30		98.98 ± 5.17		93.62 ± 7.36		94.20 ± 10.61	
DBP (mmHg)	0 ug	73.25 ± 9.83		55.86 ± 2.87		53.34 ± 4.40		53.25 ± 5.10	
	5 ug	70.73 ± 7.60		55.66 ± 7.96		49.89 ± 7.46		52.57 ± 5.09	
	10 ug	66.15 ± 7.70		52.23 ± 5.62		48.94 ± 3.19		53.31 ± 5.00	
	20 ug	64.50 ± 6.78	*	51.54 ± 4.53		47.67 ± 2.20		52.18 ± 3.55	
	30 ug	60.45 ± 6.21	*†	49.45 ± 5.92	*†	46.62 ± 2.83		47.67 ± 3.38	
MBP (mmHg)	0 ug	86.94 ± 9.22		67.01 ± 2.64		64.66 ± 4.55		65.08 ± 6.02	
	5 ug	84.75 ± 4.72		72.31 ± 7.35		64.14 ± 8.65		66.99 ± 6.01	
	10 ug	81.94 ± 4.94		70.03 ± 3.83		64.39 ± 5.04		70.48 ± 7.50	
	20 ug	80.16 ± 4.43		68.79 ± 3.24		64.28 ± 1.96		68.95 ± 6.75	
	30 ug	75.68 ± 3.70		65.96 ± 4.96		62.29 ± 4.14		63.18 ± 4.61	
HR (bpm)	0 ug	315.07 ± 29.28		327.55 ± 10.77		304.45 ± 34.31		294.68 ± 31.23	
	5 ug	304.79 ± 15.65		310.50 ± 28.22		295.90 ± 40.74		303.17 ± 28.08	
	10 ug	334.05 ± 13.94		333.11 ± 32.30		299.89 ± 43.12		316.43 ± 31.25	
	20 ug	348.71 ± 13.65		355.37 ± 39.98		328.57 ± 17.43		338.24 ± 22.14	
	30 ug	354.24 ± 9.28		374.11 ± 45.38	†	338.15 ± 9.10		357.13 ± 47.34	*

Table 2. **Anatomical and echocardiographic data of moderately-contused rodents.** LVIDd, left ventricular internal diameter during diastole; LVIDs left ventricular internal diameter during systole; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; Q, cardiac output; E, transmitral filling velocity during early diastole; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. Data is displayed as mean ± SD. *p≤ .05 dose vs. 0ug dose; †p≤ .05 dose vs. 5ug dose; ǂp≤ .05 dose vs. 10ug dose; and ᵀᵀp≤ .05 moderate vs. uninjured control group differences.

Discussion

Summary of findings

This is the first study to demonstrate that incomplete high thoracic contusion spinal cord injury results in abnormal CV control during an active exercise challenge. This abnormal hemodynamic response to exercise, manifested as the inability to maintain tight control over blood pressure, progressively worsens with time post-injury even though the animals do not display deficits at rest. Further, using echocardiography, we have shown that while there is attenuated systolic function at five weeks, this dysfunction is transient and largely recovers by ten weeks post-injury suggesting that cardiac decline is not solely responsible for the poor blood pressure control during exercise in our chronic incomplete SCI model.

Lack of CV control at rest and in response to exercise challenge

Cardiovascular control (or lack thereof) following high thoracic SCI has been investigated extensively in clinical settings, concluding that patients generally present with resting bradycardia and hypotension (West *et al.* 2012, West *et al.* 2013). Bouts of OH and AD are also common, and can be a limiting factor in rehabilitation efforts (Harkema *et al.* 2008, Weaver *et al.* 2012). However, preclinical studies investigating the effects of high thoracic SCI on hemodynamic stability have provided varied results, most likely due to the heterogeneity of the study conditions. For instance, in agreement with clinical data, a study by West et al (West *et al.* 2015) showed that complete transection of the upper thoracic spinal cord resulted in persistent hypotension beginning one week after injury. Conversely, we have found that animals with both mild and moderate injuries retain the ability to

control blood pressure within normal limits while at rest. Other laboratories have also reported that rodents with incomplete injuries retain blood pressure values that are not significantly different from pre-injury measurements (Maiorov *et al.* 1998, Mayorov *et al.* 2001). Reasons for this disparity likely lie in the fact that incomplete contusion injury spares, at the very least, a small proportion of the sympathetic preganglionic neurons important for CV control and the descending medullary axons innervating those neurons.

Despite normal hemodynamic control at rest, the animals in this study were unable to maintain CV output during instances of increased cardiopulmonary demand. Incomplete contusion to the upper thoracic cord resulted in severe bradycardia during swimming exercise acutely and large oscillations in blood pressure chronically. Under normal conditions, rodents respond to the four-minute swim challenge with increases in HR and BP that are sustained throughout the exercise session. One week after injury, rodents were unable to generate an increase in HR, and any pressor responses were blunted. The bradycardic response to exercise (i.e. HR Excursion) at this early time point likely results from neurogenic shock, in which the sympathetic circuitry is unable to effectively elicit increases in cardiac rate. Clinical reports of neurogenic shock primarily occur within the first couple of days following injury (Guly *et al.* 2008, Mallek *et al.* 2012, Summers *et al.* 2013); however, studies looking at this condition due so in the resting state, and little is known about how the system responds to instances of increased cardiopulmonary demand acutely.

Blood pressure variability, assessed on a swimming pool lap-by-lap basis (i.e. BP Excursion) was significantly greater for moderately-contused animals at nearly all time points when compared to pre-injury measurements. The inability to control BP within a

narrow range is a significant pathology that has been shown to negatively impact cardiac and vascular structure and function. For instance, rodent studies examining the effects of recurrent bouts of AD show that labile blood pressure results in vascular hyper-responsiveness to α-adrenoceptor activation (Arnold *et al.* 1995, Alan *et al.* 2010). Likewise, periods of elevated blood pressure during pregnancy can induce acute hypersensitivity to various vasopressors and chronic endothelial cell dysfunction despite the resolution of preeclampsia following delivery (Gant *et al.* 1973, Chambers *et al.* 2001, Agatisa *et al.* 2004). As such, epidemiological evidence suggests that women who experienced preeclampsia are at greater risk for hypertension and subsequent CVD than those who remained normotensive during pregnancy (Jonsdottir *et al.* 1995, Hannaford *et al.* 1997). While the swimming exercise challenge does not elicit maximum blood pressure values that would be deemed clinically detrimental, the mere "swing" in blood pressure is worth noting and deserves further investigation as repetitive elevations in arterial pressure may induce shear injury to the vascular endothelium resulting in subsequent CV complications.

Temporal Assessment of Cardiac Structure and Function

Numerous studies, both clinical and preclinical, have shown that high level SCI results in reduced systolic function (CO, SV, EF) and attenuated heart rate responses at rest (Kessler *et al.* 1986, West *et al.* 2014). Similarly, the present results illustrate a progressive decline in cardiac function that reaches significance at five weeks post-injury. However, this decline is transient; by ten weeks post-SCI rodents with moderate T3 contusion injuries present with cardiac flow indices (SV, CO) that are not different from pre-injury

measurements. In agreement with what others have noted in clinical settings, contusion injury to the upper thoracic cord leads to decreased ventricular diameter in our animals at ten weeks post-SCI (Kessler *et al.* 1986, West *et al.* 2012). Given the sustained reduction in EDV at later time points and the propensity for reduced blood pressure during echocardiography, it is reasonable to suggest that the reduction in ventricular diameter is due to chronic volume and pressure unloading of the heart. Typically, the loss of sympathetic tone below the level of the lesion and reduced hindlimb activity leads to attenuated pre-load and stroke volume, which contributes to an altered Starling curve and impaired contractility over time. However, given that our animals have chronic flow indices that are not different from pre-injury measurements and the deposition of collagen is minimal, it appears as though the left ventricle is able to function properly by ten weeks post-injury.

Further, cardiac function and the ability to augment HR after thoracic SCI is heavily dependent on the level of the lesion and, thus, the number of spared sympathetic fibers in and around the injury epicenter. As such, tetraplegic patients present with lower resting heart rates than individuals in the able-bodied population and those with paraplegia (Zhu *et al.* 2013). The critical sympathetic outflow modulating cardiac function arises from the T1 to T5 segments, with the majority of axons residing at the T1 level. In the current study, the rostral spread of the contusion injury did reach the T1 segment. However, only the dorsal columns were affected and the interomediolateral cell columns appeared intact following histological analysis. While the proportion of spared sympathetic fibers and the degree of decentralization was not assessed, it is reasonable to suggest that the sufficient numbers of sympathetic preganglionic neurons responsible for cardiac contractility

remained and were able to deliver the appropriate sympathetic drive to the heart myocardium for appropriate maintenance of CV function at the ten week time point. Therefore, the reduction in EDV could be a consequence of reduced sympathetic tone in vascular beds below the lesion, an increase in the heart/body mass ratio, or a combination of both.

Responses to Dobutamine Stress Testing

Dobutamine stress echocardiography is a clinical tool that can be used to investigate cardiac responses in the presence of enhanced sympathetic activation. Following high level SCI, spinal circuitry responsible for increasing HR and the force of ventricular contraction is disrupted, leading to blunted cardiac responses especially during times of increased cardiopulmonary demand such as exercise (West *et al.* 2014). Dobutamine, a beta-1 agonist, therefore can be used as a surrogate of autonomic modulation to investigate how the heart responds to exercise provocation in a dose-dependent, controlled manner irrespective of damaged spinal cord circuitry.

In uninjured rodents, both male and female, Dobutamine administration has been shown to elicit an increase in CO and EF with a concurrent decrease in EDV and ESV (Plante *et al.* 2005). In this study, Dobutamine infusion resulted in an increased EF due in part to a decrease in ESV at all time points assessed. Interestingly, at one week post moderate contusion animals responded to low doses of Dobutamine with augmented SV and CO. The mechanisms for this acute response are likely multifactorial; however, it is reasonable to suggest that neurogenic shock and the general lack of sympathetic output in the resting state are contributors. As suggested above, the presence of neurogenic shock

acutely after high thoracic injury is likely responsible for the blunted HR responses noted during exercise one week following SCI in our rodents. This argument is strengthened by the finding that the administration of Dobutamine, which restored sympathetic tone to the heart, was able to partially augment the chronotropic ability of the myocardium resulting in increased HR responses and flow indices. At five weeks post-SCI, Dobutamine administration continues to elicit exaggerated responses (SV) in our animals. Although no study to date has examined the role of cardiac beta-receptors following high thoracic SCI, it is likely that changes in the number and/or sensitivity of these receptors is partially responsible for this phenomenon. Given that alpha-adrenergic receptors in the vascular system develop increased sensitivity to norepinephrine over time post-injury, it is reasonable to suggest that similar changes are occurring in cardiac beta-receptors (Mathias *et al.* 1976, Yeoh *et al.* 2004, Brock *et al.* 2006). By ten weeks post-injury, responses to Dobutamine infusion were equivalent to baseline measures suggesting that the system adapted, either centrally or peripherally, to reduced SNS activity and/or altered cardiac mechanics in the wake of reduced pre-load of the heart.

Exercise and rehabilitation are important aspects of patient care in the SCI community. While there have been numerous accounts of improved CV function following exercise in the chronic period, few studies have investigated the effects of acutely implemented exercise on CV function. Acute exercise/training should be investigated given the high degree of central nervous system plasticity early post-injury and the rapid decline in CV structure and function in the wake of limited mobility. In a preclinical study already mentioned, acute passive hindlimb cycling was shown to improve many aspects of CV health, including resting hemodynamics and responses to experimentally-induced AD

(West *et al.* 2014, West *et al.* 2015). While these findings suggest potential avenues for improving patient health in the clinic, more information is needed about other forms of exercise/rehabilitation and their effects, both negative and positive, on CV health and function in spinal cord injured patients. Body weight-supported treadmill training (BWSTT) is often used in the clinic and has been shown to improve a wide range of dysfunction including allodynia, micturition, and glucose intolerance (Hutchinson *et al.* 2004, Phillips *et al.* 2004, Ward *et al.* 2014). However, the timing and intensity of this type of rehabilitation is critical. The purpose of the present study was to determine if a newly injured autonomic system was capable of eliciting appropriate CV responses to exercise and to better understand the impact of acute training on CV outcomes. Here we show that rodents with acute T3 contusions cannot effectively mount or maintain appropriate BP and HR during instances of increased cardiopulmonary demand. Therefore, robust exercise strategies may meet with failure if initiated too early in the recovery process. Further, given the substantial spontaneous recovery of hindlimb function and trunk stability exhibited by rodents with SCI, exercise paradigms must surpass the animal's innate ability to retrain themselves in their cages. Indeed, the temporal progression of cardiac decline and subsequent stabilization of function in concert with the recovery in stepping ability as assessed by the BBB supports this notion. With improvements in over-ground locomotion comes increased volume and pressure loading of the heart, which in turn creates a new steady-state for CV mechanics. The application of acute training programs, therefore, must take into account the ever evolving system and adapt exercise prescription accordingly.

Concluding remarks

In summary, we have shown, for the first time that rodents with incomplete, high thoracic injuries are unable to effectively maintain CV control during an active exercise challenge despite having normal CV parameters at rest. The presentation of hemodynamic dysfunction evolves temporally, and deficits in CV control are likely due to a combination of central and peripheral mechanisms. Additionally, cardiac decline in these animals is transient, demonstrating improvements in the chronic phase (5-10 weeks) likely reflecting the influence of recovery in over-ground locomotion and volume/pressure loading of the left ventricle. Further, our results highlight the substantial spontaneous recovery of hindlimb function and trunk stability in concert with stabilized/improved CV function, even following high thoracic contusion injuries. With improved hindlimb movement (BBB scores >10) comes increased venous return and pressure loading of the heart, which in turn, improves CV mechanics by 10 weeks post-injury. Thus, the application of acute training programs should take into account the plasticity present in both central and autonomic circuitry and the temporal evolution of spinal/neurogenic shock and recovery.

CHAPTER III

ABNORMAL CARDIOVASCULAR CONTROL DURING ACTIVE EXERCISE CHALLENGE FOLLOWING INCOMPLETE, LOW THORACIC SCI

Introduction

Cardiovascular (CV) dysfunction is a major contributor to the increased morbidity and mortality experienced by the spinal cord injury (SCI) patient population (Warburton *et al.* 2007, Inskip *et al.* 2012). Autonomic changes related to the level and severity of the lesion greatly affect the extent of CV dysfunction following injury (Weaver *et al.* 2012). Specifically, disruption of the sympathetic circuitry involved in CV control and homeostasis can lead to systemic and/or orthostatic hypotension (OH) and autonomic dysreflexia (AD), both of which make activities of daily living and participation in activity-based rehabilitation difficult (Harkema *et al.* 2008, Weaver *et al.* 2012). Further, these complications are exacerbated by the extended periods of physical inactivity following SCI, leading to lifestyles that do not support healthy cardiac or vascular function. Risk factors associated with sedentary lifestyles and subsequent cardiovascular disease (CVD), including glucose intolerance and alterations in body composition are, as a result, higher in SCI patients (Devillard *et al.* 2007, Myers *et al.* 2007, Bauman and Spungen 2008), emphasizing the need to discover ways to counteract these maladaptive changes.

The segmental organization of the sympathetic component of the autonomic nervous system (ANS) is particularly important in determining the effect of SCI on CV function. While the parasympathetic component of the ANS is extraspinal via the vagus nerve, the sympathetic preganglionic neurons that provide excitatory drive to the heart and upper body vasculature reside within the spinal cord at thoracic levels T1 to T4/5 (Hou and Rabchevsky 2014). Thus, the majority of animal studies investigating the impact of SCI on CV and autonomic function utilize complete spinal transections of the upper thoracic spinal cord. While these endeavors are critical to our understanding of CV and cardiac pathology after injury, there is a large population of patients with lesions at lower thoracic levels that suffer from debilitating bouts of OH, resting tachycardia, and cardiac insufficiency during exercise (Inskip *et al.* 2009). Incomplete paraplegia constitutes nearly half of the SCI patient population, with many patients having lesions between T5-6 and L2. While these injuries spare sympathetic fibers responsible for cardiac function, they disrupt the autonomic modulation of fibers supplying vasculature in the splanchnic bed and lower extremities. These vascular beds are critical for overall CV homeostasis as the gut receives nearly 60% of the total cardiac output at rest and holds approximately one-third of the total blood volume at any given time (Kreulen 2003). The mesenteric arterial system contributes significantly to the overall vascular resistance and thus is crucial for maintaining arterial pressure, in particular during instances of increased cardiopulmonary demand (i.e. exercise) in which splanchnic vasoconstriction shunts the necessary blood volume to working musculature (Kreulen 2003). Accordingly, understanding how maladaptive changes in the autonomic control of these two major vascular beds (splanchnic and lower

extremity) contribute to the observed CV dysfunction and subsequent disease is essential to speed the development of effective therapeutics for SCI patients.

Spinal cord injury forces patients abruptly into a period of very low physical activity, and many are truly sedentary for weeks or months post-injury. This inactivity results in substantial physical deconditioning (Nash 2005) and maladaptive vascular remodeling below the lesion (Thijssen *et al.* 2012). These changes, including reduced vessel diameter and blood flow, impaired endothelial function, and altered shear stress, occur very rapidly during periods of immobility typical of the SCI patient population (Boot *et al.* 2002, de Groot *et al.* 2006, Thijssen *et al.* 2012). Fortunately, many studies have shown that various exercise paradigms, even when implemented chronically post-SCI, can partially ameliorate the maladaptive vascular consequences of SCI (Thijssen *et al.* 2005, Thijssen *et al.* 2006). It is likely that physical activity implemented acutely after injury will help prevent the progressive decline in CV function typical of SCI and improve patients' life expectancy. However, to our knowledge no study has examined how the CV system responds to exercise provocation acutely after low thoracic injury, or if the system is capable of eliciting the appropriate CV responses allowing the benefits of exercise to be obtained.

The goal of the present study was to investigate CV responses to an active exercise challenge in adult female Sprague-Dawley (SD) rats with moderate T10 contusion injuries that disrupt the sympathetic drive to splanchnic and lower extremity vasculature. These lesions represent the most commonly used model of incomplete SCI in the rodent, and a common but not predominant human SCI (DeVivo and Chen 2011). Using implantable telemetry devices to acquire arterial blood pressure and heart rate (HR) data from

conscious, freely moving rodents, we assessed CV function before and for ten weeks after injury while the animals were at rest in their cages and during dynamic bouts of swimming exercise challenge. Dobutamine stress echocardiography was also employed to assess changes in cardiac structure and function in the presence of a beta-adrenergic agonist following prolonged periods of reduced mobility and attenuated volume/pressure loading of the left ventricle. We hypothesized that incomplete contusion to the lower thoracic spinal cord would result in altered hemodynamic responses to exercise challenge acutely after injury due, in part, to physical deconditioning in the peripheral vasculature and decreased loading of the heart. However, with time post-injury, improvements in hindlimb function and the subsequent increase in-cage activity typical of our injury model would reverse the effects of deconditioning, leading to improved hemodynamic control during exercise challenge.

Methods

Ethical Approval

All animal care and surgical procedures were performed in accordance with the NIH Guidelines and with the approval of the University of Louisville Institutional Animal Care and Use Committee.

Experimental Design

Experiments were conducted on adult female SD rats (250-300g; Harlan Laboratories, Indianapolis, IN, USA). Prior to injury, animals were implanted with telemetry devices to deliver measurements of arterial blood pressure and electrocardiogram

(ECG) data (Data Sciences International®, St. Paul, MN; C50-PXT or HD-S11 transmitters). Experiments were completed in two phases in order to maximize the number of animals with telemetry devices. Phase I animals (n= 4) were assessed weekly for ten weeks after injury to look at acute and chronic responses to an exercise challenge. Phase II animals (n=4) were also followed for 10 weeks post-SCI; however, swimming assessments were not performed at one week post-contusion. Pre-injury (baseline) recordings were collected for three weeks prior to SCI. Rats were then subjected to a 25 g/cm moderate injury contusion at the T10 spinal level using the NYU Impactor (Mascis, Rutgers University).

Telemetry Implantation

All animals were instrumented with C50-PXT (Phase II) or HD-S11 (Phase I) transmitters (Data Sciences® International, St. Paul, MN) for *in vivo* measurement of arterial pressure and heart rate (HR) as described previously (Brockway *et al.* 1991). Briefly, under isoflurane anesthesia (2% in oxygen), a ventral midline incision was made in the skin and abdominal wall. The body of the transmitter was placed within the peritoneal cavity and sutured to the abdominal wall musculature. The pressure sensing cannula was inserted into the abdominal aorta slightly above the bifurcation of the iliac arteries and advanced rostrally to the point where the left renal vein courses over the aorta. The pressure catheter was fixed in place using a small amount of VetBond tissue adhesive (3 M™ Vetbond™ Tissue Adhesive, St. Paul, MN). The two biopotential leads were subcutaneously sutured in place under the 12th left rib and over the right pectoralis major muscle for ECG signal recordings in a Modified Lead II configuration (Data Sciences®

International *public technical notes in webpage*). The abdominal wall musculature and skin were closed in layers using 4-0 nylon and 4-0 silk sutures, respectively. Post-operative care included daily injections of gentamicin sulfate for 7 days (20 mg/kg, SC), twice-daily injections of buprenorphine for 3 days (0.03 mg/kg, SC; and as needed for pain management thereafter), and twice-daily 5ml boluses of lactated ringers for three days (and as needed for hydration thereafter). Animals were allowed to recover for 7-10 days following device placement, after which pre-injury recordings of arterial blood pressure and ECG were collected at rest and during exercise challenge. In the event that a rat showed signs of peritonitis due to the transmitter implantation, daily doses of the non-steroidal anti-inflammatory ketoprofen (5 mg/kg SC) and additional gentamicin sulfate were administered until symptoms resolved.

Exercise Challenge Recording Protocol

Following recovery from implantation, rats were re-introduced to the swimming pool and testing conditions. Swimming has been used as both rehabilitation exercise and as an assessment of hindlimb function following SCI in rodents (Smith *et al.* 2006, Gonzenbach *et al.* 2012). For the purposes of this study, swimming was used as a form of exercise to challenge the cardiopulmonary system and assess cardiovascular control after SCI. Briefly, swim assessments consisted of a four-minute session in which the rat is repeatedly placed at one end of a 5-ft long plexiglass pool and encouraged to swim to the opposite end where they exited via a padded ramp. Pool temperatures are maintained at 33-35 degrees C to give rats incentive to exit the pool but also to avoid problems associated with drastic drops in core body temperature and spasticity after injury. Uninjured rats can

easily swim upwards of 45 laps in a typical session. Beat-by-beat arterial pressure and HR were collected at 1000 Hz during periods of rest and in response to the exercise challenge. In-cage recordings of arterial pressure and HR were acquired before swimming (four minutes) and during exercise recovery (6-10 minutes). Baseline measurements were made 3 times per week for 3-4 weeks.

Spinal Cord Injury

Approximately five weeks after device placement, rats were given T10 contusion injuries using the NYU Impactor. Each animal was anesthetized with a Ketamine (50 mg/kg)/Xylazine (0.024 mg/kg)/ Acepromazine (0.005 mg/kg) cocktail (IP) and given glycopyrrolate (0.08 mg/kg, IM) prior to the contusion procedure. A dorsal midline incision was made in the superficial muscle overlying the T7 – T12 vertebrae. A single level laminectomy was made at the T9 vertebral level. Using clamps applied to the T8 and T10 spinous processes, the spine was immobilized and positioned for impact. The NYU Impactor was then used to deliver moderate (25 g/cm) weight drop contusion injuries with no impactor dwell time. The muscle and skin overlying the injury was sutured in layers and antibiotic ointment was applied to the incision. Injured animals were monitored on heating pads until they recovered from the anesthesia. Rats were then doubly housed in standard cages for the remainder of the study. Post-operative care consisted of daily injections of gentamicin sulfate for 7 days (20 mg/kg, SC), twice-daily injections of buprenorphine for 3 days (0.03 mg/kg, SC; and as needed for pain management thereafter), and twice-daily 5ml boluses of lactated ringers for three days (and as needed for hydration

thereafter). Manual bladder expression was conducted three times a day until reflexive voiding was re-established.

CV Analysis and Behavioral Assessments

CV data was collected using the PONEMAH® 5.0 software package and DataQuest Acquisition hardware (Data Sciences® International, St. Paul, MN). Initial mean blood pressure (MBP) and pressure-derived HR data analyses were performed in LabChart version 8.0 (ADInstruments, Colorado Springs, CO). Mean blood pressure and HR Excursion during swimming was assessed using a custom Excel macro. Briefly, HR Excursion was determined by calculating the difference in the mean HR from the first to the last 15 seconds of the entire swim session. Mean blood pressure Excursion was assessed on a lap-by-lap basis and calculated as the average difference between the peak and trough values for each lap. Louisville Swim Scale (LSS, weeks 2-5 and 10) and BBB (weeks 1-10) assessments were performed to track locomotor recovery.

In Vivo Cardiac Echocardiography and Dobutamine Stress Testing

In a subset of moderately contused animals (Phase I, n=4), echocardiographic assessments (pre-injury and weeks 1, 5, and 10 post-SCI) were performed using a high resolution ultrasound machine (VisualSonics Vevo® 3100) and transducer (MX250S, 24 MHz) designed for preclinical studies. Rats were anesthetized with isoflurane inhalation (1.75% in oxygen), the thorax was shaved, and the animal was placed in dorsal recumbency. Body temperature, HR, and arterial pressure were monitored using the telemetry system as previously described. Prior to Dobutamine infusion, standard measures

of left ventricular structure and function were obtained using M-mode echocardiography in the parasternal short-axis view (SAX) at the mid-ventricular level. Once an acceptable view of the heart was obtained, the transducer was secured in a stereotaxic stand to minimize variation in image capture (VisualSonics) during the Dobutamine infusion protocol. Thereafter, the tail vein was cannulated using a 25-gauge butterfly needle and secured using surgical tape. Dobutamine was intravenously infused at progressively increasing doses (5, 10, 20, and 30 µg/kg/min) using an automated perfusion pump (KD Scientific, Holliston, MA). Each dose was infused for four minutes, after which M-mode imaging was performed before advancing to the next dose. Results for five cardiac cycles during expiration were averaged for comparison using the VEVO® LAB software. Arterial pressure and HR measures for the final minute of each infusion dose were analyzed using LabChart version 8.0 (ADInstruments, Colorado Springs, CO).

Study Termination and Histological Analysis

Upon completion of the study, rats were euthanized with an overdose of sodium pentobarbital (50 mg/kg IP), transcardially perfused with phosphate buffer and fresh dissected to remove the heart and spinal cord. The SC was post-fixed with 4% paraformaldehyde and cryopreserved in 30% sucrose. Spinal cord tissue was sectioned at 30 µm in six sets and assessed for white matter sparing in and around the epicenter (Magnuson *et al.* 2005, Smith *et al.* 2006). The heart was excised to determine the ratio between body mass and heart mass for each animal. An additional set of age-matched animals were used as non-injured controls (CON, n=8) for anatomical comparisons.

Statistical Analysis

Data from phase I and II animals were combined for statistical analysis as there were no differences between the groups in key behavioral, cardiovascular, or histological measures (MBP and HR, BBB, and SWM). Behavioral assessments (BBB and LSS) were analyzed using repeated measures analysis of variance (RM ANOVA) for time. Cardiovascular parameters during in-cage rest and swimming were analyzed as raw data with mixed model (fixed effects) ANOVA. Post hoc t-tests were completed with Bonferroni correction. Following normalization to femur length, echocardiography data was analyzed using repeated measures analysis of variance (RM ANOVA) for comparisons in dose responses across time. Post hoc t-tests were completed with Tukey HSD. Terminal histological measures were compared with Independent t-tests between means with equal or unequal variance, as appropriate, followed by Bonferroni correction for multiple comparisons. Statistical analyses were performed with SPSS (v22, Chicago, IL). Telemetry blood pressure data is shown as mean ± standard deviation (SD) and significance was set at $p \leq .05$. Ultrasound data is shown as mean ± standard deviation (SD) in the text and illustrated as mean ± standard error of the mean (SEM) in figures. Ultrasound data significance was set at $p \leq .05$.

Results

T10 contusion results in substantial tissue damage in and around the injury epicenter

Moderate contusion to the low thoracic spinal cord resulted in substantial white matter damage in and around the injury epicenter. The percent of spared white matter, assessed as darkly stained compact tissue ranged from approximately 1-6 percent, and

averaged 3.125 percent overall (Table 4). Locomotor assessments were executed at the beginning of each week prior to any swimming exercise challenge (Figure 9A). Timewise comparison revealed significant differences between week one assessments and all chronic time points (Figure 9A; Week 1 vs. Weeks 3 and 4, $p \leq .05$; Week 1 vs. Weeks 5-10, $p \leq .001$). Animals did not regain consistent weight-supported stepping or forelimb-hindlimb coordination. Toe clearance also remained poor. Unassisted swimming assessments (Figure 9B), evaluated weekly starting two weeks after injury, revealed a severe drop in function post-SCI with no improvements over time. As shown by the mean LSS score of 3.5, moderately-contused animals rely solely on their forelimbs for forward propulsion and had great difficulty stabilizing their trunk during swimming for the entirety of the study.

Low thoracic contusion does not disrupt resting cardiovascular hemodynamics

Incomplete moderate contusion of the lower thoracic spinal cord did not alter resting cardiovascular hemodynamics over time (Table 3). Mean blood pressure and HR were similar to pre-injury measurements at all time points assessed.

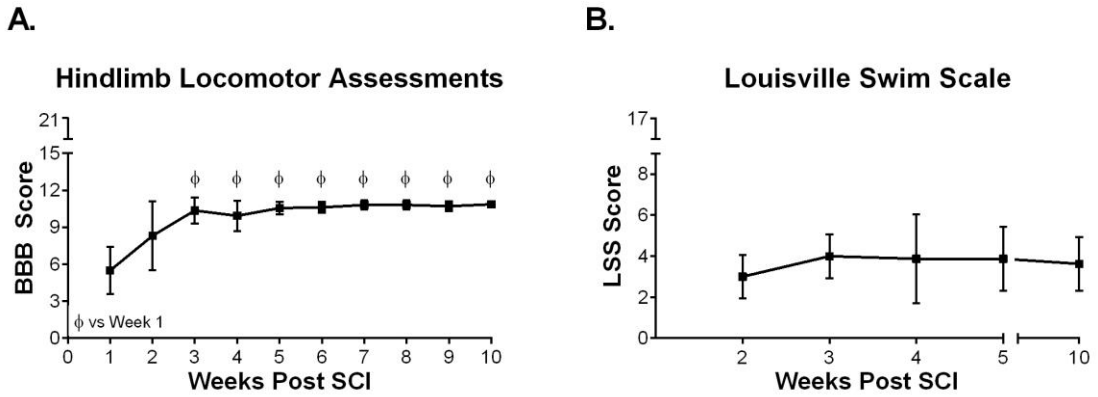

Figure 9. **Moderate T10 contusion results in attenuated locomotor function. (A)** Open field locomotor scores following moderate T10 contusion for ten weeks post-SCI. **(B)** Weekly performance during swimming assessments beginning two weeks post-SCI. No improvements were noted over time. Statistical significance was assessed using Mixed Model ANOVA with Bonferroni *post hoc* t-test. Data is displayed as mean ± SD and statistical significance was set as $^{\phi}p \leq .05$ vs. week 1.

Hemodynamics during In-Cage Rest

	Pre-Injury	Week 1	Week 2	Week 3	Week 4	Week 5	Week 10
MAP	120.74 ± 3.69	120.46 ± 3.53	123.61 ± 2.63	125.43 ± 7.00	126.00 ± 3.80	124.31 ± 3.15	121.38 ± 4.98
HR	445.97 ± 19.48	474.47 ± 29.14	440.87 ± 21.12	442.71 ± 21.80	442.72 ± 24.63	441.83 ± 29.19	426.10 ± 27.73

Table 3. **Hemodynamics during In Cage Rest before and after moderate T10 contusion.** MBP, mean blood pressure; HR, heart rate. Data is displayed as mean ± SD.

Responses to acute exercise challenge are altered following low thoracic contusion

CV responses to an exercise challenge were evaluated weekly before and after low thoracic SCI. During pre-injury assessments, there was a modest pressor response (increase of 6.27 ± 1.67 mmHg, or 105.2 %) at the initiation of swimming that was maintained throughout the four-minute session. Heart rate was also increased (increase of 38.91 ± 9 bpm, or 108.8 %) and remained quite stable for the duration of the exercise challenge. One week following moderate contusion, animals were still able to mount a modest pressor response during swimming exercise. Conversely, the HR dropped significantly as the swimming session progressed and remained low for at least several minutes after the exercise challenge had ceased. Representative traces of MBP and HR during Rest, Swimming, and Exercise Recovery are shown at baseline and acutely post-T10 contusion (Figure 10A, B. Pre-injury and one week post-SCI).

The bradycardic response to swimming exercise, measured as HR Excursion, is significantly greater in magnitude at one week verses almost all later time points after injury (Figure 11D; Week 1 vs. Pre-injury and Weeks 2 and 4, $p \leq .05$; Week 1 vs. Weeks 5 and 10, $p \leq .01$). Pressor responses to the exercise challenge at one week after injury were similar to pre-injury measurements. Following the cessation of swimming, rodents with incomplete low thoracic injury experienced exertional hypotension during the exercise recovery period one week following SCI that was significantly more severe than at all other time points (Figure 11C; Week 1 vs. Pre-injury and Weeks 2, 3, 5, and 9, $p \leq .01$; Week 1 vs. Week 4, $p \leq .05$).

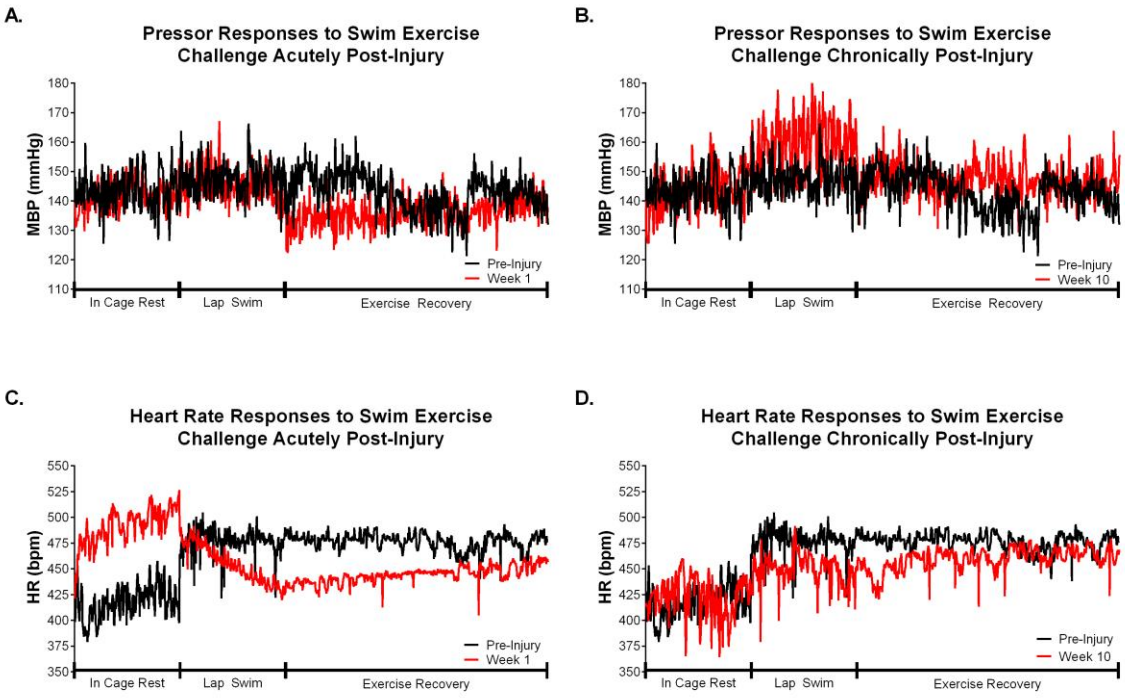

Figure 10. **Rodents with low thoracic contusion are unable to maintain cardiovascular control during swimming exercise challenge.** Representative MBP **(A)** and HR **(C)** responses to swimming exercise challenge before (black lines) and one week post-T10 moderate contusion (red lines). Note the drastic fall in HR from the beginning to the end of the four minute swimming exercise challenge. Representative MBP **(B)** and HR **(D)** responses to swimming exercise challenge before (black lines) and 10 weeks post-T10 moderate contusion (red lines). Note the elevated pressor response to swim challenge at ten weeks post-SCI. Data has been down sampled from 1000 Hz to display one data point per second. Individual recordings of In Cage Rest, Lap Swim, and Exercise Recovery are displayed as one continuous MBP or HR trace.

Lack of CV control during exercise challenge persists for many weeks following moderate low thoracic SCI

Deficits in CV function during an exercise challenge persisted for many weeks following a moderate T10 contusion. Most notably, oscillatory changes in MBP occurring on a lap-by-lap basis increased substantially in amplitude over time post-injury. Visually, this is illustrated as large saw-tooth patterns during the swim challenge in representative hemodynamic traces (Figure 10C) and is quantified as MBP Excursion (Figure 11A; Pre-injury vs. Week 2, $p \leq .05$; Pre-injury vs. Weeks 3-5 and 10, $p \leq .01$). Furthermore, the average MBP during the four-minute exercise challenge at 10 weeks was significantly greater than at pre-injury and week 1 (Figure 11B; Pre-injury vs. Weeks 3-5 and 10, $p \leq .05$; Week 1 vs Weeks 3-5 and 10, $p \leq .05$). Average HR varied considerably and measurements were not significantly different during exercise or exercise recovery over time following contusion (Figure 11E-F).

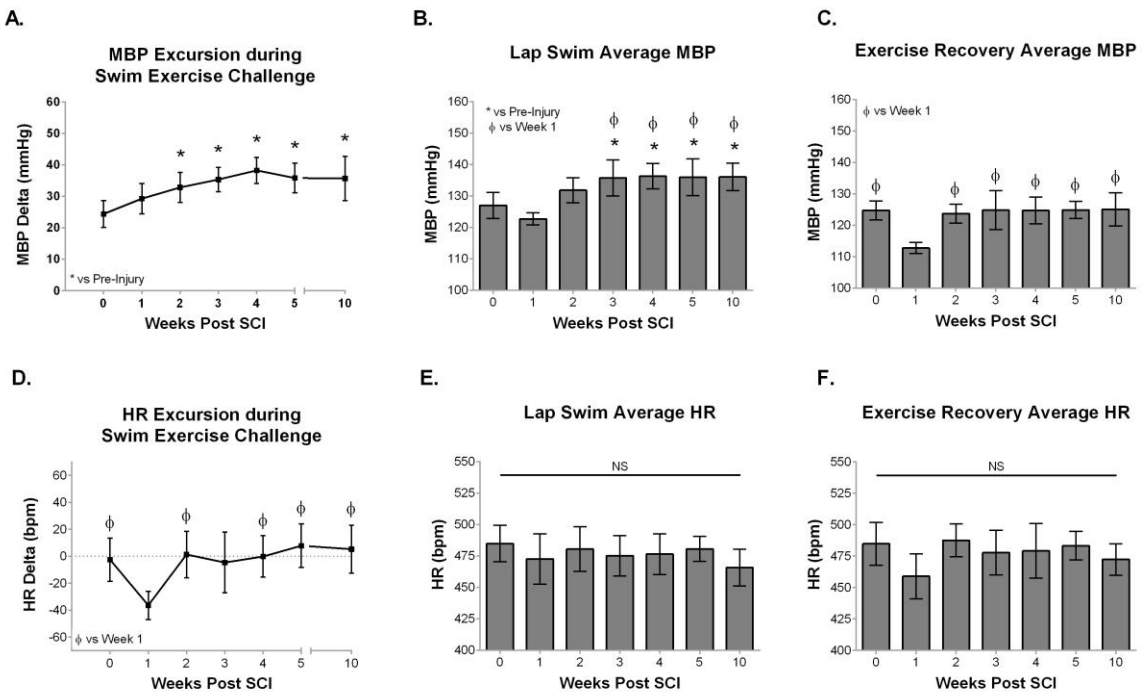

Figure 11. **The inability to regulate blood pressure control in response to exercise challenge persists for many weeks following T10 contusion. (A)** Average MBP Excursion measured prior to SCI and each week following injury. Oscillatory changes in MBP during each swim lap are averaged for each time point. The inability to maintain MBP during exercise challenge increased with time post-injury. **(B)** Average MBP during the four-minute swim session. Note the increased pressor response chronically after injury. **(C)** Average MBP during the Exercise Recovery period. Note the post-exertional hypotension one week after injury. **(D)** HR Excursion during the four-minute swim session. Note the drastic drop in HR from the beginning to the end of swimming acutely after injury. **(E)** Average HR during the four-minute swim session. **(F)** Average HR during the Exercise Recovery period. Statistical significance was assessed using Mixed Model ANOVA with Bonferroni *post hoc* t-test. Data is displayed as mean ± SD and statistical significance was set as *$p \leq .05$ vs. pre-injury and $^{\phi}p \leq .05$ vs. week 1.

Echocardiography and Dobutamine Stress Testing

Blood pressure measurements (systolic, diastolic, and mean blood pressure) during echocardiography were similar to pre-injury parameters at all time points assessed after injury (Table 4), indicating that pressor responses in the presence of isoflurane anesthesia were not disrupted by low thoracic contusion. Heart rate collected during echocardiography revealed transient tachycardia one week after injury that was significantly greater than week ten measurements (Table 4, Week 1 vs. Week 10, $p = .042$). Flow indices, left ventricular dimensions, and measures of diastolic function were similar to pre-injury measurements at all time points assessed after contusion.

The effects of Dobutamine infusion on cardiac function following contusion were similar to pre-injury measurements. Hemodynamic responses to Dobutamine during pre-injury and Week 10 assessments are graphically represented in Figure 12G-H (all time points in Table 4). Systolic, diastolic, and mean blood pressure were not significantly affected by increasing concentrations of Dobutamine infusion. Conversely, Dobutamine administration resulted in significantly higher HR measurements at the 30-ug dose prior to SCI and one week following injury (Table 4, Pre-injury and Week 1 doses, all comparisons $p \leq .05$).

Rodents also experienced a dose-dependent decrease in end-systolic volume (ESV) with a concurrent increase in ejection fraction (EF) at all time points assessed (Table 4 and Figure 12D, F: Pre-injury and Week 10, $p \leq .05$). End-diastolic volume (EDV) was also decreased by increasing concentrations of Dobutamine prior to SCI and five weeks post-contusion (Table 4 and Figure 12E: Pre-injury and Week 10, $p \leq .05$). Like pre-injury measurements, Dobutamine administration resulted in a dose-dependent increase in cardiac

output (CO) following low thoracic contusion (Table 4 and Figure 12C: Pre-injury and Week 10, $p \leq .05$; Note, Week 5, $p = .054$ approaches). Stroke volume (SV) was also increased in the presence of Dobutamine at one and ten weeks after SCI (Table 4 and Figure 12A: Week 10, $p \leq .05$). There was a dose-dependent reduction in the left ventricular internal diameter (LVID) during systole and diastole at all time points assessed (Table 4 and Figure 12A, B: Pre-injury and Week 10, $p \leq .05$). No differences were noted in body mass between T10 SCI and uninjured control groups ($p = .635$). However, heart mass and the ratio between heart and body mass were significantly higher in T10 contused animals verses uninjured, age-matched controls (Table 4; $p \leq .001$).

Anatomical Data

	T10 MOD SCI	Uninjured Control	KEY
Body Mass	304.25 ± 25.63	298.88 ± 18.06	* Dose vs. Dose 0ug, p ≤ .05
Heart Mass	1.1797 ± 0.0559 ᵀᵀ	1.0022 ± 0.0963	† Dose vs. Dose 5ug, p ≤ .05
Heart/Body Mass Ratio	0.0039 ± 0.0002 ᵀᵀ	0.0034 ± 0.0004	ǂ Dose vs. Dose 10ug, p ≤ .05
Spared White Matter (%)	3.1250 ± 1.7595	N/A	ᵀᵀ p ≤ .001

Echocardiographic Data

Dimensions	*Dose*	Pre-Injury	Week 1	Week 5	Week 10
LVIDd (mm)	0 ug	7.31 ± 0.24	7.26 ± 0.17	7.09 ± 0.47	6.95 ± 0.31
	5 ug	7.09 ± 0.49 *	7.11 ± 0.15	6.88 ± 0.32	6.99 ± 0.19
	10 ug	6.69 ± 0.46 *†	6.99 ± 0.19	6.66 ± 0.36 *	6.77 ± 0.24
	20 ug	6.56 ± 0.30 *†	6.99 ± 0.18	6.65 ± 0.29 *	6.84 ± 0.20
	30 ug	6.45 ± 0.53 *†	6.90 ± 0.15 *	6.61 ± 0.35 *	6.65 ± 0.26 *†
LVIDs (mm)	0 ug	4.27 ± 0.09	4.17 ± 0.55	4.07 ± 0.38	4.00 ± 0.51
	5 ug	3.13 ± 0.30 *	3.50 ± 0.32 *	3.25 ± 0.40 *	3.26 ± 0.36 *
	10 ug	2.37 ± 0.20 *†	2.74 ± 0.67 *†	2.48 ± 0.55 *†	2.31 ± 0.25 *†
	20 ug	2.08 ± 0.40 *†	2.53 ± 0.47 *†	2.20 ± 0.44 *†	2.23 ± 0.54 *†
	30 ug	1.99 ± 0.44 *†	1.91 ± 0.62 *†ǂ	1.97 ± 0.24 *†	2.00 ± 0.37 *†
EDV (µl)	0 ug	282.18 ± 20.48	277.58 ± 14.84	264.11 ± 38.12	251.95 ± 25.56
	5 ug	263.95 ± 39.18	264.29 ± 12.73	245.95 ± 24.90	255.18 ± 15.49
	10 ug	231.43 ± 34.16 *	255.16 ± 16.06	229.02 ± 27.11 *	237.42 ± 18.94
	20 ug	220.90 ± 22.85 *†	254.76 ± 15.29	227.78 ± 21.76 *	242.89 ± 16.10
	30 ug	213.52 ± 39.30 *†	247.52 ± 12.01	225.27 ± 25.90 *	227.59 ± 20.18
ESV (µl)	0 ug	81.94 ± 4.04	78.90 ± 24.65	73.51 ± 16.03	71.53 ± 23.15
	5 ug	39.10 ± 9.04 *	51.40 ± 11.62 *	43.30 ± 12.94 *	43.37 ± 11.59 *
	10 ug	19.81 ± 4.22 *	30.14 ± 18.44 *†	23.24 ± 13.96 *	18.67 ± 5.06 *†
	20 ug	14.73 ± 7.37 *†	23.96 ± 11.15 *†	17.16 ± 8.41 *†	18.17 ± 11.72 *†
	30 ug	13.47 ± 7.03 *†	12.94 ± 8.55 *†	12.42 ± 3.45 *†	13.38 ± 6.69 *†

Systolic Function	*Dose*	Pre-Injury	Week 1	Week 5	Week 10
SV (µl)	0 ug	200.25 ± 18.84	198.68 ± 24.00	190.60 ± 23.93	180.42 ± 12.47
	5 ug	224.85 ± 32.54	212.89 ± 12.84	202.66 ± 19.68	211.81 ± 19.75 *
	10 ug	211.62 ± 32.00	225.01 ± 7.63	205.78 ± 22.88	218.75 ± 19.66 *
	20 ug	206.17 ± 22.85	230.79 ± 4.44 *	210.62 ± 16.94	224.72 ± 16.85 *
	30 ug	200.05 ± 32.58	234.58 ± 4.33 *	212.85 ± 22.69	214.21 ± 22.92 *
EF (%)	0 ug	70.88 ± 1.93	71.63 ± 8.26	72.36 ± 2.66	71.98 ± 6.29
	5 ug	85.23 ± 2.06 *	80.59 ± 3.95 *	82.51 ± 4.20 *	82.95 ± 4.64 *
	10 ug	91.41 ± 1.42 *	88.45 ± 6.26 *†	90.05 ± 5.25 *†	92.10 ± 2.36 *†
	20 ug	93.34 ± 3.19 *†	90.76 ± 3.71 *†	92.62 ± 3.28 *†	92.57 ± 4.72 *†
	30 ug	93.97 ± 2.26 *†	94.89 ± 3.26 *†ǂ	94.57 ± 1.07 *†	94.04 ± 3.26 *†
Q (ml min-1)	0 ug	62.92 ± 10.11	67.40 ± 6.20	58.09 ± 7.74	53.62 ± 12.85
	5 ug	74.22 ± 17.21	73.97 ± 4.95	63.15 ± 7.00	60.44 ± 8.92
	10 ug	77.11 ± 18.67	82.26 ± 5.86 *	68.00 ± 7.61	67.11 ± 8.10
	20 ug	80.09 ± 15.14 *	88.54 ± 4.02 *	71.25 ± 5.70	69.66 ± 7.07 *
	30 ug	87.19 ± 32.85 *	92.21 ± 6.04 *†	72.24 ± 1.81	69.05 ± 10.81 *

Diastolic Function	*Dose*	Pre-Injury	Week 1	Week 5	Week 10
E (cm s-1)	0 ug	67.58 ± 22.34	69.73 ± 8.75	77.37 ± 25.57	83.15 ± 13.80

Hemodynamics	*Dose*	Pre-Injury	Week 1	Week 5	Week 10
SBP (mmHg)	0 ug	105.83 ± 7.59	104.09 ± 8.57	101.41 ± 14.00	102.97 ± 9.19
	5 ug	109.60 ± 7.26	107.27 ± 7.58	100.86 ± 9.99	104.77 ± 7.72
	10 ug	109.73 ± 8.22	106.45 ± 6.15	99.17 ± 8.32	102.49 ± 7.31
	20 ug	111.63 ± 14.35	103.69 ± 5.67	97.43 ± 7.69	104.15 ± 5.02
	30 ug	108.98 ± 15.56	101.08 ± 5.60	94.63 ± 6.48	99.75 ± 5.52
DBP (mmHg)	0 ug	59.93 ± 14.13	62.05 ± 1.23	63.16 ± 6.29	61.08 ± 5.05
	5 ug	64.94 ± 7.17	59.77 ± 2.23	57.92 ± 4.76	56.25 ± 6.44
	10 ug	62.72 ± 7.23	56.98 ± 3.91	54.72 ± 2.39	52.73 ± 4.01
	20 ug	64.87 ± 11.25	55.08 ± 3.47	53.95 ± 1.83	54.29 ± 3.81
	30 ug	63.53 ± 11.81	53.99 ± 3.96	52.56 ± 1.40	52.62 ± 1.21
MBP (mmHg)	0 ug	75.02 ± 11.12	76.06 ± 2.91	75.91 ± 8.85	75.04 ± 6.31
	5 ug	79.83 ± 6.99	75.61 ± 1.46	72.23 ± 6.42	72.42 ± 6.79
	10 ug	78.39 ± 7.32	73.47 ± 1.98	69.53 ± 4.21	69.32 ± 4.95
	20 ug	80.46 ± 12.11	71.29 ± 1.52	68.44 ± 3.65	70.91 ± 3.36
	30 ug	78.68 ± 12.88	69.69 ± 2.17	66.59 ± 2.83	68.33 ± 2.12
HR (bpm)	0 ug	311.07 ± 40.96	331.18 ± 32.57	300.48 ± 54.59	285.30 ± 40.48
	5 ug	323.36 ± 39.19	345.64 ± 18.25	309.96 ± 42.18	285.33 ± 35.50
	10 ug	354.44 ± 42.39	362.48 ± 19.28	328.32 ± 40.06	302.67 ± 32.83
	20 ug	383.93 ± 44.49 *†	380.15 ± 21.98	337.56 ± 35.33	302.55 ± 39.32
	30 ug	393.18 ± 41.64 *†	391.43 ± 29.84 *	340.28 ± 34.81	321.58 ± 28.04

Table 4. **Anatomical and echocardiographic data of moderately-contused rodents.** LVIDd, left ventricular internal diameter during diastole; LVIDs left ventricular internal diameter during systole; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; Q, cardiac output; E, transmitral filling velocity during early diastole; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. Data is displayed as mean ± SD. *$p \leq .05$ dose vs. 0ug dose; $^{\dagger}p \leq .05$ dose vs. 5ug dose; $^{\ddagger}p \leq .05$ dose vs. 10ug dose; and $^{\tau\tau}p \leq .05$ moderate vs. uninjured control group differences.

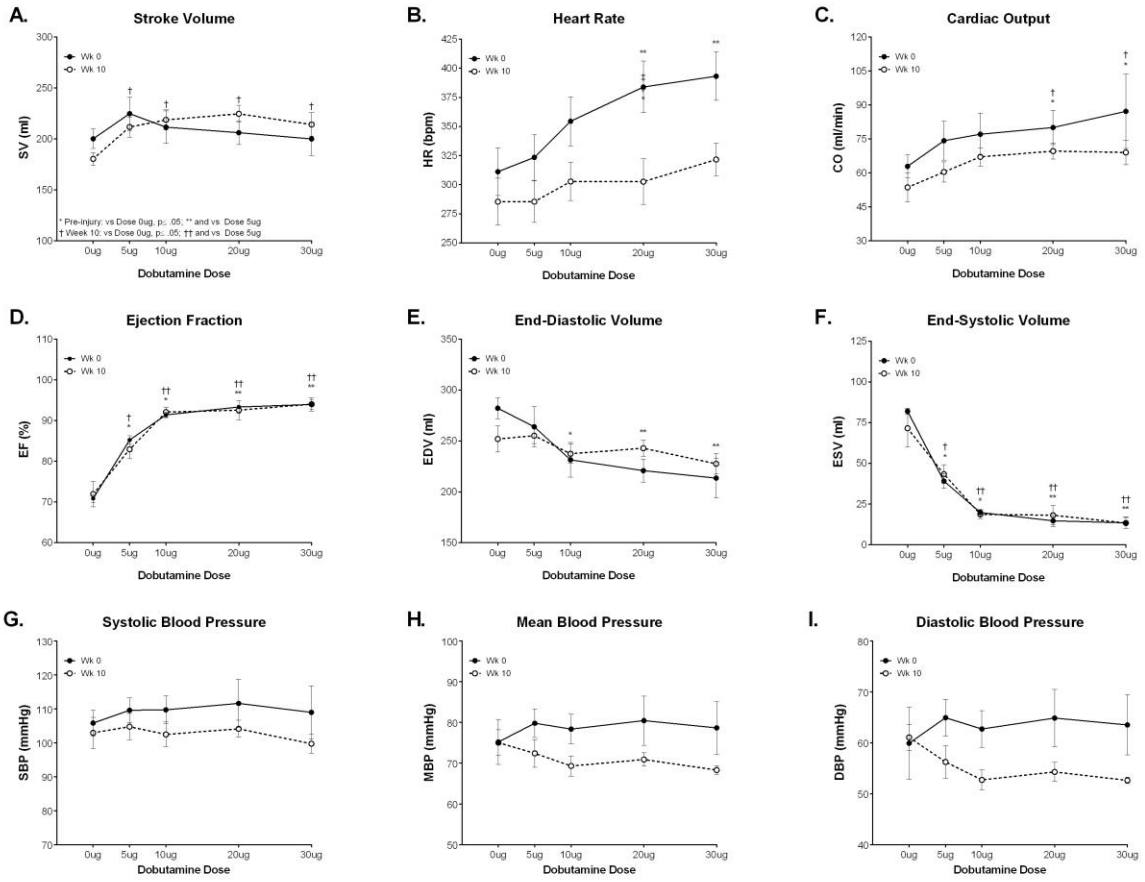

Figure 12. **Cardiac structure and function is not impaired at rest or in the presences of Dobutamine following high thoracic contusion**. There were no differences in echocardiographic parameters over time post-injury. Echocardiographic responses to increasing concentrations of Dobutamine infusion prior to and ten weeks after moderate T10 SCI are represented in **A-F**. Pressor responses in the presence of isoflurane anesthesia are displayed in **G-I**. Data is displayed as mean ± SEM. Pre-injury significance: *p≤ .05 vs. Dose 0ug and **p≤ .05 vs. Dose 0 and 5ug. Week 10 significance: †p≤ .05 vs. Dose 0ug and ††p≤ .05 vs. Dose 0 and 5ug.

Discussion

Summary of findings

For the first time, we have demonstrated that low thoracic contusion injuries result in abnormal CV control during instances of increased cardiopulmonary demand in a rodent model of incomplete SCI. Specifically, following T10 contusion, rats experience drastic drops in HR acutely and large fluctuations in MBP chronically during swim exercise challenge. Interestingly, these deficits were not noted while the animal was at rest, suggesting that under normal hemodynamic conditions there is adequate residual sympathetic innervation to the CV system following moderate T10 contusive SCI to maintain MBP and HR. Further, echocardiography revealed that even in the wake of reduced mobility and unloading of the left ventricle, cardiac structure and function was not significantly impacted by low thoracic contusion. Thus, lack of hemodynamic stability during active exercise challenge was not the result of cardiac decline, and was likely due, in part, to abnormal autonomic control of vascular structures below the lesion. This finding is strengthened by the fact that the heart responds similarly to pre-injury measurements in the presence of Dobutamine.

The presence of neurogenic shock

The findings from the present study suggest that the normally interdependent control of MBP and HR is decoupled following a T10 contusion. The primary goal of the CV system is to maintain arterial pressure within a narrow range during instances of varied cardiopulmonary demand. Interestingly, oscillatory changes in MBP during lap swimming do not elicit compensatory modifications in HR. Acutely after injury (one week), animals

experienced a dramatic fall in HR during the four minutes of lap swimming, despite exhibiting a normal pressor response. In intact rodents, CV responses to swimming result in an increase in HR, thereby enhancing cardiac output to account for the increased metabolic demand of working musculature. These adjustments in cardiac rate are accomplished through central nervous system activity. Failure to increase HR in response to exercise challenge following acute injury can be explained by a general hypo-responsiveness of the sympathetic nervous system (SNS), termed neurogenic shock. Generally, neurogenic shock is thought to primarily affect patients with cervical and high thoracic lesions due to the disruption of supraspinal input to sympathetic neurons innervating the heart (T1 – T5). However, it has been reported that patients with lesions below this critical outflow experience abnormal CV control acutely due to this condition (Guly *et al.* 2008, Mallek *et al.* 2012). Likewise, previous studies in our lab have shown that incomplete contusion to the T3 spinal cord also results in attenuated cardiac chronotrophy during exercise challenge acutely (Harman et al., woo woo), suggesting that bradycardia is due to a general blunting of the SNS and an inability to increase sympathetic tone to the myocardium, independent of lesion level. Reported instances of neurogenic shock primarily occur within the first couple of days following injury. However, studies looking at this condition due so in the resting state, and, until now, it has been unknown how the system responds to instances of increased cardiopulmonary demand.

Progressive decline in CV control

Over time, rodents with incomplete contusion injury begin to display progressively larger oscillations in MBP during swimming exercise challenge (i.e. MBP Excursion).

Reasons for this pressure lability during exercise are likely multifactorial; however, changes occurring in the vascular wall due to reduced activity/immobility in conjunction with partial denervation to peripheral vascular structures below the lesion are likely responsible.

Numerous groups have highlighted the rapid changes that occur in vascular structure following periods of immobility. For instance, both human and rodent studies have shown that the diameter of the femoral artery decreases substantially following extreme inactivity, such as SCI or lower limb immobilization (Sugawara *et al.* 2004, de Groot *et al.* 2006). Importantly, these changes can be seen as early as one week after lower limb casting (Sugawara *et al.* 2004), and have largely plateaued by three weeks after complete SCI (de Groot *et al.* 2006). Inward arterial remodeling is localized to regions below the lesion, and is primarily a response to reduced metabolic demands of the lower limb musculature (West *et al.* 2013). During the first week following injury, animals in our study had very limited hindlimb function as measured by the BBB (slight to extensive movement of the hip, ankle, and/or knee joints). While this was not assessed directly, one could suggest that reduced mobility in the wake of hindlimb denervation resulted in a decrease in diameter of lower extremity vessels. If so, acute structural changes to minimize wall shear stress could account for the ability of the animals to maintain BP during swimming exercise challenge.

As time progressed, however, T10 moderately-contused animals lost the ability to regulate pressor responses during exercise, illustrated as increased MBP excursion with each swim lap. Given that cardiac performance during dobutamine challenge was similar at all time points assessed, pressure lability was likely due to abnormal vascular compliance

during increased blood pressure and flow. In addition to compromising vasculature of the lower extremities musculature, the loss of myogenic tone following low thoracic contusion likely disproportionately affects vasoreaction in the mesenteric arterial bed. Sympathetic innervation to the gut vasculature originates in spinal segments T6 – L2. Due to the rostral/caudal spread of the injury epicenter, contusion at the T10 level disrupts large portions of this autonomic control, typically extending from the T8/T9 to T11/T12 depending on the impact severity. As the lesser splanchnic nerve is composed of fibers from the T10-T11 spinal segments, sympathetic innervation to the midgut (all of the small intestine and the proximal half of the large intestine) was undoubtedly impaired. This loss of, or reductions in tonic vasoconstriction to the gut likely results in blood accumulation, inefficient redistribution of blood to musculature, and the oscillating pattern of BP noted during exercise challenge.

Interestingly, increased pressure lability coincided with improved performance on the BBB. This is counterintuitive given that exercise training has been shown to be beneficial in many facets of patient health, particularly in improving CV performance after SCI. While exercise programs are generally believed to positively impact vascular structure and function in chronic SCI patients (Gerrits *et al.* 2001, Ditor *et al.* 2005, Thijssen *et al.* 2006), many of these studies have utilized aggressive rehabilitation regimens consisting of functional electrical stimulation (FES) of the lower extremities and clinical data delivers mixed results on the effects of such exercise on the ability of the vascular wall to accommodate changes in blood pressure and flow. For instance, six weeks of FES-hybrid training appeared to normalize FMD responses in the common femoral artery (Thijssen *et al.* 2006), whereas only four weeks of training was not effective (Thijssen *et al.* 2005). As

such, the amount and type of activity performed on a daily basis in our animals may not have been sufficient to overcome diminished sympathetic modulation of vascular tone and correct endothelial dysfunction typical of SCI. It is also possible that, given the increased activity of the hindlimbs at chronic time points, structural characteristics of the vasculature (arterial diameter, wall thickness, etc.) have largely returned to pre-injury baseline. Although not assessed in the current study, vessel architecture that is reminiscent of the normal condition may make maintaining BP more difficult in the absence of sympathetic modulation of myogenic tone. Further, it must be mentioned that there are many factors affecting myogenic tone in peripheral vasculature in addition to direct sympathetic activation by the autonomic nervous system. Thus, the contribution (or lack thereof) of circulating hormonal modulators such as endothelin, vasopressin, and angiotensin may contribute to BP lability during exercise challenge.

The inability to maintain MBP consistently throughout the exercise challenge is worrisome given that extreme fluctuations in arterial pressure can further damage the vascular endothelium, thereby increasing CVD risk (Fry 1968). As such, it has been shown that following T3 transection, repetitive increases in arterial pressure during experimentally-generated AD leads to exacerbation of SCI-induced hypersensitivity to phenylephrine in the superior mesenteric artery (Alan *et al.* 2010). Therefore, exercise regimens that induce oscillating pressure responses, such as those we have shown here, should be implemented with caution as repetitive elevations in blood pressure may induce shear injury to the vascular endothelium further exacerbating vascular and CV dysfunction.

Concluding remarks

To date, most animal studies examining CV function after injury have utilized high thoracic, full spinal transection models and have focused on instances of exaggerated system breakdown such as AD. However, most clinical injuries are incomplete and relatively little is known about the temporal progression of dysfunction that occurs following low thoracic injuries, especially in response to instances of increased cardiopulmonary demand. It is generally accepted that following injuries below the T5 spinal segment, there remains sufficient supraspinal control over the heart and upper body vasculature such that the CV system is able to respond appropriately to baroreceptor mediated reflexes, maintain CV homeostasis, and thus limit clinically significant hemodynamic manifestations of system dysfunction (Teasell *et al.* 2000). Severe low level lesions principally elicit dysfunction related to OH, resting tachycardia, and a diminished ability to appropriately respond to the demands of physical activity (Inskip *et al.* 2009). In agreement, we observed that incomplete low thoracic contusions, in which there is considerable white matter damage at the injury epicenter, rodents were unable to maintain CV control when forced to respond to the physical demands of exercise. Cardiac structure and function at rest was not significantly impacted by low thoracic contusion. Further, cardiac performance during exercise challenge was likely normal given that the heart responds similarly to pre-injury measurements in the presence of Dobutamine stress echocardiography. The normal cardiac control following low thoracic injury in this study is likely due to significant improvements in hindlimb function, as increased activity would lead to enhanced pressure and volume loading of the heart. Therefore, pressure lability during exercise is likely a consequence of altered vascular structure/function in the wake

of reduced sympathetic tone to the mesenteric and lower extremity vasculature. As CVD continues to be the leading cause of increased morbidity and mortality following SCI (Warburton *et al.* 2007), more insight regarding the effects of exercise and exercise training on vascular function is warranted to improve the lives of spinal cord-injured patients.

CHAPTER IV

EFFECTS OF ACUTE EXERCISE TRAINING REHABILITATION ON THE SEVERITY OF AUTONOMIC DYSREFLEXIA FOLLOWING INCOMPLETE SCI

Introduction

Patients living with spinal cord injury (SCI) experience a vast array of autonomic and somatic dysfunction. While the majority of clinical and preclinical research has focused on improving locomotor impairments after injury, recent surveys suggest that patients rank the recovery of autonomic function as a higher priority than the ability to walk again (Anderson 2004). In particular, therapies that target cardiovascular (CV) abnormalities, such as orthostatic hypotension, are deemed very important for everyday living and improved quality of life for SCI patients.

The level and completeness of injury largely determine the clinical presentation of CV pathology following injury, with cervical and high thoracic lesions constituting the most severe phenotypes (Weaver *et al.* 2012). As such, patients with lesions above the T6 spinal segment, in which supraspinal control of sympathetic preganglionic neurons of the heart and upper body vasculature is disrupted, often experience episodic hypertensive crises known as autonomic dysreflexia (AD). While the development of AD is likely multifactorial, the loss of bulbospinal regulation on sympathetic fibers and injury-induced aberrant plasticity within the lumbosacral spinal cord and peripheral nervous system are contributing factors (Teasell *et al.* 2000). Autonomic dysreflexia is most often evoked by

noxious visceral stimuli from the bowel or bladder, and results in exaggerated sympathetic outflow below the level of the lesion, extreme vasoconstriction, and subsequent centrally-unopposed hypertension (Cragg and Krassioukov 2012). Episodes of AD are characterized by upper body flushing and sweating, headache, and reflex-mediated bradycardia. Although somewhat uncommon, severe bouts of AD can result in life-threatening conditions such as myocardial infarction and intracranial bleeding (Wan and Krassioukov 2014). Symptoms of AD are present in over 90% of complete tetraplegic patients (Curt *et al.* 1997), and episodes of AD prove to be a large factor hindering rehabilitation and recovery post-injury (Harkema *et al.* 2008, Krassioukov *et al.* 2009, Weaver *et al.* 2012).

In addition to the inherent autonomic disruption following injury, abrupt and persistent denervation-induced immobility creates a physiological condition that is not conducive to maintaining adequate CV health or function. Numerous studies have shown that the sedentary lifestyles typical of SCI patients leads to substantial cardiac and vascular deconditioning and atrophy, further contributing to the overall decline in physical fitness and patient health (Kessler *et al.* 1986, Nash *et al.* 1991, de Groot *et al.* 2006, Thijssen *et al.* 2011, Thijssen *et al.* 2012). The temporal profile of CV system demise following SCI illustrates that changes occurring in the CV end-organs (i.e. the heart and vasculature) relate to the length of time patients remain sedentary after injury (Thijssen *et al.* 2012, West *et al.* 2015). As such, the implementation of physical activity paradigms to counteract deconditioning remains an important component of patient care. While many groups have highlighted the benefits of physical activity and training programs following injury (Jacobs *et al.* 2001, de Groot *et al.* 2003, Ditor *et al.* 2005, Ditor *et al.* 2005, Thijssen *et al.* 2006, Harkema *et al.* 2008, Tawashy *et al.* 2010), the majority of clinical studies have utilized

rehabilitation paradigms in the chronic stages of SCI. This is problematic given that the decline in physical fitness in SCI patients is progressive. The length of time that a patient remains immobile post-SCI and the degree to which their lifestyles become sedentary have great ramifications on CV outcomes. Few studies have attempted to implement exercise rehabilitation during the acute phase of injury, a time in which CV function has not declined to any substantial degree and the potential for adaptive neuronal plasticity within the spinal cord is high.

Some of the only studies to examine the effects of exercise training acutely post-SCI were conducted by West and colleagues (West *et al.* 2014, West *et al.* 2015). They showed that passive hindlimb cycling (PHLC) rehabilitation implemented six days post-T3 transection resulted in improved cardiac function, attenuated responses to experimentally-induced AD (via colorectal distension, CRD), and reduced factors associated with cardiovascular disease (CVD) risk. While these endeavors are critically important to our understanding of CV pathology following SCI, most clinical injuries are anatomically incomplete and the degree to which residual sympathetic fibers can contribute to proper CV function following acute exercise-induced plasticity of CV circuits is not well understood. Many studies have shown that acute exercise rehabilitation can favorably impact the residual spinal circuitry responsible for locomotion (Barbeau and Rossignol 1987, de Leon *et al.* 1998), effects that are likely mediated through various neurotrophic growth factors (Vaynman and Gomez-Pinilla 2005, Sandrow-Feinberg and Houle 2015). Further, there is a large body of experimental evidence for inherent plasticity within the respiratory system following cervical hemisection (Lane *et al.* 2009), of which can be strengthened or enhanced following specific respiratory muscle training (Sapienza and

Wheeler 2006). Conversely, loss of function studies have shown that activities such as acutely-implemented wheelchair immobilization and stretching physical therapy have the capacity to negatively impact spinal reorganization and produce locomotor deficits that last into the chronic phase (Caudle *et al.* 2011, Caudle *et al.* 2015). Given that CV abnormalities such as AD have been linked to aberrant sprouting in central circuits, primarily mediated through calcitonin gene-related peptide positive ($CGRP^{+}$) fibers in the lumbar cord (Krenz *et al.* 1999, Cameron *et al.* 2006), additional information pertaining to the combined effects of early exercise on CV fitness and adaptive plasticity of CV circuits is warranted.

Cardiovascular disease continues to be implicated in increased morbidity and mortality in the SCI patient population (Warburton *et al.* 2007, Inskip *et al.* 2012). Acutely-timed exercise interventions are necessary to curtail CV decline, enhance CV control, and improve the life-expectancy of SCI patients. The aim of the present study, therefore, was to investigate whether various modalities of acutely-implemented exercise rehabilitation strategies have the capacity to improve resting hemodynamic parameters and attenuate pressor responses to colorectal distension (i.e. AD) following a clinically-relevant, incomplete injury of the upper thoracic cord. Active swimming exercise or PHLC rehabilitation was implemented eight days following severe T2 contusion. After 3.5 weeks of training, animals were instrumented with telemetric devices to assess hemodynamic control and AD severity. We hypothesized that abrupt and persistent immobility combined with disruption of sympathetic modulation of CV end-organs following severe T2 contusion would lead to disordered CV control (i.e. AD), similar to that of a complete transection. Rodents that received swimming exercise therapy would develop improved hemodynamic control at rest given the active nature of swimming and its ability to

sufficiently challenge the CV system (see results from previous chapters). Further, we hypothesized that both active swim exercise and PHLC rehabilitation would be effective in reducing the severity of experimentally-induced AD; however, PHLC would produce more robust outcomes due to its positive effects on neuronal plasticity in the lumbar circuitry.

Methods

Ethical Approval and Experimental Design

All animal care and surgical procedures were performed in accordance with the Canadian Council for Animal Care. Ethics approval were granted by the University of British Columbia.

All experiments were conducted on adult male Wistar rats weighing 250-300 grams (Harlan Laboratories, Indianapolis, IN, USA). Rodents were socially housed and maintained on a reverse 12 hour day/night schedule. Animals were initially divided into three groups: non-exercised, uninjured control (CON, n= 6), non-exercised, incomplete T10 SCI (T10-CON, n= 6), or incomplete T2 SCI (n=18). T2 contusion animals were then randomly assigned to one of the following experimental cohorts: non-exercised T2 SCI (T2-CON, n=7), T2 SCI plus passive hind-limb cycling (T2-CYC, n=5), or T2 SCI plus active swimming exercise (T2-SW, n = 6). Rehabilitation strategies were initiated eight days post-SCI in the training groups and lasted for 3.5 weeks. At the termination of the study, all animals were instrumented with telemetric devices to assess resting hemodynamic parameters and pressor responses to experimentally-induced autonomic

dysreflexia (via colorectal distension, CRD). Animals were then transcardially perfused for tissue collection and processing.

Spinal Cord Injury

Following acclimation to the researchers and testing/exercise facilities, severe 400kD contusion injuries with a 5 second dwell were delivered to SCI animals at their respective levels using the Infinite Horizons device (IH, Precision Systems & Instrumentation [PSI], Lexington, KY). Three days prior to SCI, rats were prophylactically treated with enrofloxacin (Baytril; 10 mg kg^{-1}, s.c., AVP). On the day of surgery, rats were anesthetized with isoflurane (5% in induction chamber and maintenance with 2.5%, 1.5-2 L/min oxygen flow), administered buprenorphine (0.02 mg kg^{-1}, s.c.), enrofloxacin (10 mg kg^{-1}, s.c.), and warmed lactated ringers (5mL, s.c). For T2 contusion injuries, a dorsal midline incision was made in the superficial muscle overlying the C7-T3 (T8-T11 for T10 injuries) vertebrae. A single level laminectomy was performed at the T2 (T9 for T10 injuries) vertebral level. Following impact, the muscle and skin were closed in layers with 4-0 myocryl and 5-0 prolene sutures, respectively. Rats were given an additional bolus of lactated ringers (5mL, s.c.) and allowed to recover in a temperature-controlled environment (33°C, Animal Intensive Care Unit, HotSpot for Birds, Los Angeles, CA). Post-operative care consisted of daily injections of enrofloxacin (10 mg kg^{-1}, s.c.), twice-daily injections of buprenorphine (0.02 mg kg^{-1}, s.c.), and twice-daily 5ml boluses of lactated ringers for three days following surgery. Manual bladder expression was conducted three to four times per day until reflexive voiding was re-established. Rats were weighed and monitored daily for two weeks following SCI, and three times per week thereafter. Animals were socially

housed unless prohibited by aggressive behavior and provided with an enriched standardized diet as previously described (Ramsey *et al.* 2010).

Rehabilitation Interventions

Weekly BBB assessments were performed to track each rat's locomotor recovery beginning one week after injury. For exercised animals, locomotor assessments were conducted in the morning, prior to rehabilitation training.

Passive Hind-limb Cycling

Eight days following contusions, T2-CYC animals began PHLC rehabilitation. Training lasted for 30 minutes each day, five days a week for 3.5 weeks (Monday through Friday) using a customized cycle ergometer. Cycle training has been used extensively in SCI rehabilitation and details are available elsewhere (Houle *et al.* 1999, West *et al.* 2014). Briefly, rats were horizontally suspended on a leather sling equipped with two holes cut for their hindlimbs. Their hind paws were secured to pedals with parafilm and gauze padding to minimize skin abrasions. Animals were cycled at a frequency of 0.5 Hz in accordance with previous studies (West *et al.* 2015). Cereal treats were given to rats during the cycling to encourage compliance.

Active Swimming

Swimming has been used as both an exercise rehabilitation modality and as an assessment technique for locomotor recovery following SCI in rodents (Smith *et al.* 2006, Gonzenbach *et al.* 2012). Eight days after injury, T2-SW animals were re-introduced to the

swimming pool to begin rehabilitation. Animals completed six swim sessions, five days per week for 3.5 weeks (Monday through Friday). Briefly, each swim session consisted of assisted lap swimming (either by tail or trunk support) in which the rat was repeatedly placed at one end of a 5-ft long plexiglass pool and encouraged to swim to the opposite end where they exited the water via a padded ramp. Each swim session lasted five minutes for a total of 30 minutes of exercise rehabilitation per day. Pool temperatures were maintained at 33-35 degrees C to give rats incentive to exit the pool but also to avoid problems associated with drastic drops in core body temperature and spasticity. Uninjured rats can easily swim upwards of 50 laps in a typical swim session. Injured rats rarely use their hind-limbs for forward propulsion; as such, swimming exercise following severe contusion in our animals is mainly a forelimb rehabilitation modality.

Blood Pressure Assessment

Carotid cannulation of the sensing device was performed under isoflurane anesthetic (5% in induction chamber and maintenance with 2.5%, 1.5-2 L/min oxygen flow). Briefly, following administration of warmed lactated ringers (5mL, s.c), a 3 cm incision was made in the skin between the shoulder blades. Using blunt-tipped dissection scissors, a small subcutaneous pocket was created to hold the body of the device (model TRM54P, Millar Inc. Auckland, New Zealand). The pressure-sensing catheter tip was tunneled subcutaneously around the neck and exteriorized along the ventral midline. Care was taken to preserve the nerve plexus overlying the carotid artery during dissection. The rostral segment of the carotid artery was occluded to allow cannulation of the pressure sensor. The catheter was secured within the vessel using 4-0 silk suture. The skin was

closed using 5-0 prolene sutures and rats were administered an additional bolus of lactated ringers (5mL, s.c.). Rats recovered in a temperature-controlled environment (33°C, Animal Intensive Care Unit, HotSpot for Birds, Los Angeles, CA) for 90 minutes, after which they were moved to the testing environment for acclimation. Following acclimation (30 minutes), baseline hemodynamics were acquired for ten minutes. Heart rate was derived from the beat-to-beat Arterial blood pressure (BP) recording using LabChart, version 8.0 (ADInstruments, Colorado Springs, CO). Severity of AD was assessed using at least 3 bouts of CRD (noisy recordings removed), a procedure that is commonly administered in the laboratory (Alan *et al.* 2010, Ramer *et al.* 2012, West *et al.* 2015). Colorectal distension was not performed in uninjured control animals. Animals were unrestrained and freely moving in their home cages during the CRD procedure. To invoke CRD, a small, deflated plastic balloon was rectally inserted a distance of 1.5cm (balloon-tip of a Swan-Ganz catheter; 10mm in length). Blood pressure and HR were allowed to stabilize (about 10 minutes), after which a second baseline recording was made to ensure that hemodynamic parameters were not altered due to the insertion of the catheter. Once BP returned to pre-insertion levels, the balloon was infused with 2mL of air over ten seconds and distension was maintained for one minute. Sequential bouts of CRD were performed with at least 10 minutes in between sessions to allow hemodynamics to return to baseline values. Raw, unfiltered beat-to-beat BP and HR data were averaged over 1-second intervals for each CRD trial. Baseline (pre-distension, one minute) hemodynamic parameters were averaged for each session to determine resting, unprovoked BP and HR. Hemodynamic traces during CRD with movement artifact were removed from the analysis and the remaining one-minute responses to CRD were averaged for each animal. Absolute change in systolic

blood pressure (SBP), maximum SBP value, and percent SBP increase from baseline were determined for each animal and experimental group averages were enumerated for statistical analysis. The time needed to return to resting hemodynamic variables (in seconds), from the point of deflation, was also determined for each session and averaged for each animal/experimental group. Briefly, second-by-second hemodynamic data was assessed during the recovery period and compared to pre-distension baseline. Time to recovery was calculated using a custom-made macro that determined the time for hemodynamic measurements to recover within 5 mmHg (SBP, Diastolic BP, and Mean BP) or 10 bpm (HR) of pre-distension values for at least 10 consecutive seconds. The total time to recovery (recovery of all four variables) was also determined and averaged for each animal/group.

Study Termination and Histological Analysis

Upon completion of the study, rats were anesthetized with isoflurane (5% in induction chamber and maintenance with 2.5%, 1.5-2 L/min oxygen flow) to allow removal of the transmitter device. The caudal end of the cannulated carotid artery was occluded using additional 4-0 silk suture and the catheter was explanted. Rats were then transcardially perfused with phosphate-buffered saline (PBS) at 100 mmHg using a hand-held sphygmomanometer and customized setup, followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer at 80 mmHg. The epicenter and surrounding penumbra were removed, post-fixed in 4% PFA for 24 hours, followed by 48 hours in 20% sucrose for cryoprotection. The epicenter sections (C7-T6 or T7-T12) were cryosectioned at 30 µm and assessed for white matter sparing in and around the epicenter (Magnuson *et al.* 2005).

Statistical Analysis

Behavioral assessments (BBB) were analyzed with nonparametric Mann-Whitney U tests (T10-CON, T2-CON, T2-CYC, and T2-SW). Post hoc t-tests were completed with Wilcoxon Ranked Sums. For BBB, the left and right hindlimbs were averaged if there was no significance difference between them. Cardiovascular (CRD/AD) parameters were analyzed as both raw data and data normalized to individual pre-distension measurements with nonparametric Mann-Whitney U tests. Post hoc t-tests were completed with Wilcoxon Ranked Sums. Comparisons were made between original experimental groups and then re-analyzed based on terminal hindlimb performance. All animals with T2 contusion, regardless of treatment condition, were stratified according to terminal BBB scores: BBB <6 (T2-LOW) or BBB ≥6 (T2-MOD) and compared to T10-CON. Statistical analyses were performed with SPSS (v22, Chicago, IL). Significance was set at $p \leq .05$. Data is presented in the text as means ± standard deviation (SD) and represented graphically using means ± standard error (SEM).

Results

Severe thoracic contusion significantly impacts hindlimb locomotor function

Locomotor assessments were executed at the beginning of each week starting seven days post injury. For T2-SW and T2-CYC animals, BBB assessments were performed prior to their daily exercise training session. Animals displayed a wide range of locomotor abilities within and between the T2 experimental groups. Most animals remained in the early stage of locomotor recovery in which there was no weight support of the hindlimbs (BBB < 8), regardless of whether or not exercise rehabilitation was implemented. There were no

differences noted between the groups at the terminal time point (Figure 13A). All T2-SCI groups performed better on the BBB than T10-CON one week after injury (data not shown; $p \leq .05$ for all comparisons), and T2-SW animals continued to have better hindlimb function at weeks 2 and 3 versus T10-CON (data not shown; $p \leq .05$). Timewise comparisons showed significant hindlimb improvements over time in T2-SW, T2-CON, and T10-CON (Figure 13B; T2-SW: Week 1 vs Weeks 2, 3, 4, and 5, $p \leq .05$; T2-CON: Week 1 vs. Weeks 4 and 5, $p \leq .05$; T10-CON: Week 1 vs. Weeks 4 and 5, $p \leq .05$). However, cycle-trained animals did not show improvements in BBB performance at any time points assessed.

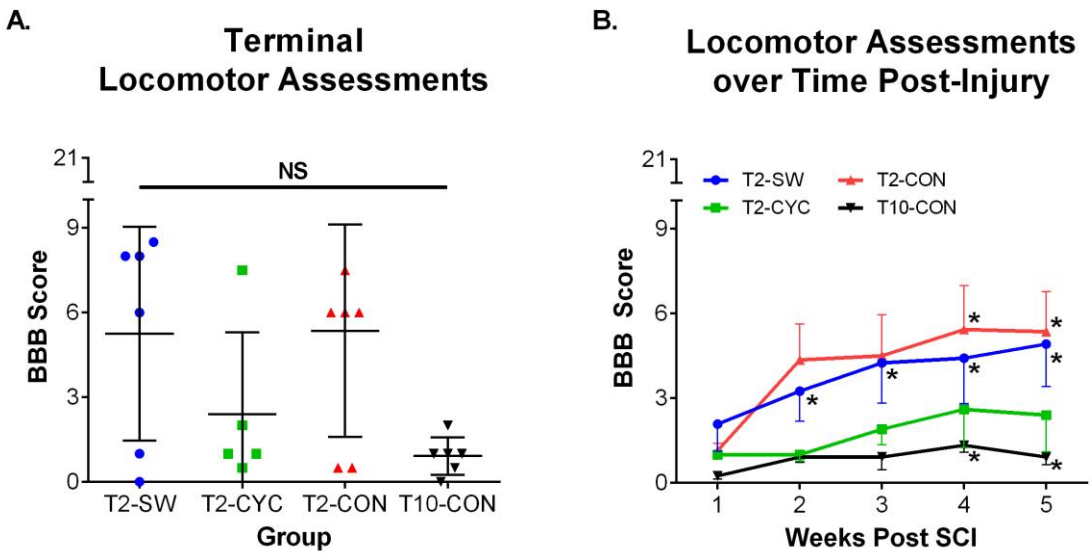

Figure 13. **Severe contusion of the spinal cord causes austere deficits in hindlimb locomotor function. (A)** Terminal performance on the BBB hindlimb locomotor scale. No differences were noted between groups. **(B)** Improvements in BBB scores over time following T2 or T10 contusion as compared to Week 1 scores. Data is displayed as mean ± SEM and significance was set at *$p \leq .05$.

High thoracic contusion results in diminished cardiovascular parameters at rest

Hemodynamics measurements were computed prior to CRD for all SCI conditions and compared to uninjured controls (CON) at rest. Severe contusion of the T10 spinal cord resulted in resting tachycardia that was significantly greater compared to CON and all T2-injured animals, regardless of exercise intervention (Figure 14D; T10-CON vs CON, p= .047; vs T2-SW, p= .018; vs T2-CYC, p= .05; vs. T2-CON, p= .045). No differences were noted in SBP, DBP, or MBP between CON and T10-CON animals. Both T2-CYC and T2-CON exhibited significantly reduced blood pressure parameters in comparison to both CON and T10-CON (Figure 14A-C; SBP: T10-CON vs T2-CON, p= .006; T10-CON vs T2-CYC, p= .014; CON vs T2-CYC, p= .05; DBP: T10-CON vs T2-CON, p= .018; T10-CON vs T2-CYC, p= .027; CON vs T2-CON, p= .045; CON vs T2-CYC, p= .05; MBP: T10-CON vs T2-CON, p= .006; T10-CON vs T2-CYC, p= .14; CON vs T2-CON, p= .018; CON vs T2-CYC, p= .014). However, swimming exercise rehabilitation (T2-SW) appeared to normalize resting blood pressure values (SBP, DBP, and MBP), as they were not different from CON or T10-CON animals (Figure 14 A-C).

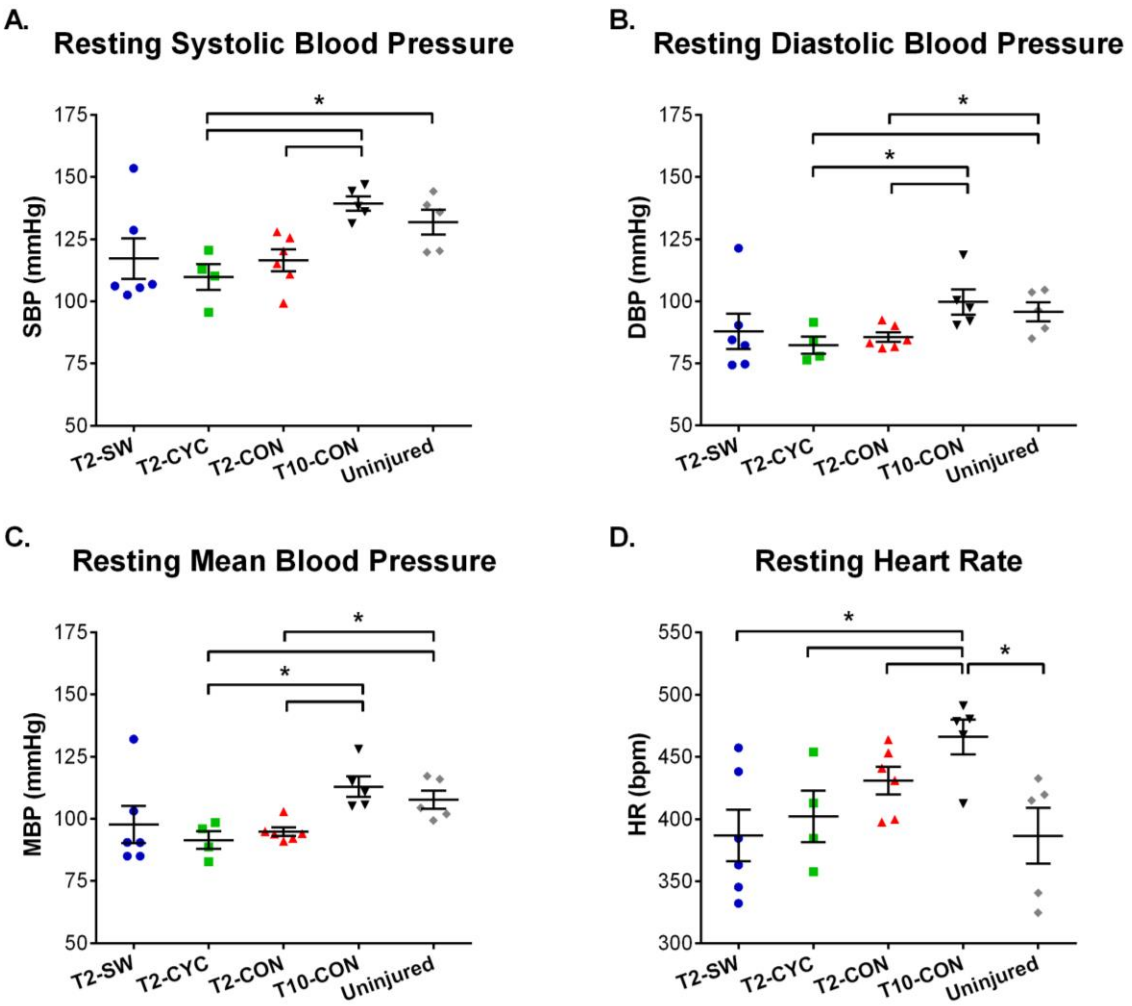

Figure 14. **Swimming exercise rehabilitation partially restores resting hemodynamic control following severe contusion to the upper thoracic spinal cord.** Resting hemodynamic parameters were measured prior to the CRD protocol five weeks post-SCI. T2-CON and T2-CYC animals exhibited significantly reduced systolic **(A)**, diastolic **(B)**, and mean **(C)** blood pressure as compared to T10-CON and uninjured control animals. No differences were noted in pressor values between T2-SW and T10-CON and uninjured control animals**.** Severe T10 contusion caused resting tachycardia that was significantly greater than all other groups **(D)**. Data is displayed as mean ± SEM and significance was set at *$p \leq .05$.

Exercise-associated rehabilitation did not attenuate the pressor responses to colorectal distension following T2 contusion.

Pressor responses to CRD were observed in all contusion groups, irrespective of lesion level. However, the magnitude of responses were much greater in high thoracic T2-SCI animals than in animals which received low thoracic T10 contusion.

Lower thoracic SCI induced only modest pressor responses to CRD(Figure 15B, T10-CON absolute increase in SBP was less than 30 mmHg). Conversely, high thoracic contusion induced much greater pressor responses to CRD, with T2-SW, T2-CYC, and T2-CON exhibiting average increases in SBP of 67 mmHg, 61 mmHg, and 46 mmHg, respectively (Figure 15A; T2-SW vs T10-CON, $p= .037$; T2-CYC vs T10-CON, $p= .028$; T2-CON vs T10-CON approached significance, $p= .063$). Absolute changes in DBP and MBP from rest was also significantly greater in T2-SW and T2-CYC compared to T10-CON (DBP and MBP data not shown, all comparisons $p\leq .05$). Neither active forelimb swimming nor passive hindlimb cycling exercise rehabilitation attenuated the degree of autonomic dysreflexia during CRD as there were no differences between T2-SCI groups.. T2-SCI group responses to the CRD protocol are illustrated in Figure 15A.

When expressed as a percentage SBP increase from baseline hemodynamic measurements, all T2-SCI groups exhibited significantly greater pressor responses to CRD than T10-CON animals (Figure 15C; all comparisons $p\leq .05$).

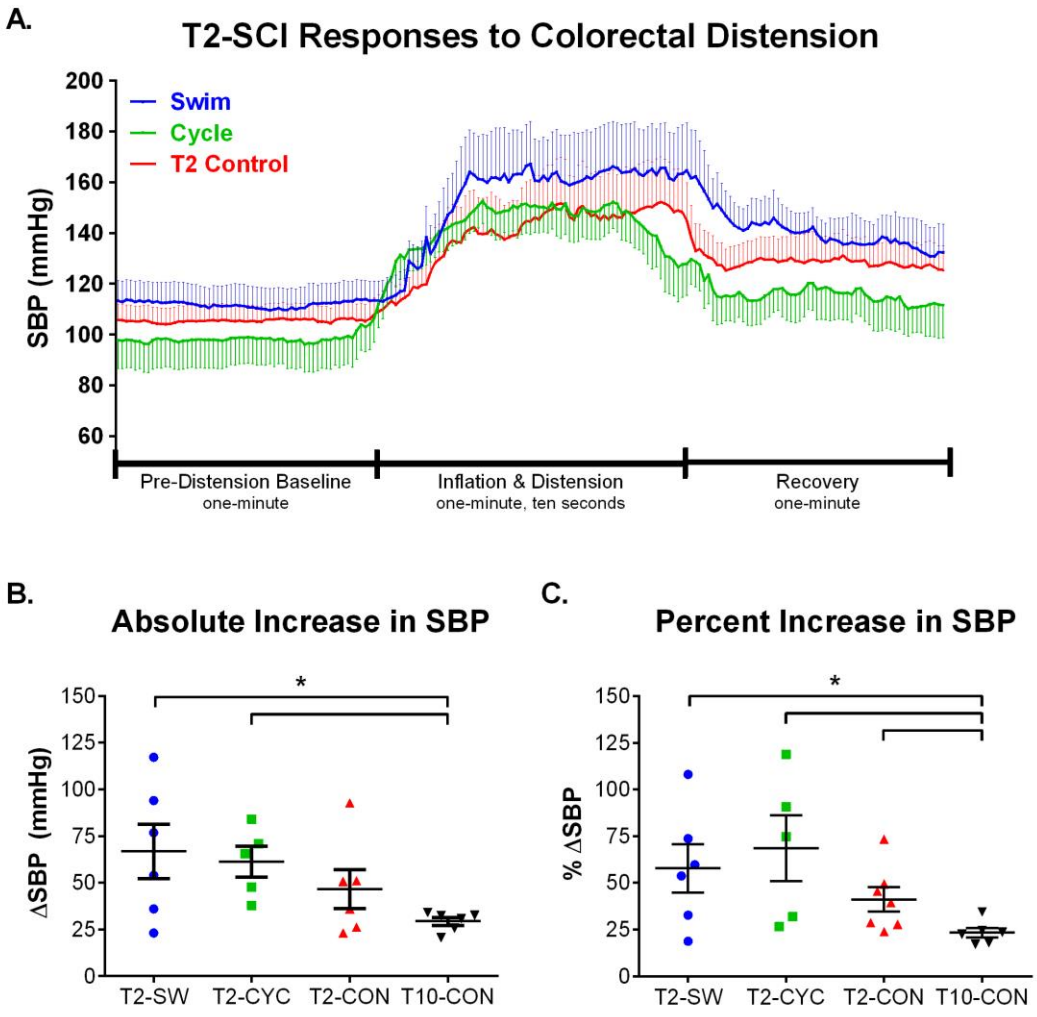

Figure 15. **Exercise rehabilitation initiated acutely after T2 contusion did not attenuate the pressor response to colorectal distension.** All T2-SCI groups experienced significantly greater pressor responses to CRD than T10-CON. **(A)** Time-locked group average SBP data during pre-distension baseline, inflation and distension, and recovery. Data is down sampled from 1000 Hz to one data point per second **(A)**. The absolute increase in SBP was greater in exercised animals than T2-CON and T10-CON **(B)**. Also, SBP rise during CRD, as expressed as a percentage of baseline SBP, was significantly higher in all T2-SCI groups, independent of exercise rehabilitation, as compared to T10-CON **(C)**. Data in **B-D** is represented as mean ± SEM and significance is set at *p≤ .05.

Responses to CRD correspond to hindlimb performance on the BBB

Pressor responses varied considerably between T2-SCI animals, irrespective of experimental condition. Therefore, responses to CRD were re-analyzed based on terminal performance on the BBB scale. Based on the results of a cluster analyses, all T2-SCI animals that received scores less than 6 on the BBB (indicating slight or extensive movement of one or two hindlimb joints only) were reclassified into the Low BBB group (T2-LOW). T2-SCI animals with scores equal to or greater than 6 (movement of all three hindlimb joints, with at least extensive movement of two of those joints) were placed into the Moderate BBB group (T2-MOD). T10 CON animals remained in their original cohort and group comparisons were executed as previously described.

There was a clear distinction in locomotor ability between T2-LOW and T2-MOD groups (Figure 16B). T2-LOW animals had significantly reduced hindlimb function five weeks after incomplete T2 contusion, in which no animal scored above a 3 indicating, at most, extensive movement of two hindlimb joints. Conversely, BBB scores for T2-MOD animals ranged from 6 to 11, with the majority of animals displaying sweeping ability in one or both of the hindlimbs. As before, T10-CON animals had very little hindlimb function at the terminal time point and scores were similar to T2-LOW animals.

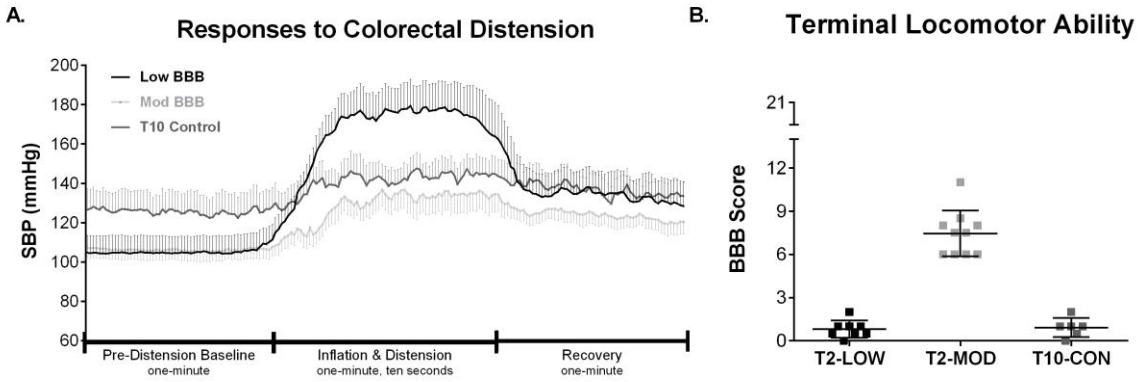

Figure 16. **Pressor responses to colorectal distension are correlated with the recovery of locomotor function following severe T2 contusion.** Animals with T2-SCI were re-classified according to terminal (week 5) performance on the BBB scale. Rodents with poor locomotor recovery (T2-LOW) had significantly higher pressor responses to colorectal distension than T2-MOD and T10-CON. **(A)** Time-locked group average SBP data during pre-distension baseline, inflation and distension, and recovery. Data is down sampled from 1000 Hz to one data point per second **(A)**. **(B)** Terminal BBB scores of T2-SCI animals categorized into LOW and MOD hindlimb performance. Data is represented as mean ± SEM and significance is set at *p≤ .05.

Similar to previous analyses, T10 severe contusion caused resting tachycardia that was significantly higher than T2-LOW and T2-MOD (data not shown; T10-CON vs T2-LOW, p= .039; T10-CON vs T2-MOD, p= .009). Second-by-second group hemodynamic responses to the CRD protocol are illustrated in Figure 16A. Note that in T2-LOW, CRD caused an exaggerated pressor response, where SBP rose dramatically higher than both T2-MOD and T10-CON groups (Figure 17A: T2-LOW vs T2-MOD, p= .026; T2-LOW vs T10-CON, p= .007). The change in DBP and MBP were also significantly higher in T2 animals with low BBB scores (Figure 17B and C, respectively; DBP: T2-LOW vs T2-MOD, p= .003; T2-LOW vs T10-CON, p= .005; MBP: T2-LOW vs T2-MOD, p= .003; T2-LOW vs T10-CON, p= .005). Changes in SBP as a percentage of pre-distension baseline measures were significantly higher in all T2 animals as compared to T10-CON groups (Figure 17E: T2-LOW vs T10-CON, p= .005; T2-MOD vs T10-CON, p= .023). There was also a trend for T2-LOW animals to have higher SBP responses to CRD than T2-MOD, although this did not reach statistical significance (Figure 17E: T2-LOW vs T2-MOD, p= .051). The average maximum SBP value reached during the CRD protocol was significantly higher in T2-LOW as compared to T2-MOD animals (Figure 17F; T2-LOW vs T2-MOD, p= .021).

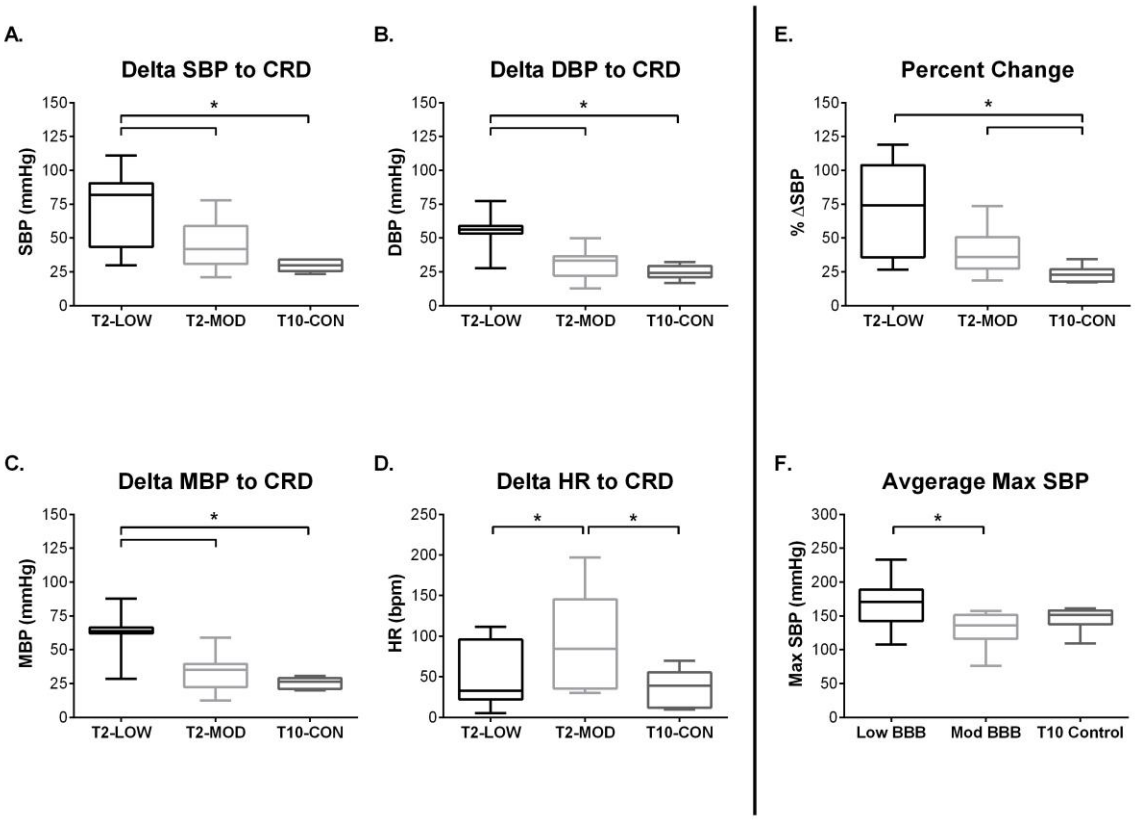

Figure 17. **Severity of autonomic dysreflexia is greater in rodents with limited hindlimb recovery.** Pressor responses to colorectal distension were significantly greater in T2-LOW. The absolute change in SBP **(A)**, DBP **(B)**, and MBP **(C)** was significantly greater in T2-LOW than T2-MOD and T10-CON. The maximum SBP during CRD was also greater in T2-LOW vs T2-MOD **(F)**. Heart rate responses to the distension protocol were significantly greater in T2-MOD compared to all other groups **(D)**. Rodents with T10 contusion injuries did not experience significant pressor responses to CRD, as expressed as a percentage of baseline SBP, in comparison to both T2-SCI groups **(E)**. Significance is set at *p≤ .05.

Time recovered to achieve hemodynamic stability following CRD

Pressor and heart rate responses to CRD were monitored and assessed for at least ten minutes following deflation of the swan-ganz catheter balloon. The time needed, in seconds, for each animal to return to pre-distension hemodynamic parameters (SBP, DBP, MBP, and HR) was calculated and compared across groups. No differences were noted during comparison of the original experimental groups (Figure 19E, F: T2-SW, T2-CYC, T2-CON, and T10-CON). However, when terminal locomotor ability was used to stratify T2-SCI animals, group differences revealed that rodents with greater pressor responses to CRD required more time to recover resting hemodynamic stability. T2-LOW had significantly greater recovery times for all parameters assessed than T10-CON (Figure 18 A-D; SBP, p= .02; DBP, p= .028; MBP, p= .02; and Total Recovery, p= .01). In comparison to T2-MOD, T2-LOW also required significantly longer time to establish pre-distension DBP and MBP (Figure 18B, C; DBP, p= .001; MBP, p= .003).

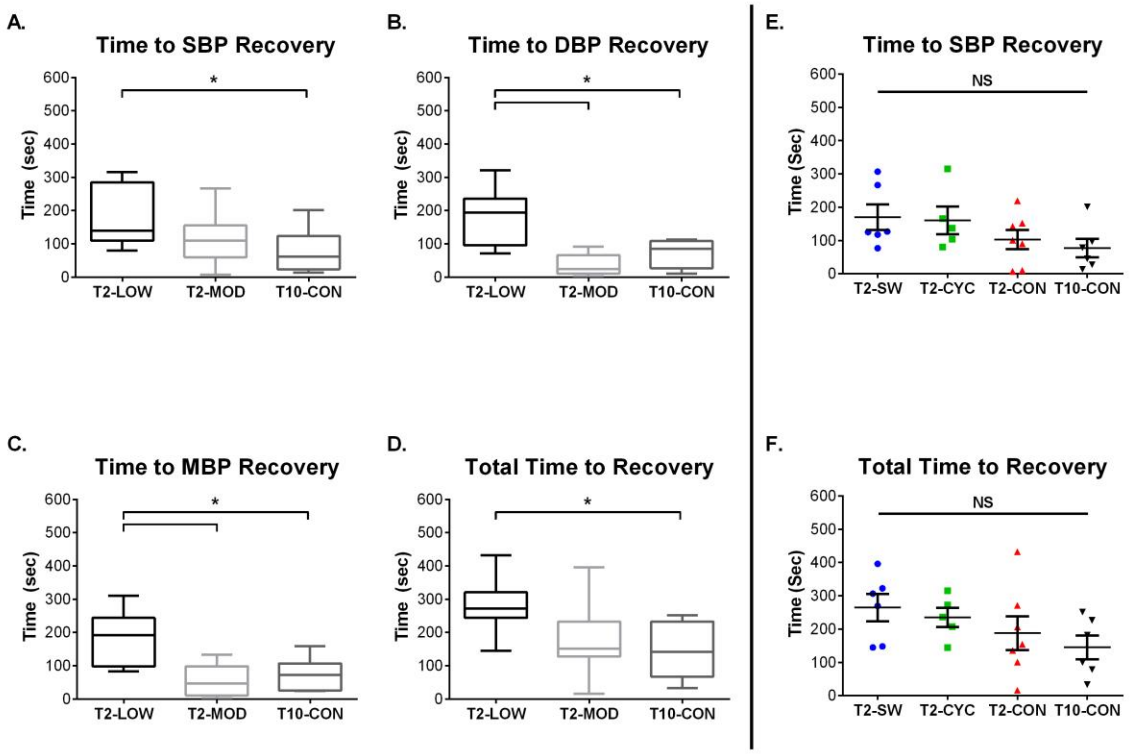

Figure 18. **Rodents with more severe pressor responses to colorectal distension require longer recovery periods to reach resting hemodynamic parameters.** The time needed for animals to reestablish pre-distension hemodynamic parameters was calculated in seconds. T2-LOW had significantly longer time to SBP **(A)**, DBP **(B)**, MBP **(C)**, and Total **(D)** recovery than T10-CON animals. T2-LOW also had significantly greater DBP and MBP recovery times than T2-MOD. No differences were noted between T2-MOD and T10-CON. No differences were noted in any of the recovery variables when animals were analyzed in their original experimental groups **(E, F)**. Original experimental group data is in **E, F** and is represented as mean ± SEM. Significance is set at *p≤ .05.

Discussion

Summary of findings

The findings of the present study illustrate that severe contusion to the T2 thoracic spinal cord results in episodes of unstable blood pressure regulation and homeostasis. Similar to studies of transection SCI, rodents experienced instances of autonomic dysreflexia during experimentally-induced colorectal distension. The recovery of CV control, or lack thereof, was independent of acute exercise rehabilitation, as neither swimming nor PHLC attenuated the pressor response to CRD. Instead, disordered CV control and severity of AD appeared to be a related to sparing at the injury epicenter and/or recovered hindlimb function.

The development of therapeutic interventions to reduce the incidence of CVD in SCI patients continues to be a main priority for clinicians and scientists alike. Chronic implementation of various rehabilitation strategies, such as functional electrical stimulation (FES) cycling, has proven to be beneficial in improving vascular function (Ditor *et al.* 2005, Tordi *et al.* 2009) and aerobic capacity (DiCarlo *et al.* 1983, de Groot *et al.* 2003) in the SCI community. However, to our knowledge, there have been no studies that have sought to resolve the burden or severity of AD in chronic, high level lesioned patients. This is troublesome given the vast majority of SCI individuals experience regular bouts of AD that effect everyday living and quality of life (Lindan *et al.* 1980, Elliott and Krassioukov 2006).

Passive-hindlimb cycling rehabilitation

Passive cycling rehabilitation is an attractive therapeutic option over FES cycling in that it does not require extensive equipment and is not subject to the various contraindications and side-effects of electrical stimulation (Ashley *et al.* 1993). Passive hindlimb cycling has been examined extensively in the preclinical SCI literature as a rehabilitation modality for not only locomotor impairments, but also for cardiovascular dysfunction and neuropathic pain. Previous work by West and colleagues has illustrated the many CV benefits of acute PHLC rehabilitation, including a reduction in AD severity and attenuated aberrant nociceptor fiber sprouting in lumbar circuitry (West *et al.* 2014, West *et al.* 2015). In the current study, PHLC failed to eliminate pressor responses to CRD following incomplete contusion to the upper thoracic spinal cord. The lack of improvement in CV control following cycle training may be due to differences in the duration of daily training bouts (30 verses 60 minutes). However, we find this unlikely given that only 15 minutes of PHLC training has been shown to produce augmented levels of various neurotrophic factors important for adaptive neuronal plasticity in the lumbar spinal cord, which in turn contributes to sensory and motor reflex recovery following complete SCI (Cote *et al.* 2011). Alternatively, one possible explanation for the failure of PHLC to attenuate the severity of AD in the current study may be related to the level of locomotor recovery our rats experienced. Animals in the T2-CYC group, on average, developed at least extensive movement of one hindlimb joint. Given this ability, the passive nature of the cycling was, on occasion, contested by volitional hindlimb kicking. Therefore, the traditional benefits gained through repetitive and predictable cyclic patterns of afferent stimulation may not been achieved in this study. Further, some of the animals in this group

developed small pressure sores on the hindlimbs that were aggravated/inflamed during the cycling sessions. While not directly assessed during this study, noxious stimulation and periods of increased inflammation may have been counterproductive to the cycling rehabilitation and further contributed to AD severity (Gris *et al.* 2004, Weaver *et al.* 2006, Marsh and Flemming 2011). Indeed, work by Grau et al. has shown that intermittent nociceptive stimulation, not unlike that experienced by some of our rodents, results in reduced neurotrophic activity and attenuated locomotor recovery following complete SCI (Garraway *et al.* 2011).

Active swimming exercise

Active aerobic exercise modalities have traditionally been preferred over passive rehabilitation for their effects on CV and respiratory systems (Ballaz *et al.* 2008, Hellsten and Nyberg 2015). However, denervation-induced paralysis of the trunk and lower limbs in combination with blunted hemodynamic responses to exercise following high thoracic SCI makes this type of rehabilitation difficult in the SCI community (Van Loan *et al.* 1987). Forms of active exercise have, therefore been limited to rehabilitation using solely the upper extremities. This is problematic, however, as there is general consensus that there is not enough upper limb mass to reap cardioprotective benefits of arm ergometry alone (Davis *et al.* 1987, Gates *et al.* 2002, West *et al.* 2012). Unlike arm ergometry, however, swimming exercise recruits trunk as well as upper extremity musculature following thoracic SCI. Previous studies (Aim 1) have shown that four minutes of swim exercise sufficiently challenges the CV system following incomplete T3 contusion in female rodents. Therefore, we predicted that swimming rehabilitation following T2 contusion

would be an appropriate form of aerobic challenge to bring about positive CV enhancements. Indeed, results of the current study illustrate that 3.5 weeks of swimming exercise (30 minutes per day, 5 days per week) normalized resting hemodynamic parameters to uninjured control levels (SBP, DBP, and MBP) following severe contusion to the upper thoracic cord. Unfortunately, pressor responses to CRD were not attenuated following this protocol. Reasons for this likely relate to swimming being a purely forelimb propulsion task, with no contributions from the hindlimbs after injury. While this was not investigated directly in the current study, exercise paradigms that fail to deliver predictable afferent stimulation to the lumbosacral spinal cord are unlikely to elicit favorable plasticity in central circuitry important for the development and maintenance of AD. Results of this study, therefore, highlight the importance of exercise specificity in generating desired CV outcomes following SCI.

The intensity of pressor responses to CRD varied considerably in T2-SCI animals following contusion to the upper thoracic spinal cord. In an effort to better understand the effects of incomplete contusion on the development and severity of AD, T2-SCI animals were reanalyzed based on terminal performance on the BBB scale. Following this, a clear relationship emerged between the pressor responses to experimentally-induced CRD and the degree of hindlimb locomotor ability. Previous studies have shown that a significant correlation exists between sparing at the lesion epicenter and motor outcomes following a high thoracic impact of this severity (Basso *et al.* 1996). Further, neuronal sparing and the anatomical changes that occur as a result of an injury of this magnitude (both at the lesion epicenter, in the lumbosacral spinal cord, and in the ventrolateral medulla), significantly influence the phenotype of CV dysfunction during terminal assessments (Squair *et al.*

2016). Likewise, we have shown here that T2 incompletely injured animals with very limited hindlimb motor recovery (LOW BBB group) had exacerbated pressor responses to CRD and longer recovery times that were independent of exercise rehabilitation. These findings are likely due to the lack of sympathetic sparing at the injury epicenter and reduced CV stimulus in the wake of low locomotor performance. Although not assessed, the lack of plantar paw placement (and thus appropriate afferent stimulation to the lumbar spinal cord) may have contributed to inappropriate sprouting and plasticity of lumbosacral circuits, further exacerbating pressor responses to CRD. Moderately functioning rodents (MOD BBB group) had CV responses not significantly different than T10-SCI in which the pressor responses to CRD are considered to be within normal limits, owning to the preservation of a large portion of sympathetic preganglionic neurons critical for CV control. These data are in agreement with other studies in which a strong correlation exists between injury severities and the resulting CV control at rest and in response to provocation (Weaver *et al.* 2001, Squair *et al.* 2016).

Concluding Remarks

The beneficial effects of exercise training after SCI have been noted numerous times in both clinical and preclinical settings. Therapeutic interventions such as wheelchair and arm ergometry have been shown to produce positive changes that include increased aerobic fitness, improved blood lipid profiles, and increased muscle mass and strength in chronic SCI patients (DiCarlo *et al.* 1983). Yet, the decline in physical fitness following injury is progressive and the length of time a patient remains immobile largely impacts CV structure and function (Thijssen *et al.* 2006, Thijssen *et al.* 2012). Acute implementation

of exercise paradigms are ideal because they have the ability to take advantage of inherent spinal plasticity and prevent maladaptive remodeling of both central and peripheral structures. However, the timing of exercises initiation and the intensity of exercise programs are critical factors, for starting rehabilitation efforts too early can negatively impact recovery efforts (Laird *et al.* 2009, Smith *et al.* 2009). Further, given the substantial spontaneous recovery of hindlimb function and trunk stability exhibited by rodents with SCI, exercise paradigms must surpass the animal's innate ability to retrain themselves in their cages. Indeed, data from this study supports this notion. With improvements in over-ground locomotion and plantar placement of the hindlimbs comes increased volume and pressure loading of the heart and appropriate afferent stimulation of the lumbar circuitry, which in turn creates a new steady-state for CV mechanics. This is also true for the clinical population. The heterogeneity of patients' injury parameters and the level of immobility following injury will undoubtedly influence the impact of rehabilitation strategies. The application of acute training programs, therefore, must take into account the ever evolving system and adapt exercise prescription accordingly.

CHAPTER V

CONCLUDING REMARKS

Spinal cord injury is a devastating life event that results in extreme somatic, autonomic, and sensory dysfunction. To date, the majority of clinical and preclinical research has concentrated on the recovery of locomotor function in the wake of paralysis below the level of the lesion. However, secondary complications, most notably those of the autonomic nervous system, continue to encompass the leading causes of mortality and morbidity in the SCI community. Specifically, CVD occurs earlier and is more robust in SCI patients than in the able-bodied community, differences which cannot be solely explained by traditional risk factors (lipid profiles, age, obesity, smoking status, etc.) (Whiteneck *et al.* 1992, Cragg *et al.* 2012).

Damage to the neural circuitry important for CV regulation and output are inherently disrupted by the primary insult, and injury-induced modifications in both central and peripheral pathways in the days to months following injury exacerbate and potentiate CV decline (Dampney 1994, Furlan *et al.* 2003). Particularly, changes occurring in sympathetic neurons and medullary control centers lead to disordered hemodynamic states and extreme system collapse, notably episodes of systemic or orthostatic hypotension and reflex-mediated hypertension (i.e. AD) (Teasell *et al.* 2000, Weaver *et al.* 2001, Weaver *et al.* 2006). Further, reduced mobility subsequent to paralysis below the lesion leads to

lifestyles that are not advantageous for sustaining adequate cardiac or vascular health. As such, numerous studies have highlighted the decline in cardiac mechanics and reduction in ventricular contractility as well as changes occurring in vascular tissue due to the sedentary lifestyles characteristic of SCI patients (Kessler *et al.* 1986, Nash *et al.* 1996, de Groot *et al.* 2006, de Groot *et al.* 2006). Spinal cord injury represents the most extreme example of immobility, and alterations in cardiac as well as vascular structures likely underlie the increased risk of CVD experienced by this patient cohort.

The body of work presented here has sought to allow a better understanding of the lack of hemodynamic control and the temporal progression of CV dysfunction in rodents with a clinically-relevant contusive thoracic SCI. Heretofore, most preclinical studies have employed complete transection models of the upper thoracic cord to investigate extreme breakdown of CV function (i.e. autonomic dysreflexia). However, most clinical injuries are incomplete and little attention has been given to the temporal progression of CV decline that may lead to bouts of AD or pressor insufficiencies during every day living. Further, given the inherent immobility associated with SCI patients, strategies to counteract deconditioning of CV end-organs and the subsequent deterioration in CV health are warranted.

For the first time, we have shown that incomplete contusion injuries result in altered blood pressure and heart rate control during exercise challenge that is independent of lesion level. There is a temporal dichotomy in mechanism of CV control in which responses to exercise acutely after injury are vastly different from those in the chronic period. Further, following attempts to remedy CV dysfunction with acutely-implemented exercise-associated training, we have discovered that the various CV benefits achieved are specific

to the rehabilitation modality employed and heavily dependent on sparing at the injury epicenter and/or recovery of hindlimb ability.

Relating preclinical findings back to the clinic

Very few studies examining CV function after injury have employed SCI models that mimic the clinical population. Transection SCI models have traditionally been preferred over contusion injuries due to the lack of variability in CV outcomes between animals and for its ability to produce austere CV complications (Hou *et al.* 2013). However, most clinical injuries are incomplete and there is great heterogeneity among SCI patients with regards to CV outcomes and recovery (Furlan *et al.* 2003). Further, due to the anatomical location of preganglionic sympathetic neurons important for cardiac function, most labs examine CV effects of SCI using injury models that only disrupt circuitry in the T1-T5 levels. Work presented in this dissertation emphasizes the importance of using various models of injury to gain a better understanding of CV dysfunction in SCI patients. We have shown that profound deficiencies in CV control exist during instances of increased cardiopulmonary demand following incomplete lesions, even though the animals appear to have normal CV function at rest. Importantly, this disordered control is present in animals with high (T3) and low (T10) spinal cord injuries.

Hemodynamic control during periods of rest

The majority of preclinical studies to date have examined hemodynamic control at rest and during provocation, such as CRD, in rodents with complete transection of the upper thoracic spinal cord. It is generally accepted that this type of lesion produces profound CV

instability that is characterized by persistent hypotension similar to what occurs acutely in the clinic and in chronic high-level lesion SCI patients (West *et al.* 2015). However, others have found that transection SCI models do not always deliver this type of hemodynamic presentation, and in reality the lack of CV control in rodent models of SCI appear to exist along a spectrum of dysfunction. For instance, in a study by Rabchevsky et al., complete transection of the T3 spinal cord produced transient hypotension that resolved to pre-injury values within two weeks of the injury (Rabchevsky *et al.* 2012). Preclinical studies of incomplete SCI also deliver contrasting results with regards to the presence of resting hemodynamic instability both acutely and chronically after injury. A recent study by Squair and colleagues showed that both moderately- and severely-injured (T3, 200 vs 400 kdyn IH) rodents presented with reduced resting SBP and MBP values five weeks post-contusion (Squair *et al.* 2016), whereas others have reported no change in hemodynamic profiles over time (Maiorov *et al.* 1998, Mayorov *et al.* 2001). Reasons for this disparity likely relate to 1) the degree of tissue sparing in and around the injury epicenter, and 2) the proportion of healthy sympathetic preganglionic neurons available to participate in proper CV mechanics following various injury modalities. Likewise, we have shown that the injury parameters, most notably the severity of the injury impact, greatly influence hemodynamic outcomes following incomplete contusion of the thoracic spinal cord. Results from the studies presented in this dissertation suggest that both mild (12.5 g/cm NYU) and moderate (25 g/cm NYU) contusive SCI models do not generate enough spinal cord tissue damage to induce lasting deficits in CV control while rodents are resting in their cages. Further, aside from slight tachycardia in T3 moderately-contused animals one week after injury, no differences were noted in high (T3) versus low (T10) thoracic contusion injury models,

suggesting that there was sufficient sympathetic sparing to minimize overt signs of system dysfunction in awake, freely moving animals. Severe contusion of the upper thoracic spinal cord (aim 2, 400 kdyn IH), however, did lead to persistent and significant hypotension at five weeks post-injury. These animals also regained very little hindlimb function implying that the degree of tissue damage in and around the injury epicenter may be important for recovery of various types of function following SCI, not just locomotor outcomes (Basso *et al.* 1996, Squair *et al.* 2016). Given the varying hemodynamic profiles of animals in the studies reported here, it is worthwhile to consider whether or not mild and moderate contusive injury models appropriately represent spinal cord-injured patients in the clinic and whether or not they are important for studies of clinical translation.

People are active creatures… What about CV control during activity and exercise?

Examining hemodynamic regulation during periods other than at rest is crucial for improved quality of life for SCI patients. The primary purpose of the CV system is to maintain blood pressure within normal limits during instances of *varied* cardiopulmonary demand. Previous studies have noted unstable blood pressure control during and following exercise training in individuals with autonomic dysfunction such as SCI (Claydon *et al.* 2006, Low *et al.* 2012). This is troublesome, for poor hemodynamic control limits patients' abilities to partake in rehabilitation and community-based sporting activities, further contributing to the decline in CV health and fitness (Harkema *et al.* 2008, Krassioukov and West 2014). Few studies have attempted to characterize hemodynamic responses during exercise challenge, and little is known about whether or not a newly-injured system has the capacity to stimulate CV end-organs to produce the necessary output needed to support

acute rehabilitation efforts. Data in this dissertation shows that rodents with both high and low incomplete thoracic injuries are unable to maintain hemodynamic control acutely after injury, even during short bouts of exercise challenge. This lack of control persists into the chronic period, despite the significant recovery of hindlimb function and exhibiting normal resting CV responses without signs of overt system breakdown (such as AD).

Comparing hemodynamic responses to exercise challenge in low verse high SCI

All moderately-injured animals experienced hemodynamic dysregulation during and following exercise challenge, regardless of whether responses were from T3 lesioned rodents or rodents with lower thoracic injuries. One week following contusion, animals experienced a drastic fall in mean HR during active swimming exercise challenge that was significantly greater than pre-injury measurements and measurements made at chronic time points (see figures 7 and 11 in previous chapters). Interestingly, the temporal pattern of responses was very similar between high and low lesion conditions, as there were no differences between the groups (Figure 19B). The bradycardic phenotype during exercise challenge likely reflects the transient anatomical and functional changes occurring in sympathetic preganglionic neurons shortly after injury (Krassioukov and Weaver 1996) that lead to states of general sympathetic hypo-activity and neurogenic shock. The finding that both low and high thoracic injuries result in this type of dysfunction is not surprising given that the presence of neurogenic shock has been noted across a wide spectrum of injuries in the clinic (Guly *et al.* 2008, Mallek *et al.* 2012, Summers *et al.* 2013).

Blood pressure responses to swimming exercise challenge were also altered following moderate thoracic contusion. Acutely after injury, animals with high level

lesions experienced exertional hypotension that was significantly greater in magnitude than measurements at nearly all other time points assessed (see Figure 7). After week 1, the mean blood pressure achieved during exercise returned to pre-injury values and was not statistically different at any time point assessed. On the contrary, animals that received low T10 moderate contusion did not present with blunted blood pressure responses at any time after injury. In fact, after Week 2, the average four-minute blood pressure response to swimming exercise challenge was greater in magnitude than pre-injury assessments (see Figure 11). This enhanced pressor response to exercise in T10 rodents is clear upon direct comparison between high and low lesion groups. Animals that received T10 injuries had significantly higher mean blood pressure responses to swimming during the first four weeks of assessment after injury (Figure 19C; note: post-SCI weekly data is normalized to pre-injury measurements; *$p \leq .05$). Further, both injury groups developed a heightened lack of blood pressure control during swimming challenge over time. Quantified on a lap-by-lap basis, moderately-injured rodents exhibited large swings in mean blood pressure that were significantly greater than pre-injury measurements (MBP Excursion, see Figures 7 and 11). Low lesioned animals began to express this lack of hemodynamic control two weeks after injury, whereas animals with high thoracic SCI did not exhibit this troubling phenotype until the three week time point. Despite T10 animals displaying larger mean values of blood pressure during exercise, there were few differences in MBP Excursion between high and low lesioned animals (Figure 19A, *$p \leq .05$).

Normal hemodynamic responses to the cessation of exercise include a slight reduction in mean blood pressure due to centrally-mediated decreases in sympathetic activity and local vasodilation (Halliwill *et al.* 2013). In individuals with autonomic

disorders, post-exertional hypotension can be severe and lead to syncopal events (Forjaz *et al.* 2000). Results from this study illustrate that short bouts of exercise following incomplete SCI can also lead to episodes of post-exertional hypotension in the acute time period after injury. Previous studies have shown that transient hypotension is common following arm-ergometry exercise in cervical SCI patients, but not in individuals with thoracic injuries (Claydon *et al.* 2006). On the contrary, we show that rodents with both high and low thoracic lesions have reduced blood pressure during the recovery period following active forelimb swimming exercise in the first couple of weeks post-injury (see Figures 7 and 11; T3 MOD: weeks 1 and 2 vs pre-injury, *$p \leq .05$; T10 MOD: week 1 vs pre-injury, *$p \leq .05$). While many factors contribute to the development of post-exercise hypotension in SCI patients, the degree of sympathetic nervous system dysfunction and unopposed vagal tone are likely responsible. As such, upon comparison of hemodynamic responses from high and low thoracic conditions during the Exercise Recovery phase, we have shown that rodents with high (T3) lesions have significantly lower pressure responses than rodents with T10 injuries. (Figure 19D, T3 vs T10 Moderate SCI: Weeks 2-5, *$p \leq .05$; note: post-SCI weekly data is normalized to pre-injury measurements). This suggests that the proportion of residual sympathetic fibers available to contribute to blood pressure control is important for hemodynamic maintenance following exercise challenge in incompletely injured rodents.

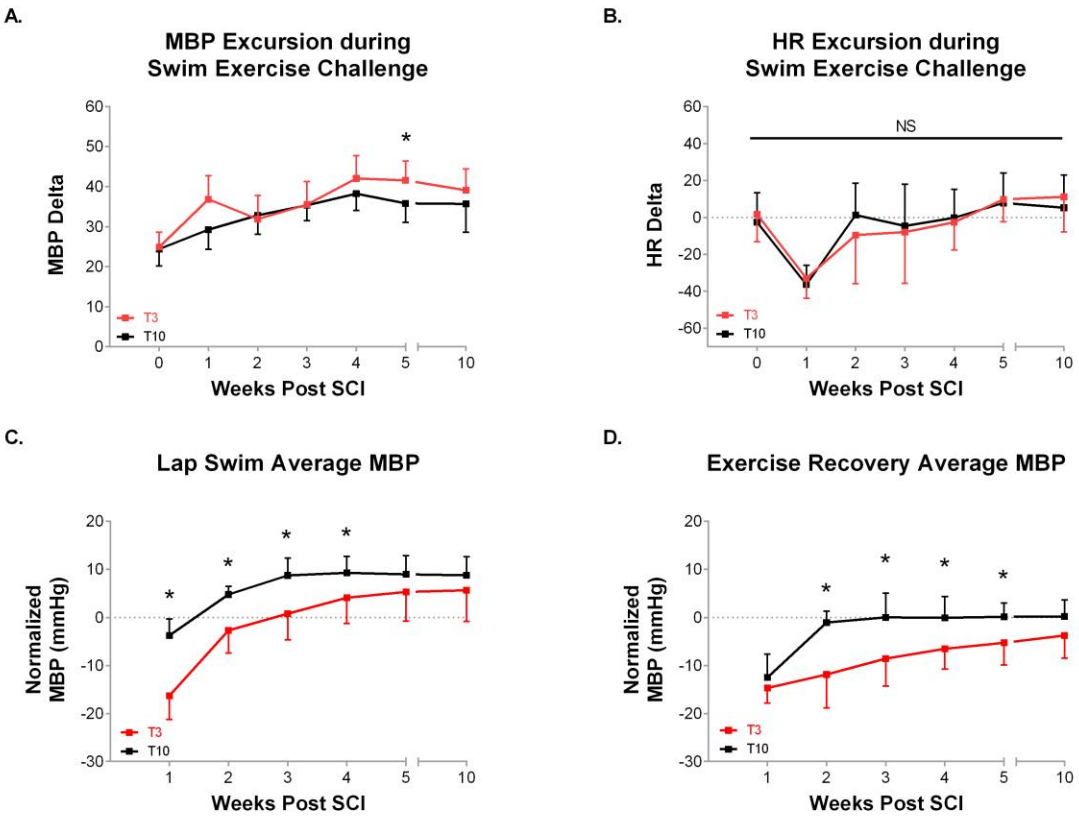

Figure 19. **Hemodynamic responses to swim exercise challenge following high and low moderate SCI.** **(A)** Average MBP Excursion during swimming exercise challenge. Differences between T3 and T10 SCI were noted only at 5 weeks post-injury. **(B)** HR Excursion during the four-minute swim session. Note the drastic drop in HR from the beginning to the end of swimming acutely after injury in both T3 and T10 SCI groups. **(C)** Average MBP during the four-minute swim session. Animals with low thoracic injuries were able to generate significantly greater pressor responses during the first four weeks after injury compared to T3 MOD. **(D)** Average MBP during the Exercise Recovery period. Note the higher blood pressure values after low thoracic SCI. Statistical significance was assessed using Mixed Model ANOVA with Bonferroni *post hoc* t-test. Data was normalized to pre-injury measurements in **C** and **D**. All data is displayed as mean ± SD and statistical significance was set as *p≤ .05.

Contusive SCI results in limited hindlimb recovery and function

The level of hindlimb locomotor recovery during over-ground stepping was comparable between rodents with high and low thoracic lesions in Aim 1 (see chapters II and III) (Figure 20B). Weekly locomotor assessments were performed prior to swimming exercise challenge in moderately-injured animals and results illustrated that, on average, animals were unable to regain consistent weight-supported stepping or forelimb-hindlimb coordination. Toe clearance also remained poor in T3 and T10 injured rodents.

Locomotor assessments during unassisted swimming (Figure 20C), evaluated weekly starting two weeks after injury, revealed a severe drop in function post-SCI with no improvements over time in either T3 or T10 lesioned animals. Most moderately-contused animals relied solely on their forelimbs for forward propulsion and had great difficulty stabilizing their trunk during swimming for the entirety of the study. Differences in swimming scores were noted at three and ten weeks post-SCI between T3 and T10 groups, with high lesioned rodents performing slightly better than animals with T10 injuries. Terminally, rodents with moderate T3 injuries typically had superior hindlimb kicking ability than animals with low thoracic lesions, which was reflected in the average group score. Reasons for this disparity likely relate to the percentage of gray matter sparing in and around the epicenter (data not shown). Comparison of white matter sparing in T3 and T10 SCI groups is shown in Figure 20A.

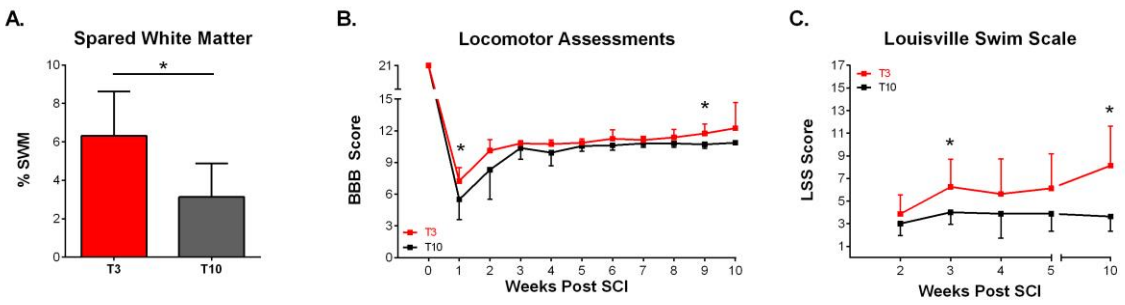

Figure 20. **Comparison of anatomical and behavioral data from high and low incomplete thoracic SCI groups. (A)** Average percentage of spared white matter in T3 and T10 animals following moderate SCI. **(B)** Timewise comparison of hindlimb locomotor ability during over-ground stepping as measured by the BBB scale. **(C)** Timewise comparison of unassisted swimming ability in T3 and T10 moderately-contused animals. Assessments were analyzed using Independent t-tests between means with equal or unequal variance, as appropriate, followed by Bonferroni correction. All data is displayed as mean ± SD and statistical significance was set as *p≤ .05.

The spontaneous recovery in CV control experienced by our animals at rest was clearly not enough to support periods of hemodynamic stress. Thus, studies examining the effects of SCI and the benefits of various exercise programs on CV function should consider evaluating improvements in hemodynamic control that go beyond the resting state.

Given that moderately-contused animals have near normal cardiac structure and systolic function chronically (as demonstrated by echocardiography assessments), the lack of hemodynamic control during and following exercise challenge likely stems from insufficient vascular reaction due to reduced mobility and altered sympathetic modulation. Evidence from both clinical and preclinical studies supports this notion, for vascular remodeling occurs very rapidly post-SCI and is present in tetraplegic and paraplegic patients alike (Olive *et al.* 2003, Laird *et al.* 2008, Thijssen *et al.* 2008, Thijssen *et al.* 2012). Our findings suggest a commonality between high and low lesions that should be considered when developing therapeutic interventions for CV recovery. Most labs examine only cardiac or only vascular consequences of injury, and most assessments are completed in the resting state. Our ability to examine many facets of CV function (cardiac function at rest and in response to Dobutamine infusion, hemodynamic responses during and after active exercise challenge, and hemodynamic parameters during In Cage Rest) has provided additional insight into the CV system as a whole, which is essential to remedy CV decline in all SCI patients, irrespective of lesion level. Further, future studies must acknowledge the diversity among SCI patients and take the characteristics of the clinical population into consideration when planning preclinical studies. Doing so will foster the best chance at

effective translation, especially when considering the implementation of rehabilitation efforts.

Using acute exercise training to attenuate autonomic dysreflexia

The majority of preclinical research has focused on elucidating the mechanisms of extreme system dysfunction, notably AD, and in some cases have sought to attenuate symptoms of AD using exercise rehabilitation following transection injury (Laird *et al.* 2009, West *et al.* 2015). Conversely, clinical studies have largely ignored therapeutic attempts at ameliorating this potentially deadly complication of SCI, possibly because patients that generally enroll in experimental trials are in the chronic phases of disease progression and the steady-state of hemodynamic control has already been established. Most clinical studies attempt to recondition peripheral structures (the heart and vasculature), but fail at improving central neural mechanisms of CV function. In an effort to alter the trajectory of CV dysfunction and improve hemodynamic control, studies of the second aim in this dissertation pursued avenues of acute exercise rehabilitation following severe, incomplete contusion. Acute implementation of rehabilitation is ideal given the high level of inherent plasticity within neural circuitry of the spinal cord within the first couple of weeks post-injury (Murakami *et al.* 1992, Fouad and Tetzlaff 2012). Additionally, given that the decline in physical fitness is progressive, early exercise training is believed to prevent the decline in cardiac and vascular structure/function typical of SCI, leading to improved CV homeostasis.

Severe contusion lesions were employed in the second aim of this dissertation in lieu of mild and moderate injuries for their ability to produce profound CV dysfunction

(importantly AD) that lasts into the chronic period (Squair *et al.* 2016). Pilot studies performed in the Magnuson lab suggest that rodents with moderate high thoracic contusions do not consistently develop the typical symptoms of AD during experimental colorectal distension procedures. In fact, at ten weeks post moderate contusion, only two of four rodents with T3 moderate injuries responded to CRD with exaggerated pressor responses typical of an AD event (Krassioukov *et al.* 2003, Hou *et al.* 2013, West *et al.* 2015) (see Figure 21).

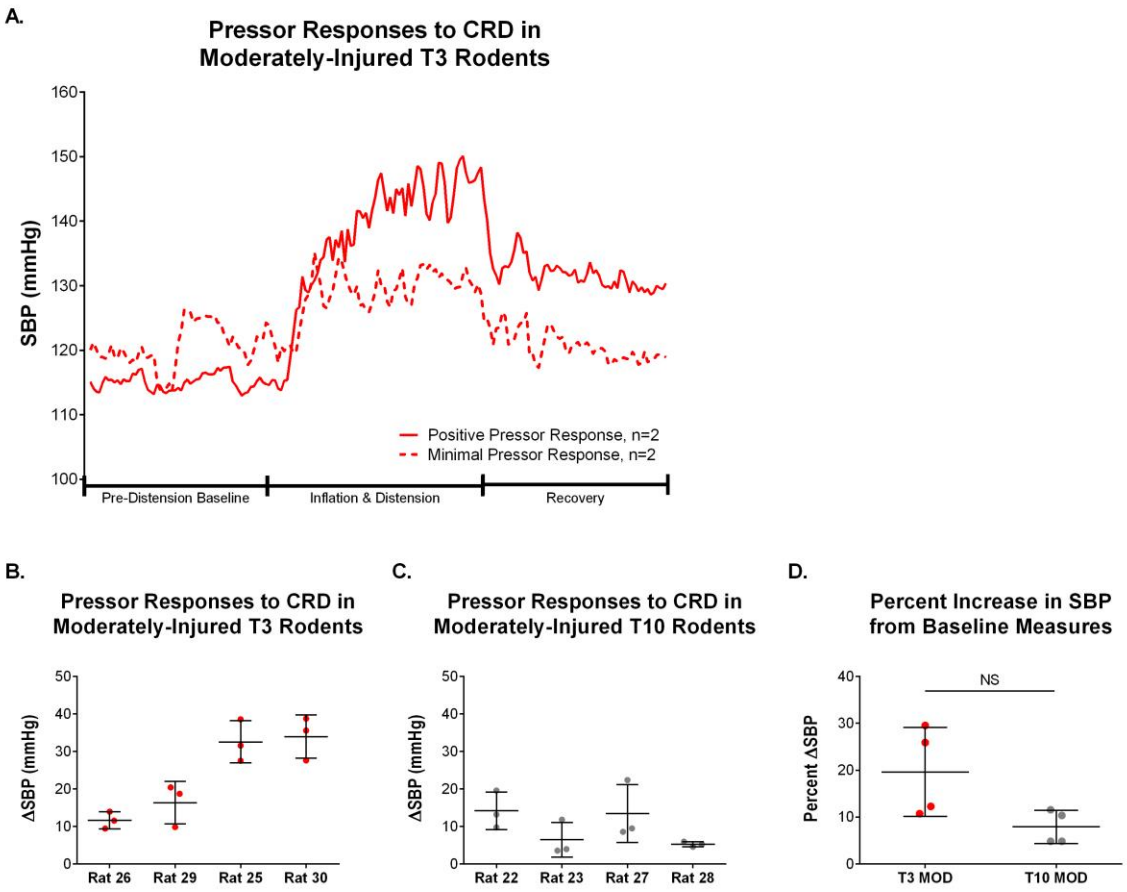

Figure 21. **Pressor response to colorectal distension following moderate contusive SCI.** **(A)** Time-locked group average SBP data during pre-distension baseline, inflation and distension, and recovery in moderate T3 rodents who did (solid red line) and did not (dashed red line) display pressor responses to CRD ten weeks post-SCI. Absolute changes in SBP during each CRD trial for T3-MOD **(B)** and T10-MOD **(C)** animals. Note that only two out of four rodents with T3 injuries displayed exaggerated responses to CRD. All animals with T10-MOD injuries displayed minimal changes in SBP. **(D)** Group comparison of the percent increase in SBP from baseline measurements in T3 and T10 injured animals. Data was analyzed using Independent t-tests between means with equal or unequal variance, as appropriate, followed by Bonferroni correction. All data is displayed as mean ± SD and statistical significance was set as *p≤ .05.

Effects of acute exercise training post-SCI

While the severity of AD was not positively influenced by PHLC or active swimming in Aim 2 studies, rodents that received swim exercise training regained hemodynamic control while at rest that was similar to uninjured animals. While improvements were minimal, it appears as though swimming may be an appropriate form of aerobic challenge to bring about positive CV enhancements after SCI. These data stress the importance of exploring all facets of CV health following therapeutic interventions, in addition to overt dysfunction such as AD. Data in the second and third chapter of this dissertation supports this notion in which moderately-injured animals with normal resting hemodynamic control were unable to effectively elicit appropriate CV responses to exercise challenge

Previous accounts of acute PHLC rehabilitation report improved hemodynamic regulation and reduced AD severity following T3 transection. We were unable to mimic this level of recovery in our animals, likely due to the variability in recovery of hindlimb function and its effect on the exercise mechanics (volitional kicking while strapped into the foot pedals of the bike), aberrant plasticity within the lumbosacral cord, and neuronal sparing between animals. Importantly, therapies that are likely to exacerbate inflammatory pathways or increase inappropriate nociceptive afferent feedback to the spinal cord may actually potentiate aberrant sprouting and the increase the severity of AD in the clinic. While not directly assessed during our study, noxious stimulation and periods of increased inflammation may have been counterproductive to the cycling rehabilitation process and further contributed to AD severity (Gris *et al.* 2004, Weaver *et al.* 2006, Marsh and Flemming 2011). Previous work by Jim Grau has shown that intermittent nociceptive

stimulation, similar to the afferent feedback received by some of our animals, resulted in reduced neurotrophic activity and attenuated locomotor recovery following complete SCI (Garraway *et al.* 2011).

Body weight-supported treadmill training, in which the harness produces transient body abrasions, or excessive stretching physical therapy are two potentially harmful rehabilitation strategies that could exacerbate certain aspects of CV dysfunction in the clinic, and implementation of these types of rehabilitation modalities should proceed with caution. The application of acute training programs must take into account not only the heterogeneity among subjects, but also the level of spontaneous recovery/reorganization in motor and sensory systems that could contribute to mechanisms of CV control (spasticity, changes in level of denervation, etc.) and adapt exercise prescription accordingly. Knowledge of activities that could potentially impair recovery of CV function must be considered and those activities should be evaluated for their therapeutic efficacy. There is likely not a "one shoe fits all" model of rehabilitation for SCI patients and preclinical research should be performed accordingly. The best results will likely stem from acutely-implemented, combinatorial rehabilitation efforts in which exercises that sufficiently stimulate the cardiopulmonary system are combined with activities that deliver consistent and rhythmical afferent input to lumbosacral circuits. Active arm cycling that propels the lower extremities on a stationary bike is an example. Indeed, a recent study published by Chris West claimed improvements in CV function following active upper and passive lower body exercise rehabilitation in a cervical SCI patient (West *et al.* 2015). However, as this was a case study, more information is needed in order to authenticate the validity of this type of training.

Conclusions

The work presented in this dissertation has provided additional insight into the physiological mechanisms of CV dysfunction typical of SCI patients. Herein we provide evidence of disordered CV control in rodents during provocation and exercise challenge using clinically-relevant models of contusive SCI at various levels of the spinal cord. Information regarding the application of acutely implemented exercise rehabilitation and its effects on resting hemodynamic stability and on the severity of AD is provided and results suggest that a cohesive, all-inclusive approach to rehabilitation is needed to prevent CV decline and ameliorate CV consequences of SCI.

In total, my findings advocate for acutely-implemented exercise training programs, either volitional or applied, which not only sufficiently stress the cardiovascular system but also provides appropriate afferent feedback to the injured spinal cord. Rehabilitation programs beginning early after injury that incorporate lower body electrical stimulation and/or eccentric contractions of paralyzed musculature in combination with active upper arm ergometry could be ideal and should be tested in the clinical population.

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APPENDIX I

LIST OF ABBREVIATIONS AND SYMBOLS

AB	Able-Bodied
AD	Autonomic Dysreflexia
ANS	Autonomic Nervous System
BBB	Basso , Beattie, and Bresnahan
BP	Blood Pressure
BPM	Beats per Minute
BWSTT	Body-Weight Supported Treadmill Training
$CGRP^{+}$	Calcitonon Gene-Related Peptide
cm	Centimeter
CO	Cardiac Output
CRD	Colorectal Distension
CV	Cardiovascular
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
E	Transmitral Filling Velocity
ECG	Electrocardiogram
EDV	End-Diastolic Volume
EF	Ejection Fraction
EPR	Exercise Pressor Response
ESV	End-Systolic Volume

FES	Functional Electrical Stimulation
ft	Foot
g	Gram
HR	Heart Rate
HRV	Heart Rate Variability
Hz	Hertz
IML	Interomediolateral Cell Column
IP	Intraperitoneal
kg	Kilogram
L	Liter
LSS	Louisville Swim Scale
LVIDd	Left Ventricular Internal Diameter during Diastole
LVIDs	Left Ventricular Internal Diameter during Systole
MAP	Mean Arterial Pressure
MBP	Mean Blood Pressure
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
mmHg	Millimeter of Mercury
NGF	Nerve Growth Factor
OH	Orthostatic Hypotension
PBS	Phosphate-Buffered Saline

PFA	Paraformaldehyde
PHLC	Passive Hindlimb Cycling
Q	Cardiac Output
RM ANOVA	Repeated Measures Analysis of Variance
s	Second
SAX	Short-Axis
SBP	Systolic Blood Pressure
SC	Subcutaneous
SCI	Spinal Cord Injury
SCT	Spinal Cord Transection
SD	Sprague-Dawley
SD	Standard Deviation
SEM	Standard Error of the Mean
SNS	Sympathetic Nervous System
SPN	Sympathetic Preganglionic Neuron
SV	Stroke Volume
SWM	Spared White Matter
TPR	Total Peripheral Resistance
µg	Microgram
µl	Microliter
µm	Micrometer

CURRICULUM VITAE

Kathryn A. Harman

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PERSONAL INFORMATION

Date of Birth: **November 10, 1985**
Place of Birth: **Louisville, KY**
Citizenship: **United States**

EDUCATION

University of Louisville, School of Medicine, Louisville, KY
Ph.D. Anatomical Sciences and Neurobiology **2013 - 2016**

University of Louisville, School of Medicine, Louisville, KY
M.S. Anatomical Sciences and Neurobiology **2010 - 2013**

University of Louisville, Louisville, KY
B.S. Psychology with a Minor in Biology **2004 - 2009**

RESEARCH EXPERIENCE

University of Louisville, Department of Anatomical Sciences & Neurobiology
Predoctoral Research **2010-2016**

Dissertation Project: "EVALUATING CARDIOVASCULAR DYSFUNCTION DURING INCREASED ACTIVITY AND EXERCISE REHABILITATION FOLLOWING INCOMPLETE THORACIC SPINAL CORD INJURY IN THE ADULT RAT"

Research Focus: My research was focused on cardiovascular decline following incomplete spinal cord injury in adult rats. I used telemetry hardware to assess hemodynamic control in awake, moving animals during periods of rest and exercise challenge. I also employed high resolution ultrasound to track cardiac and vascular structure and function in response to injury. My studies revealed that mild, moderate, and severe contusive spinal cord injury produces a myriad of deficits, including lack of hemodynamic control during instances of increased cardiopulmonary demand and bouts of autonomic dysreflexia. Further, exercise rehabilitation implemented acutely after injury did not fully attenuate cardiovascular dysfunction. This work has established methods for examining autonomic demise using clinically relevant injury models and has provided insight into therapeutic interventions that can be translated to the clinical population of injured individuals.

Techniques: Small animal surgical procedures and recovery (including spinal cord contusions and trasection surgeries, subcutaneous electrocardiogram/electromyogram sensor placement, and abdominal aorta/carotid artery cannulation of telemetry sensors), echocardiography with and

without dobutamine infusion, vascular ultrasound, immunohistochemistry, analysis of various physiological data (using LabChart, Ponemah, VEVO Lab, MatLab), and various behavioral assessments (BBB, Louisville Swim Scale)

University of British Columbia, International Collaboration on Repair Discoveries
Predoctoral Research **2015**

Research Focus: The effects of active and passive exercise training on cardiovascular dysfunction following incomplete spinal cord injury

Techniques: Small animal surgical procedures and recovery (including spinal cord contusions surgeries, pressure myography, pressure-volume loop catheter implantation, and carotid artery cannulation of telemetry sensors), experimentally-induced autonomic dysreflexia procedures

University of Louisville, Biology Department
Undergraduate Research **2009-2011**

Research Focus: The role of estrogen and progesterone in multiple sclerosis symptomology

Techniques: Participant enrollment, participant informed consent and HIPAA authorization, various data analysis

HONORS

- Graduate Student Travel Awards (3 Awards), University of Louisville **2013-2016**
- Friends for Michael Foundation Scholar, University of Louisville **2014-2015**
- ICORD Scholarship for International Trainees, University of British Columbia **2015**
- 15th Biannual ISNR Graduate Student Travel Award, University of Louisville **2013**
- Graduate Student Teaching Academy Certificate, University of Louisville **2013**
- Psi Chi Honors Society, University of Louisville **2009**
- Dean's List (3 Awards), University of Louisville **2007-2009**
- Dean's Scholar (2 Awards), University of Louisville **2006-2007**
- Dean's List (2 Awards), University of Kentucky **2004-2005**

PROFESSIONAL MEMBERSHIPS AND ACTIVITIES

- Society for Neuroscience Member **2011-2016**
- National Neurotrauma Society Member **2011-2016**
- Society for Women in Neurotrauma Research Member **2011-2016**
- Graduate Student Council Representative Proxy for the Department of ASNB **2015-2016**
- Graduate Student Council Representative for the Department of ASNB **2014-2015**
- 21st Annual KSCHIRT Symposium Keynote Speaker Organizing Committee **2014-2015**
- Graduate Student Advisory Panel for IPIBS Orientation **2014**
- Advisory Panel for Graduate Teaching Academy (GTA) Assistants Orientation **2013-2014**
- Graduate Student Council Representative Proxy for the Department of ASNB **2013-2014**
- GTA Alumni Mentor on Applying Critical Thinking Activities in the Classroom **2013**

TEACHING EXPERIENCE: COLLEGIATE ENDEAVORS

- Graduate Teaching Assistant for Medical Neuroanatomy & Neural Systems
  University of Louisville, School of Medicine **2012-2016**
- Part-time Instructor in the Department of Health and Sports Sciences
  University of Louisville, College of Education (Course: Biomechanics) **2014-2016**
- Medical Neuroanatomy Case Presentation Evaluations
  University of Louisville, School of Medicine **2015-2016**
- Medical Student Tutoring for Neuroanatomy (individual & small group)
  University of Louisville, School of Medicine **2012-2014**
- Medical Gross Anatomy Guest Instructor for Head & Neck Anatomy
  University of Louisville, School of Medicine **2014**
- Graduate Teaching Assistant Academy
  University of Louisville, Delphi Center for Teaching and Learning **2012-2013**
- Graduate Teaching Academy Micro Teaching Session
  University of Louisville (Title: Lower Respiratory System Development) **2013**

TEACHING EXPERIENCE: COMMUNITY OUTREACH

- Brain Awareness Week at the Kentucky Science Center
  Louisville Chapter of the Society for Neuroscience, University of Louisville **2015**
- Nanodays at the Kentucky Science Center
  Louisville Chapter of the Society for Neuroscience, University of Louisville **2014**

SCIENTIFIC PUBLICATIONS IN PEER-REVIEWED JOURNALS

Harman KA, Aslan SC, Wade AD, Wainwright GN, Stepp CA, Morehouse JR, Magnuson DSK (2016). Abnormal cardiovascular control during active exercise challenge following incomplete low thoracic spinal cord injury. (in preparation)

Harman KA, DeVeau KM, Aslan SC, Dudley SE, States GJ, Morehouse JR, Magnuson DSK (2016). Effects of incomplete high thoracic spinal cord injury on cardiac control and cardiovascular homeostasis during rest and exercise challenge. (in preparation)

Harman KA, DeVeau KM, Squair JW, Woods BD, West CR, Magnuson DSK, Krassioukov AV (2016). Autonomic dysreflexia persists following acute rehabilitation in rats with incomplete contusive spinal cord injury. (in preparation)

Squair JW, DeVeau KM, **Harman KA**, Poormasjedi-Meibod M, Hayes B, Liu J, Magnuson DSK, Krassioukov AV, West CR (2016). Spinal cord injury induced impairments in cardiac contractility reveal novel insight into the regulation of normal cardiac inotrophy. (in review)

DeVeau KM, **Harman KA**, Squair JW, Magnuson DSK, Krassioukov AV, West CR (2016). The effects of active versus passive exercise on cardiac function following experimental spinal cord injury. (in preparation)

PROFESSIONAL ABSTRACTS AND POSTERS

Lexington, KY
National Neurotrauma Society Symposium **2016**
Abstract Title: Autonomic dysreflexia persists following acute rehabilitation in rats with incomplete contusive spinal cord injury
Harman KA, DeVeau K, Squair JW, West C, Magnuson DSK, Krassioukov

San Diego, CA.
Experimental Biology Conference **2016**
Abstract Title: Left-ventricular pressure and volume responses to active- and passive-exercise training following experimental spinal cord injury
West CR, DeVeau KM, **Harman KA**, Squair JW, Magnuson DSK, Krassioukov AV

San Diego, CA.
Experimental Biology Conference **2016**
Abstract Title: Autonomic dysreflexia persists following acute rehabilitation in rats with incomplete contusive spinal cord injury
Harman KA, DeVeau K, Squair JW, West C, Magnuson DSK, Krassioukov A

Vancouver, British Columbia, Canada
ICORD Trainee Symposium **2015**
Abstract Title: Cardiovascular collapse following T10 spinal cord contusion
Harman KA, Magnuson DSK

Montreal, Canada
ISCoS and ASIA Joint Scientific Meeting **2015**
Abstract Title: Cardiovascular responses to an active exercise challenge following acute spinal cord injury
Harman KA, Wainwright G, Wade A, Shum-Siu A, Magnuson DSK

Louisville, KY
Kentucky Spinal Cord & Head Injury Research Trust Symposium **2015**
Abstract Title: Cardiovascular responses to an active exercise challenge following acute spinal cord injury
Harman KA, Wainwright G, Wade A, Shum-Siu A, Magnuson DSK

Louisville, KY
26th Annual Neuroscience Day Symposium **2015**
Abstract Title: A novel continuous pool for investigating cardiovascular dysfunction in spinal cord injured rats
Hoeper A, Martin E, **Harman KA**, Wainwright G, Shum-Siu A, Magnuson DSK

Louisville, KY
Research! Louisville Symposium **2014**
Abstract Title: Using high resolution ultrasound to assess cardiovascular function post spinal cord injury
DeVeau KM, Martin E, Brown R, Shum-Siu A, **Harman KA**, Tinney J, Keller B, Magnuson DSK

Pacific Grove, CA
International Symposium on Neural Regeneration **2013**
Abstract Title: Exposing latent cardiovascular dysfunction using exercise challenge
Harman KA, Stepp CA, States GJ, Shum-Siu A, Aslan SC, Magnuson DSK

Nashville, TN
National Neurotrauma Society Symposium **2013**
Abstract Title: Temporal changes in the "silent" cardiovascular dysfunction that ensues post spinal cord injury
Harman KA, Stepp CA, States GJ, Shum-Siu A, Aslan SC, Magnuson DSK

ORAL PRESENTATIONS

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Seminar Series **2016**
Title: Effects of Exercise and Exercise Training on Cardiovascular Function following Incomplete Spinal Cord Injury in Adult Rats

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Journal Club Series **2015**
Title: Topological Data Analysis for Discovery in Preclinical Spinal Cord Injury and Traumatic Brain Injury

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Seminar Series **2015**
Title: Cardiovascular Dysfunction following T10 Contusion

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Seminar Series **2014**
Title: Assessing Cardiovascular Function using Exercise: Challenges & Triumphs

Pacific Grove, CA
International Symposium on Neural Regeneration Data Blitz **2013**
Title: Exposing Latent Cardiovascular Dysfunction using Exercise Challenge

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Journal Club Series **2013**
Title: Autonomic Dysreflexia Causes Chronic Immune Suppression after Spinal Cord Injury

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Seminar Series **2013**
Title: Exercise Rehabilitation and the Silent Cardiovascular Dysfunction following SCI

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Journal Club Series **2013**
Title: Full-length Axon Regeneration in the Adult Mouse Optic Nerve and Partial Recovery of Simple Visual Behaviors

University of Louisville, Louisville, KY
Anatomical Sciences and Neurobiology Seminar Series **2013**
Title: Using Acute Aerobic Rehabilitation to Attenuate Cardiovascular Dysfunction following SCI

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Seminar Series **2012**
Title: Tools for Assessing Cardiovascular and Vascular Function after Contusive SCI

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Journal Club Series **2012**
Title: Repetitive Intermittent Hypoxia Induces Respiratory and Somatic Motor Recovery after Chronic Cervical Spinal Injury

University of Louisville, Louisville, KY
Kentucky Spinal Cord Injury Research Center Journal Club Series **2012**
Title: Exposure to Acute Intermittent Hypoxia Augments Somatic Motor Function in Humans with Incomplete Spinal Cord Injury

University of Louisville, Louisville, KY
Anatomical Sciences and Neurobiology Neural Systems Presentation **2011**
Title: Congenital Insensitivity to Pain with Anhydrosis: An Overview

University of Louisville, Louisville, KY
Anatomical Sciences and Neurobiology Department Seminar Series **2011**
Title: Activated T-cells Inhibit Neurogenesis by Releasing Granzyme B: Rescue by Kv1.3 Blockers