Vascular Changes in Spinal Cord Injured Animals With Repetitive Episodes of Autonomic Dysreflexia

by

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Abstract

Spinal cord injury (SCI) is a devastating condition that not only leads to paralysis, but also causes dramatic changes in cardiovascular function. Individuals with cervical or high thoracic SCI commonly suffer from a life threatening condition known as autonomic dysreflexia (AD). AD is characterized by episodic hypertension— an exaggerated sympathetic response triggered by irritating stimulus below the level of injury e.g. distended bladder. As a lifespan of SCI patients increases, cardiovascular-related illnesses become more prevalent. Recent studies suggest marked vascular dysfunction within the critical splanchnic vascular bed. Mesenteric arteries from rats with chronic high-thoracic SCI are hypersensitive to the $\alpha_1$ adrenoceptor agonist PE. The hypersensitivity of splanchnic vascular bed in response to PE develops over time after SCI and may contribute to the development of AD. In this dissertation, I examined the morphological changes in peripheral vasculature following repetitive episodes of AD in animals with high SCI. I hypothesized that recurrent episodes of AD will trigger an inward eutrophic remodeling in peripheral resistance arteries of SCI rats. In this study, male Wistar rats with complete spinal cord transection at third (T3) thoracic segment were utilized. At 2 weeks after the injury, AD was induced in rats with T3 SCI using CRD. 4 weeks following injury superior mesenteric (SMA) arteries and primary branches (PMA) were collected from T3 SCI-only, T3+CRD and control uninjured rats. Morphological characteristics such as media thickness, lumen diameter, wall-to-lumen ratio and wall cross sectional area (CSA) of the arteries were evaluated. Results suggest that AD induced through CRD lead to structural remodeling of PMAs, but no changes were observed in SMAs of CRD group. Media thickness, wall-to-lumen ratio significantly increased in PMAs of CRD group; lumen diameter and CSA of PMAs in CRD did not change when compared to T3 SCI-only and uninjured groups. The data support eutrophic (no change in CSA) remodeling of PMAs in CRD group, but failed to show a reduction in lumen diameter (inward changes) of these arteries. The findings of the study highlight the underlying
effect of AD on structural remodeling of vasculature following an injury.
Preface

The experiment in this dissertation was approved by the Animal Care Committee of the University of British Columbia, under certificate number A10-0129.

The cardiovascular assessment data presented in chapter 2 and 3 came from the work in preparation for publication by Dr. Chris West and Dr. Leanne Ramer.
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<th>Description</th>
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<tbody>
<tr>
<td>AB</td>
<td>Able-bodied</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AD</td>
<td>Autonomic dysreflexia</td>
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<tr>
<td>ATP</td>
<td>Adenosine 5'-triphosphate</td>
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<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>COX-1</td>
<td>Cyclooxygenase-1 (COX-1)</td>
</tr>
<tr>
<td>CRD</td>
<td>Repetitive Colorectal Distension</td>
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<tr>
<td>CSA</td>
<td>Cross-sectional area</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>CVLM</td>
<td>Caudal ventrolateral medulla</td>
</tr>
<tr>
<td>D</td>
<td>Diameter</td>
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<tr>
<td>DAP</td>
<td>Diastolic arterial pressure</td>
</tr>
<tr>
<td>DOCA</td>
<td>Deoxycorticosterone acetate</td>
</tr>
<tr>
<td>E</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>EDHF</td>
<td>Endothelium derived hyperpolarizing factor</td>
</tr>
<tr>
<td>EDRF</td>
<td>Endothelial derived relaxing factor</td>
</tr>
<tr>
<td>EH</td>
<td>Essential hypertension</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilation</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>IML</td>
<td>Intermediolateral nuclei</td>
</tr>
<tr>
<td>IMT</td>
<td>Intimal-medial thickness</td>
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<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MVC</td>
<td>Motor vehicle collisions</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NMD</td>
<td>Nitroglycerin-mediated dilation</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NTS</td>
<td>Nucleus of solitary tract</td>
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<tr>
<td>PE</td>
<td>Phenylephrine</td>
</tr>
<tr>
<td>PG12</td>
<td>Prostacyclin</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PMA</td>
<td>Primary mesenteric artery</td>
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<tr>
<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system</td>
</tr>
<tr>
<td>RVLM</td>
<td>Rostral ventrolateral medulla</td>
</tr>
<tr>
<td>SAP</td>
<td>Systolic arterial pressure</td>
</tr>
<tr>
<td>SCI</td>
<td>Spinal cord injury</td>
</tr>
<tr>
<td>SHR</td>
<td>Spontaneous hypertensive rats</td>
</tr>
<tr>
<td>SMA</td>
<td>Superior mesenteric artery</td>
</tr>
<tr>
<td>SMCs</td>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>SPNs</td>
<td>Spinal preganglionic neurons</td>
</tr>
<tr>
<td>WT</td>
<td>Wall thickness</td>
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</table>
Acknowledgments

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Dedication

To my wonderful family and friends, who have supported me through out the process and never left my side.

“If you think you made it, you’re at the wrong place, never stop”
Chapter 1: Introduction

1.1 Overview

Spinal cord injury (SCI) is a devastating condition that affect both motor-sensory and autonomic nervous system. The level and completeness of the spinal lesion determines the severity of the outcomes following the injury. Regardless of the level of injury, this condition comprises a significant challenge to both quality and quantity of life.

In comparison with able-bodied (AB) individuals, people with chronic SCI are more prone to develop cardiovascular dysfunction. In fact, recently, developments of cardiovascular disease (CVD) have become a growing concern among SCI population. People with SCI at T6 (thoracic segment 6) or above develop a condition known as autonomic dysreflexia (AD). AD is characterized by episodes of extreme hypertension that is induced by noxious or non-noxious stimulus below the level of the injury. This aspect of cardiovascular dysfunction following an injury is the focus of this dissertation. Currently, the bulk of literature has focused on the mechanisms of AD and the functional changes of vasculature following an injury. The long-term effect of this condition on morphological changes of vessels is not yet well understood. Due to the significant role that resistance arteries play in regulation of blood pressure (BP), it is of an important value to understand how the episodic raise in BP influence the morphological characteristics of these vessels following an injury.

My dissertation examines the effect of repetitive episodes of AD on the morphological changes (vascular remodeling) of peripheral vasculature following high thoracic injury in animal model of SCI. Structural and functional changes of vasculature are closely associated with one another; therefore, in the introduction, I will review the current knowledge and understanding on aspects of nervous system and cardiovascular parameters that contribute greatly to morphological and functional changes in the vasculature following an injury.
1.2 Epidemiology of SCI

SCI is a devastating condition that tremendously over shadows the quality and longevity of life of an injured individual. Risk factors such as obesity, lipid disorders, diabetes and metabolic syndrome are quite prevalent in SCI population\(^2\). Currently, advances in medicine and technology have improved the life expectancy of people with SCI. As a result, SCI patients are now more prone to face secondary complications such as cardiovascular disorders, bladder/pulmonary infections, and osteoporosis\(^3\). Abnormalities in BP, heart rate (HR) variability, limited response to exercise and arrhythmias associated with severe autonomic dysfunction following the injury contribute tremendously to an increased risk of CVD and stroke in chronic SCI patients\(^2,4\). In fact, cardiovascular complications are leading cause of morbidity and mortality in long-term SCI\(^5\).

In North America the incidence of SCI has increased in the past 30 years\(^6,7\). Recent statistics published on SCI in Canada estimated an occurrence of 1,237 new cases of traumatic SCI/year\(^8\). There were 85,556 persons with SCI in Canada in 2010\(^8\). Among this population, 43,974 are cases with traumatic SCI and 41,582 are injured due to nontraumatic causes\(^8\). Picket et al (2006) reported that between 1997-2001, motor vehicle collisions (MVC), falls and sport related injuries were among the most common causes of SCI in Canada. Based on this study, the prevalence of SCI due to MVC is highest among young and middle aged population; on the other hand, fall incidents seem to be the most frequent cause of injury in patients older than 65\(^9\). Older patients with SCI face a higher rate of secondary complications and longer hospital stay\(^10,11\). At present, there are about 300,000 people with an acute traumatic SCI living in United States and Canada; on a worldwide scale this value is close to 2.5 million\(^12\). With this rate of incidence, SCI is counted as a fairy uncommon cause of disability; however, the lifetime economic and social costs of this condition are quite high\(^12\). Unfortunately, many of these costs are spent on treatment, rehabilitation and control of secondary complications of SCI\(^3,9\).
1.3 Autonomic Control of Nervous System:

1.3.1 The Role of Supraspinal Centres in Sympathetic Control

Supraspinal centres regulate HR and BP by sending inputs to spinal sympathetic preganglionic neurons through descending pathways. After traumatic injury (high SCI), the descending pathways are interrupted leading to loss of supraspinal control over sympathetic activity. Viral tracing studies reveal that 5 main regions in the brain provide inputs for spinal preganglionic neurons (SPNs): rostral ventrolateral medulla (RVLM), rostral ventromedial medulla, caudal raphe nuclei, A5 region and paraventricular hypothalamus\textsuperscript{13,14}. Electrophysiological studies also reveal that monosynaptic connections exist between RVLM and SPN\textsuperscript{15}. The peripheral sympathetic vasomotor tone and the resting BP are primarily controlled by the oblongata region of the RVLM. Therefore, an increased activity of RVLM could be easily transmitted to the intermediolateral (IML) region of spinal cord segments between (T1-L3), where preganglionic sympathetic neurons innervating heart and vessels originate and lead to an increase in BP\textsuperscript{16-19}. For this reason, the RVLM is often referred to as sympathetic control centre, which plays an important role in cardiorespiratory reflexes and homeostasis.

RVLM receptors receive both inhibitory and excitatory inputs; the two most predominate forms of the receptors found in this region of the brain are glutamate (excitatory) and GABA (inhibitory) receptors; other neurotransmitters such as ATP, angiotensin II and enkephaline also have receptors in the RVLM\textsuperscript{20,21}. The RVLM also receives afferent innervations from various sites such as the nucleus of solitary tract (NTS), caudal ventrolateral medulla (CVLM), medullary lateral tegmental field, midline raphe nuclei, midbrain periaqueductal gray, lateral and periventricular nuclei of hypothalamus and prefrontal cortex\textsuperscript{22}. Using a graded clip compression SCI model on adult rat, Krassioukov and Fehling (1999) showed that depending on the severity of the injury, the number of neurons projecting from RVLM to spinal cord changes. They
showed that as severity of injury increases, there would be more disconnected neurons projecting from RVLM to the thoracic segment of spinal cord, which could greatly contribute to the sustained and disordered BP after the injury\textsuperscript{23}. 

1.3.2 Baroreceptor and Chemoreceptor Control of Blood Pressure

Baroreceptor and chemoreceptor afferent inputs play a major role in short term regulation of arterial BP. Of these two important reflex mechanisms, baroreceptors have received more attention due to their role in controlling arterial BP following postural change (in which venous blood return reduces) or an increase in muscle activity due to exercise\textsuperscript{24,25}. In hypertension, it is well understood that baroreceptor sensitivity will be reduced\textsuperscript{26}. Techniques such as neuroanatomical tracing, immunohistochemical analysis, gene over expression, electrophysiological and pharmacological testing on both anaesthetized and conscious animals were used to determine the pathway through which baroreceptors control BP changes\textsuperscript{27}. These studies showed that baroreceptors are generally located in the walls of carotid sinus and aortic arch as well as auricle of heart and vena cavae; they respond predominately to stretch and could act within a wide range of arterial pressure changes (50-150 mmHg)\textsuperscript{28}. In summary, an increase in arterial BP distends the baroreceptors in the above mentioned regions; these baroreceptors send afferent inputs to rostral NTS located in the dorsomedial medulla and ipsilateral portion of commissural subnucleus of NTS\textsuperscript{29}. The excitatory inputs from NTS activate CVLM through glutamatergic neurons. The activated CVLM inhibits the activity of RVLM through GABAergic inhibitory fibres\textsuperscript{27}. Since the RVLM is vasomotor centre of the body; therefore, inhibition of the excitatory projections that innervate preganglionic sympathetic neurons in IML of spinal cord, tend to reduce the sympathetic activity and lead to reduction in arterial BP and an activation of vagal nuclei\textsuperscript{30,27}. It is well established that in SCI preganglionic sympathetic neurons undergo structural alternation such a change contribute greatly to the development of cardiovascular disturbance following the injury\textsuperscript{31}. 
Chemoreceptors also play a critical role in regulating BP, sympathetic activity and ventilation\textsuperscript{32,33}. Like baroreceptors, chemoreceptors are located in aortic and carotid bodies and extend their afferent fibres to NTS. NTS send direct monosynaptic excitatory (glutamatergic) projections to RVLM\textsuperscript{33}. They are sensitive to an increased level of carbon dioxide; for this reason, they play a major role in physiological adaptation to acute or chronic hypoxia\textsuperscript{34,35}. Essentially, activation of chemoreceptor reflex pathway lead to an increase in ventilation and triggers sympathetically mediated vasoconstriction in most of the vascular beds with the exception of those in heart and brain\textsuperscript{32}.

1.3.3 The Sympathetic Control of the Splanchnic Region

In 1923, Bayliss for the first time proposed the idea that blood vessels just like any other organs in the body receives innervations from the autonomic nervous system\textsuperscript{36}. The majority of blood vessels in the body are heavily innervated by only sympathetic neurons with the exception of vessels that branch off to salivary glands, gentile erectile tissue, pulmonary system (lungs and trachea) and brain that receive innervations from both parasympathetic and sympathetic neurons\textsuperscript{37-40}.

Activation of blood vessels sympathetic neurons leads to vasoconstriction of arteries and arterioles (resistance vessels), which ultimately increase systemic vascular resistance and arterial BP, but reduces the distal blood flow. Older studies demonstrated that the stimulation effect of sympathetic neurons in larger arteries such as aorta could extend to the smooth muscle cell layer; such an effect is minute in medium sized vessels and it normally manifests itself in a form of change in vascular distensibility with little effect in pressure drop\textsuperscript{41}. BP is regulated through two important factors: the cardiac output and peripheral vascular resistance. What is striking to note is that the contribution of peripheral vasculature to BP regulation is not the same among all arterial beds in the peripheral region. For example, splanchnic system receives \textasciitilde25\% of the cardiac output through three major arteries: superior mesenteric artery, inferior mesenteric artery
and celiac artery. In normovolemic individuals, the splanchnic organs (stomach, liver, intestines, pancreases and spleen) contribute to 10% of the body weight; however, as mentioned earlier 25% of the total blood volume is confined within this particular region of the body. Further, compared to spleen, which could only supply 100 ml of blood, intestines and liver each could provide between 300-400 ml of blood. For this reason, the vasculature closely confined within the splanchnic region is known as one of the major blood reservoirs of the circulatory system.

The distribution and function of various subtypes of adrenergic receptors in the splanchnic region have been studied using various α and β adrenergic agonists such as phenylephrine (PE) and isoproterenol respectively. In general, factors such as relative density of α and β adrenoceptors in splanchnic region, the affinity of a particular neurotransmitter (catecholamines) for the specific receptor subtype, catecholamine concentration in the blood plasma, vascular tone and the blood volume play a critical role in the spectrum of changes that could be induced by catecholamines in splanchnic circulation.

In arteries, the dual control mechanisms established between perivascular nerves that supply smooth muscle layer along with endothelium is responsible for regulation of blood flow to organs. The major neurotransmitters used by sympathetic projections in mesenteric arteries are: neuropeptide Y (NPY), adenosine 5'-triphosphate (ATP) and norepinephrine (NE). Previous studies have suggested that NA and ATP could actively induce vasoconstriction in both mesenteric artery and vein; on the other hand, NPY seems to be most important in vasoconstriction of the mesenteric vein rather than the artery. ATP and NA normally work synergistically to induce vasoconstriction in mesenteric artery. NA acts on α₁ adrenoceptors and release intracellular stored Ca²⁺. Conversely, ATP acts on P2X₁-ligand-gated receptors and lead to depolarization and opening of Ca²⁺ gated channels.
Neurones seem to be also actively involved with trophic changes in both heart and vasculature. For example, studies show that an intact sympathetic outflow mediated by ATP co-transmitter is required for normal development of heart muscle\(^51\). In blood vessels, chemically-induced sympathectomy leads to an abundant synthesis of collagenous tissue on the vessels wall; implicating potential structural changes in the vasculature\(^52\). In rats, it has been shown that vasoconstriction of mesenteric artery increases following sensory denervation, an effect that has been attributed to the link exist between the sensory innervation and expression of vasoactive agents by endothelium\(^52,53\).

1.3.4 SCI and Cardiovascular Outcomes

Disrupted autonomic pathways after SCI perturbs cardiovascular homeostasis. Studies indicate that in people with SCI, there is a 200% greater chance of developing CVD\(^54\).

As described by Furlan et al (2003), the three most important contributing factors to cardiovascular control are descending vasomotor pathways, sympathetic cardiovascular control and spinal afferents\(^55\). The development of cardiovascular complications such as bradyarrhythmias, cardiac arrest, low resting BP, and orthostatic hypotension in early stages after high SCI could be potentially life-threatening events\(^56\). Many of these cardiovascular abnormalities following SCI have been well studied in both humans and animal models\(^57,58\). Despite the fact that over time many of these complications improve; however, cardiovascular control never returns to its normal baseline after the injury. The increased risk of CVD and mortality observed in patients with high SCI is often associated with the development of autonomic dysfunction following the injury\(^2,5\). Damage to sympathetic fibres originating in (T1-L3) located in the gray matter of the spine or the loss of connectivity of these fibres from higher centres in the body after trauma lead to severe alternation of autonomic control of cardiovascular system after injury\(^46,59\).
1.3.5 SCI and Autonomic Dysreflexia

Injuries at T6≥ could greatly affect the autonomic control of cardiovascular system leading to development of AD, altered circadian oscillations in BP and limited cardiovascular responses to exercise\(^4,60,61\). AD occurs in 90% of individuals with high thoracic/cervical lesion (tetraplegic individuals with complete injury)\(^62\) and it is often characterized by episodes of extreme hypertension, which is usually (but not always) accompanied by bradycardia (Figure 1)\(^63,64\). AD has been reported in people with either complete or incomplete lesions; however, data suggest that among incomplete tetraplegic SCI patients the frequency of occurrence of AD is much lower\(^62\). The exaggerated sympathetic responses that is unopposed by central inhibitory pathways along with an increase in vagal activity above the lesion site through baro-receptor-mediated mechanisms could very well explain the observed bradycardia during the onset of AD\(^64\). The pathophysiology of AD has been greatly studied in the work done by Krassioukov and colleagues. In their studies, they showed that AD could occur as early as 24 hrs following high thoracic injury; this have been greatly attributed to the loss of inhibition of spinal reflex and the disruption of bulbo spinal pathway\(^31\). Soon after day one, the severity of AD reduces, the observed reduction in AD has been related to the loss of dendritic tree and atrophy of SPNs\(^31\). Gradually dendritic branches are formed in SPNs and to some degree the normal morphological characteristics of SPNs recovers; this time point is associated with a return of intensified episodes of AD\(^31,57\). Despite the fact that bradycardia is often used as one of the major signs of AD, but there have been a number of medical reports in which tachycardia accompanied the onset of AD instead\(^63\). In such cases, the spinal reflex arc seems to be also involved with sympathetic nerve fibres of heart; in which case, inducing these fibres may lead to an increase in a HR during AD\(^64\). Generally, daily trivialities could initiate AD; bladder and bowel distension, catheterization, stimulation of skin, and even muscle spasms are among some of the best known factors leading to development of AD\(^65-69\). Interestingly, for so long it was thought that AD is
confined to the chronic stage of SCI, and the hypotensive state is more associated with the acute phase; however, it is now known that AD can also occur during the early phases of SCI. During AD, the systolic BP in humans could rise up to 250-300 mmHg, and at the same time diastolic BP will be in a range of 200-220 mmHg. Stroke, myocardial infarction, seizures and even death may occur if AD remains untreated.

The cause and effect association between BP fluctuation and morphological/functional changes in vasculature following SCI is yet to be determined. In other words, it is becoming increasingly important to determine whether the occurrence of AD triggers changes in vasculature, or changes in the vasculature below the lesion site predispose the individuals with SCI to develop AD.

1.4 Vascular Remodeling Post SCI:

1.4.1 Morphology of Blood Vessels

Most knowledge of vascular morphology comes from examining the two-dimensional histology of vascular beds. Based on these long-standing and static views of histological cross-sections, five major components of vascular structure have been identified. These structural components from outer to inner segments are as follows: tunica adventitia, external elastic lamina, tunica media, internal elastic lamina and tunica intima.

Adventitia

The outermost layer in blood vessels is the adventitia. This layer is mainly composed of connective tissues such as elastin and collagen, plus fibroblasts (the most common cells contributing to the formation of collagen), mast cells and macrophages. The adventitia also receives nerve innervations; these neurons are primarily adrenergic in nature. Faber et al (2001) showed that the adventitial layer of aorta obtained from rat possesses α1 subtypes. The density of these adrenoceptor subtypes was also shown to be equally high in adventitia when compared to smooth muscle cells (SMCs). New emerging studies suggest that despite the fact that
adventitial cells do not have contractile capabilities, the presence of adrenoceptors on these cells may have a physiological role in inducing a trophic effect via adventitia following vascular damage\textsuperscript{74}. The proportion of the vascular wall made of adventitia is quite variable in different vascular beds: for example, in cerebral arteries adventitia constitute only a small fraction of the wall structure, but in other types of vessels, adventitia can make up as much as half of the wall material\textsuperscript{75}.

**Media**

This layer is mainly composed of SMCs. In arterioles these cells are spirally oriented around the long axis of the vessel. However, in resistance arteries, where the lumen is wider and multiple layers of SMCs are present, SMCs are oriented close to perpendicular with a little diagonal offset along the axis of flow; this gives them a spring-like organization\textsuperscript{76}. This level of organization in SMCs is used to facilitate changes in vascular length in response to BP fluctuations, and reduces the pulse-wave velocity in the arterial tree. Unlike adventitia, fibroblasts are not present in the media; instead, SMCs take over the production of collagen and proteoglycans\textsuperscript{77}. Older studies have shown that the production of matrix components by SMCs significantly changes (both qualitatively and quantitatively) during hypertension, early development, atherosclerosis and angiogenesis\textsuperscript{77-80}. The internal and external elastic laminae are mainly present in large and medium sized arteries, but they disappear in smaller arteries and arterioles\textsuperscript{72}. Further, the number of SMC layers depends upon the size of the artery: for instance, the number of layers varies from 6 (in 300µm arteries) to 1 (in arterioles)\textsuperscript{81,82}. The question of how the tensile stress exerted upon vessel wall is transmitted between the cells in the media has been the point of interest in a number of studies on structural properties of the vessels. It is now known that SMCs are interconnected to one another through membranous structures, and in larger arteries anchor points exist along the plasma membrane that interact with myofilaments;
although rat small mesenteric artery does also possess such anchor points; it has yet to be
determined whether such structural attributes do manifest in other smaller arterial beds\textsuperscript{83,84}.

**Intima**

Endothelial cells are the building blocks of the intima layer. In arterioles, the single layer
endothelial cells have a thickness of about 2\textmu{}m\textsuperscript{85}. These cells in large arteries possess an
invagination of the plasma membrane that is used to communicate with arterial lumen; the
capillaries, on the other hand, demonstrate endothelial cells that share no similarities with
endothelial cells of arteries (no invaginations)\textsuperscript{86}. The basement membrane of endothelial cells
rests on the internal elastic lamina; the internal elastic lamina is well defined in larger and
medium sized arteries, but it is absent in smaller arterioles. The non-static nature of the internal
elastic laminae is known to play a critical role in altering myoendothelial junctions (structures
used to communicate between endothelial cells and SMCs) and leading to changes in vascular
function as a whole\textsuperscript{87}. The presence of myoendothelial connections is well established in arteries;
image-analysis studies showed that in mesenteric arteries of rats, the nuclei of endothelial cells
are aligned with those of SMCs\textsuperscript{76}. Interestingly, early evidence suggested that endothelial cells
could facilitate contraction of SMCs. This conclusion was primarily based on three important
lines of evidence: 1) mechanical removal of endothelial cells reduced maximum contractile
response to NE, 2) when tension was induced in the veins using either thrombin or arachidonic
acid, a reduction in strain force was observed, and 3) a reduction in anoxia-induced tension was
also apparent following NE-induced contraction\textsuperscript{88}.

**1.4.2 Morphological Characteristics of Mesenteric Vasculature**

The superior mesenteric artery (SMA) originates from the anterior surface of the
abdominal aorta and is responsible for supplying blood to the intestine and pancreas. The SMA
is characterized by the presence of well-developed laminae in media\textsuperscript{89}. In this arterial bed, media
and intima make up more than half of the wall structure, with adventitia contributing to the rest
of the wall. Although they are primarily involved in conduction of the blood, hypertension studies have highlighted structural modifications observed in this elastic artery during the established phase of hypertension\textsuperscript{89,90}. Distinct from the SMA, large mesenteric arteries are considered as muscular arteries and are characterized by well-defined external and internal laminae\textsuperscript{89}. Additionally, in rats they possess 4-6 layers of SMCs\textsuperscript{89}. Interestingly, older studies have shown that both the prehypertensive and developing hypertensive stages trigger morphological alternations in large mesenteric arteries\textsuperscript{89}; the details of this will be discussed later in this chapter.

As previously mentioned, true resistance arteries are in fact the small muscular arteries and arterioles, plus the capillaries. These vessels act as the major site where a drop in hydrostatic pressure occurs. The lumen diameter (D) of small arteries is <350\(\mu\)m and for arterioles is <100\(\mu\)m\textsuperscript{91,92}. Small mesenteric arteries and arterioles have 1 to 3 layers of SMCs and normally possess internal elastic laminae, but the external elastic laminae disappear in these arterial structures\textsuperscript{29}. Structural remodeling of these vessels is evident during developing and established phases of hypertension, which directly and greatly contributes to changes in BP values in chronic hypertension\textsuperscript{93,94}.

**1.4.3 Techniques Used to Study Vascular Morphology & Function**

Designing optimal methodology for assessing the complexity of vascular function and structure, and relating the observed changes to the pathology and prognosis of CVD, has become an invaluable part of cardiovascular studies. Below, some of the most widely used techniques in evaluating vascular structure and function will be reviewed in brief.

**Flow-mediated vasodilation**

Flow-mediated dilation (FMD) is a non-invasive technique used to assess arterial function and more specifically endothelial function\textsuperscript{95}. The basis of this technique relies on the mechanism that is used by endothelial cells to respond to shear stresses. Under normal
conditions, endothelial cells release nitric oxide (NO, a vasodilator) in response to an increase in mechanical stress; however when the endothelium is damaged as a result of cardiovascular risk factors and/or atherosclerosis, the protective effects of NO are lost\textsuperscript{96}. Modulation of vasomotor tone at the level of endothelial cells through NO is primarily under the control of ionic channels and hyperpolarization of the membrane. This ultimately leads to an increase in eNOS production (an enzyme that catalyzes the production of NO) and the release of endothelium-derived relaxing factors in response to shear from an increased blood flow in arteries\textsuperscript{95}. In the literature, FMD has mainly been used to measure the D of conduit arteries, such as vascular stress in the arm (brachial or radial artery); there are also studies in which FMD was used for femoral and posterior tibial arteries\textsuperscript{97-100}.

FMD measures the changes in vessel D between baseline and maximal points during hyperaemia\textsuperscript{97}. To measure FMD, tools such as an ultrasound machine, a linear array probe, a video system and a printer are required. In addition to endothelium-dependent FMD, the effects of endothelium-independent stimulation could be evaluated by using nitroglycerin (NTG) in a technique known as nitroglycerin-mediated dilation (NMD)\textsuperscript{101}, administered to the subjects either sublingually or through intra-arterial infusion\textsuperscript{102}. Unfortunately, FMD faces two major limitations: 1) the release of NO in response to shear stress also depends on blood viscosity, and 2) blood flow is equal to flow velocity times arterial D\textsuperscript{52,103}. Concerns exist over the fact that the stimulus and response are not independently measured, since arterial D is also used to quantify the effect of NO. This problem has been partly addressed by taking measurements at various time points\textsuperscript{52}.

**In vitro assessment of microvasculature**

To quantify structural modifications in small resistance vessels such as mesenteric arteries, techniques such as wire and perfusion-pressure myography are used. Myography techniques have the advantage of eliminating the need for histology, and thus reducing the
artefacts normally introduced as a result of tissue fixation\textsuperscript{53,104,105}. In pressure myography, morphological characteristics of the vessels, such as adventitial, medial, and intimal thickness measurements, should be normalized to the pressure difference along each side of the wall (transmural pressure)\textsuperscript{106}. This technique is widely used for assessing both functional and structural properties of smaller vessels (D and wall thickness (WT) changes). Wire myography is mostly used to assess functional properties such as endothelial function; in this technique for determining the vessel's dimensions corresponding to each transmural pressure, the values must first be normalized by use of a resting wall tension-internal circumference curve\textsuperscript{106}. The wire myograph apparatus consists of a stainless steel surface on which the vessel is placed, a force transducer, and a micrometer that measures either isometric or isotonic pressures\textsuperscript{53}. The perfusion-pressure myograph is composed of a pressure transducer, connected to the pressure pipette on which the vessel is positioned\textsuperscript{107}. In both techniques vascular dimensions are measured using a light microscope, with only a small difference that in pressure myography the microscope is also connected to a video camera. Further, to correlate the morphological alterations of vessels to their functional adaptations, intracellular ion activity, and membrane potential in the excited state, complementary techniques such as fluorescent dyes and microelectrode measurements are used accordingly\textsuperscript{108-112}. To assess endothelial dysfunction in the wire or pressure myograph, vasodilator agonists such as acetylcholine (Ach), substance P, and bradykinin are used\textsuperscript{113,114}. Laser digital Doppler technique is another non-invasive method for evaluation of the reactive response of endothelial cells in dermal microcirculation\textsuperscript{115}.

**Intimal-medial thickness**

Thus far, various approaches have been used to measure the thickness of blood vessels. Unfortunately, many of these techniques require excision of the desired vasculature; this process alone is known to induce structural alternation in the isolated vessels. Historically, McDonald (1980) calculated the vessel thickness from the measured vessel weight in water and air,
assuming that under different physiological pressures, density and volume remained unchanged\textsuperscript{116}. In another study, the thickness was calculated using direct unfixed histological sections; others fixed the tissue at a particular arterial pressure before an attempt was made to measure the arterial thickness\textsuperscript{116}. In the past, angiography was also used to show the association between WT and the D; this approach took advantage of measuring the thickness at various intraluminal pressures\textsuperscript{116}. Currently, intimal-medial thickness (IMT) measurements of the carotid artery act a surrogate marker for many CVD such as atherosclerosis; for this reason, IMT is now recognized as an important parameter to be measured in many observational studies\textsuperscript{117}. High resolution B-mode ultrasonography is a non-invasive technique used to assess the IMT in carotid arteries\textsuperscript{118}. In an older approach, Olson (1974) placed a transducer, which was equipped with pulse echo crystal, over the artery and measured the D and thickness of carotid artery. Further, in the same study, blood flow velocity was measured using the Doppler shift crystal in the transducer\textsuperscript{119}. Interestingly, in one recent study, \textit{in vivo} imaging by a slit-lamp biomicroscope, along with imaging software, was used to calculate the luminal D and WT in the ciliary artery of conscious normotensive and hypertensive rats\textsuperscript{120}.

\textbf{1.4.4 Intrinsic Vascular Response}

In general, as a result of changes in blood flow and BP, stretch and shear stress are constantly reinforced in blood vessels. In response to such changes and to maintain the integrity of basal tensile and shear forces, vessels modify their Ds; however, when such changes persist, the endothelial and SMCs undergo compensatory modulations, eventually leading to structural and functional changes in vasculature\textsuperscript{121}.

\textbf{Stretch}

Due to the nature of blood flow and shear stress, vessels permanently experience an encompassing cyclic mechanical strain known as stretch\textsuperscript{121}. Stretch response has a direct correlation with BP; this type of response is responsible for the D and thickness changes in
vasculature, which were potentially initiated through circumferential stress acting tangentially on the vessel wall. How the stretch response in the vessels transmits to SMCs and leads to contraction of these cells is still not completely understood. However, studies suggest that factors such as activation of SMCs ion channels, changes in contractile protein function in these cells, or the detection of the distension by endothelial cells could contribute to the contractile behaviour of SMCs. In one patch clamping study, it was shown that endothelial cells in culture respond to stretch by activating cation channels; the activation of these channels depolarizes the endothelial cells\textsuperscript{122}. Another study showed that the electrical signals from depolarized endothelial cells membrane could be transduced to SMCs\textsuperscript{123}. It is known that in arterioles the contribution of endothelial cells to contraction of SMCs is minute\textsuperscript{124,125}. Evidence suggests that SMCs also possess stretch-activated cation channels; among those, \(\text{Ca}^{2+}\) and \(\text{Na}^{+}\) play a critical role in depolarization of the SMC membrane. The depolarization of the cell membrane in SMCs can be achieved either through an influx of \(\text{Ca}^{2+}\) or activation of intracellular \(\text{Ca}^{2+}\) stores\textsuperscript{126,127}. A whole-cell patch clamp study on SMCs of the mesenteric artery isolated from a guinea pig showed that stretch-sensitive ion channels in SMCs change the membrane potential and trigger stretch-mediated cell response\textsuperscript{128}. Further, an \textit{in vitro} study on small arteries isolated from a rabbit ear has shown that the stretch response is mainly dependent upon extracellular \(\text{Ca}^{2+}\); and when a calcium blocker was used, stretch response showed no changes\textsuperscript{124,129}.

**Pressure**

BP exerts a perpendicular force to the endoluminal surface of the vasculature. The strain induced by BP opposes other tangential forces that affect the vessel wall in either circumferential or longitudinal directions\textsuperscript{121}. Pressure-induced vascular contractility has been greatly studied in arteries and arterioles isolated from animals, such as rat mesenteric artery, and porcine coronary arterioles, cerebral arterioles and skeletal muscle arterioles\textsuperscript{130-133}. Many studies focused on determining the role of the endothelium in the induction of pressure-induced response in vessels;
the results were somewhat controversial. For example, older studies on canine renal arteries and feline cerebral resistance arteries demonstrated that an intact endothelium is required for pressure-induced response in these arteries\textsuperscript{134,135}. However, recent studies report that the pressure-induced response, in arterial beds isolated from rats, is independent from endothelial function and represents a true myogenic response\textsuperscript{130-133}. Interestingly, resistance arteries isolated from human cerebral circulation also showed that fluctuation in pressure alone could trigger myogenic response in this type of artery; such behaviour seems to be independent from endothelial function in this arterial tree\textsuperscript{136}. Further, the role of ion channels, most particularly Ca\textsuperscript{+2}, has been also shown in a majority of the studies on pressure-induced vascular response. Reports have demonstrated the dependency of pressure-induced response to extracellular calcium; however, unlike stretch-induced response, utilization of calcium antagonists in pressure-induced response seems to be capable of inhibiting the myogenic response in the vasculature\textsuperscript{137}.

Flow

The effect of flow-mediated dilation through NO-dependent mechanisms is well documented in conduit arteries\textsuperscript{138}. However, until a decade ago, flow-mediated dilation in arterioles and resistance arteries was not fully explored. A study by Koller and Huang (1994) on skeletal muscle arterioles from both hypertensive and normotensive rats showed that in early hypertension, the co-released effect of prostaglandins and NO induced vasodilation in the arterioles. However, later on, due to the impairment of NO mechanism, flow-mediated vasodilation was reduced despite the fact the blood flow was increased\textsuperscript{139}. This study along with many others, point out the role that endothelium could potentially play in arterioles. Further, \textit{in vitro} studies suggest that endothelium-derived relaxing factor (EDRF) could also be released from smaller arterioles. For instance, an \textit{in vitro} blood-perfused juxtamedullary nephron technique has demonstrated that the continuous release of EDRF influences the efferent and
afferent arterioles in the juxtamedullary nephron; interestingly, it seems EDRF interacts with the renin-angiotensin system to control resistance in these arterioles\textsuperscript{140}.

1.4.5 Blood Flow and Vascular Changes in Able-bodied vs. SCI individuals

Following SCI, profound adaptations occur in both the central and peripheral circulatory systems. Depending on the level of injury, cardiac deconditioning and various degrees of vasomotor dysregulation may also occur\textsuperscript{141,142}. Structural alternations following SCI are often accompanied by remodeling of vasculature, specifically changes in vessel D. For example, studies on tetraplegic individuals showed that blood flow is reduced in legs following SCI, and that such a reduction is normally accompanied by a decrease in the D of the common femoral artery\textsuperscript{48,143}. Collectively, these findings seem to show that blood flow and femoral D in chronic SCI patients is 50% that of AB individuals\textsuperscript{48,144}. Interestingly, the findings of two independent studies showed that if the vascular size were corrected for the reduction of muscle mass following SCI, such a change would no longer coexist with reduction of blood flow in these arteries\textsuperscript{82,145}. Controversy also exists over reports on the changes in blood flow below the level of injury. Some studies such as the ones mentioned above suggested a change in blood flow of the legs, when comparing SCI and AB individuals; there are, however, studies which report no significant change in resting or hyperaemic blood flow of lower limbs, between injured and uninjured control groups\textsuperscript{82,145,146}.

Despite the fact that vascular properties seem to change below the level of the injury, many studies suggest that the integrity of vessels above the level of injury remains unchanged (both structurally and functionally). For instance, the D measurements of common carotid artery in AB individuals have been reported to be between 6 and 7mm: this value is very similar to what has been observed in SCI individuals\textsuperscript{80,147,148}. Studies also confirm that blood flow in the common carotid artery is very similar between SCI and AB individuals\textsuperscript{80,148,149}. Similarly, studies on the brachial artery’s D and blood flow in SCI and AB individuals revealed no
significant difference between the two groups\textsuperscript{145,150}. Interestingly, a few studies also reported a reduction in capillarization following SCI\textsuperscript{79,151}. The question of why such difference is observed in the structural properties of vasculature above and below the injury site could be partly explained through the drop in lower limb movement following the injury, and the subsequent reduction in metabolic demands (oxygen) associated with this region\textsuperscript{152}.

1.4.6 Shear Stress and Vascular Changes in SCI

In general, blood flow and shear stress are closely related to one another. Because shear stress defines the frictional force that blood cells exert upon endothelial wall; slight uncontrolled fluctuations in the shear stress could potentially damage the vessel wall. Vessels maintain a constant shear stress by dynamically changing their D through inward or outward remodeling\textsuperscript{145}. In other words, an increase in blood flow lead to an increase in a D (outward remodeling) and a reduction in a blood flow lead to decrease in a D (inward remodeling)\textsuperscript{153,154}.

Using non-invasive ultrasound techniques, Schmidt-Trucksass and colleagues evaluated changes in D, shear stress and compliance in arteries above and below the level of injury. Their work showed that the common femoral artery D and compliance was lowest in paraplegic individuals when compared to control groups; they also measured mean and peak shear stress values, which turned to be almost doubled in the SCI group\textsuperscript{155}. Later in 2002, Boot et al measured vascular D, blood viscosity and shear stress using echo-Doppler ultrasound methodology. Their study revealed no particular changes in the D and shear stress in the common carotid artery between control and SCI; however, mean and peak shear stresses were significantly greater in the common femoral artery of SCI subjects\textsuperscript{156}. Boot and associates argued that such an increase in shear stress below the level of the injury could be either as a result of high blood velocity, viscosity and/or a reduction in a D of the vessels. De Groot et al (2006) also demonstrated that both basal and peak shear rates increase significantly at 3 weeks and 6 weeks.
post-injury. Further, they suggested that the inward remodeling of vasculature following SCI is a response to changes in peak blood flow rather than resting blood flow.\textsuperscript{145}

1.4.7 Endothelial Function Following SCI

The endothelial cell layer acts as an interface between the flowing blood and the vascular wall. It is known that under constant shear stress, the structure and function of endothelial cells remain unchanged; however, under turbulent flow, activated endothelial cells lower the expression of eNOS and enhance the manifestation of vasoconstrictor and pro-inflammatory responses.\textsuperscript{157} When blood flow increases, healthy vessels increase their D by vasodilation, mediated through NO release in a process known as FMD.\textsuperscript{26} It is known that FMD reduces in the presence of hypertension, diabetes, hypercholesterolemia and smoking.\textsuperscript{24,158-160}

Generally, evaluating FMD response in upper and lower limbs is necessary for making conclusive remarks about how this parameter is changing. In SCI, this becomes particularly important, because: 1) vascular beds in the upper and lower extremity of the body respond differently to endothelium-dependent and -independent vasodilators and 2) for a given change in shear rate, vessels in arms and legs exhibit variability in dilation.\textsuperscript{161,162} When FMD values of superficial femoral arteries were evaluated between SCI and control groups, an increase was observed in the absolute and relative FMD values of the SCI group. However, when corrections were applied on calculated FMDs, no change in FMDs between SCI and control groups was reported in the superficial femoral artery.\textsuperscript{102,145,146,163} De Groot et al (2004) showed that FMD was reduced in brachial arteries of SCI, after correcting the value using $FMD/\Delta$shear rate; such a reduction is not indicative of endothelial dysfunction, however, as this value was still above the reported values of FMD in healthy individuals.\textsuperscript{95,146}

Aside from FMD, NMD has been also evaluated in few SCI-related studies. In general, it was noted that following SCI, the NMD was either enhanced,\textsuperscript{102,164,165} or did not change at all in the superficial femoral artery of SCI and control groups.\textsuperscript{146} When evaluating FMD and NMD the
structural properties of vasculature following SCI (inward remodeling) must be considered. Therefore, to reduce bias factor in analyzing the NMD outcomes, values must be presented as change in D over the change in dilator range\(^{102}\).

### 1.4.8 α-Adrenoceptor Responses Following SCI

In tetraplegic SCI individuals, the resting level of NE and epinephrine (E) and their spillover rates are significantly lower than AB subjects; this could be very well explained through reduction in sympathetic activity following the injury\(^{166,167}\). An older study done by Mathias et al (1976) showed that during the hypertensive phase in tetraplegic subjects, the level of NE increases significantly, whereas the level of E does not change much. Despite the fact that NE increases considerably during hypertensive episodes, the level of NE never surpasses the recorded NE value at rest in AB subjects\(^{168}\). Such an observation led researchers to hypothesize that the massive sympathetic response during episodes of hypertension is partly facilitated through the development of hyperresponsivity towards catecholamines at the receptor level in SCI.

Mathias and associates used NE infusion as an index to measure the peripheral α-adrenoceptor sensitivity in SCI and control groups. Evaluation of receptor supersensitivity with NE, however, is problematic in two important aspects. Firstly, the NE is known to be a non-specific postsynaptic α-adrenoceptor ligand, and secondly, in AB subjects synaptic re-uptake of NE occurs frequently; such a process to some degree may alter after SCI\(^{169}\). Follow-up studies on this subject by other research groups used PE, which is a selective agonist for α\(_1\)-adrenoceptor; this particular ligand, unlike NE, is not subject to synaptic re-uptake. Krum et al (1992) showed that when PE was infused, the tetraplegic subjects manifested an enhanced pressor response when compared to the AB group.

In animal models of SCI, hypersensitivity was examined through dose-response studies. Landrum et al (1998) showed that α-adrenoceptor hyperresponsiveness contributes somewhat to
the elevated BP in response to CRD (technique used to induce AD) a month after SCI\textsuperscript{168}. Further, in studies done by Yeoh and colleagues it was shown that supersensitivity to PE was only transiently observable in the tail artery following decentralization or SCI\textsuperscript{170,171}. In Brock et al (2006), second-order mesenteric arteries from spinalized rats (T4) showed an enhanced sensitivity to PE (evident at week 7) due to a lower rate of PE removal by NE transporters at the synaptic junction\textsuperscript{172}.

To distinguish between peripheral and central mechanisms contributing to vascular responses following SCI, Laird et al (2008) examined $\alpha$-adrenoceptor sensitivity to PE in vessels located in upper (brachial & carotid arteries) and lower extremities (femoral & renal arteries) before and after ganglionic blockade in completely transected rats. Their study suggested that following blockade to eliminate the central component, vessels in both upper and lower extremities showed vasoconstriction in response to PE in SCI animal group, though the potency was similar in SCI and control groups\textsuperscript{173}. Alan et al showed that the enhanced pressor response in mesenteric arteries of rats with SCI was not due to endothelial dysfunction (part of peripheral mechanism), as vasodilatory response mediated by acetylcholine (Ach) was similar in both SCI-only and SCI+CRD group\textsuperscript{176}. So far, other peripheral mechanisms such as changes in adrenoceptor density\textsuperscript{174}, increased reactivity of SMCs\textsuperscript{171}, increased NE release and reduced NE re-uptake at the synaptic junction\textsuperscript{171,172} have been proposed as alternative options for developed hypersensitivity at the vascular level following SCI, and more specifically AD.

1.4.9 Peripheral Resistance and Vascular tone

Blood is pushed through the circulatory system by overcoming the existing resistance to flow; this is known as systemic peripheral resistance or total peripheral resistance. Chronic BP alteration has been identified as a core contributing factor to structural remodeling in vasculature. Mean arterial pressure and venous pressure are kept within a narrow range through regulation of peripheral resistance at the level of precapillary vessels, which distribute the blood
at the right pressure and amount to capillaries\textsuperscript{175}. At the level of resistance arteries, regulation of peripheral resistance is accomplished through a combined effect of hormones, neural activity and the inherent characteristics of the vessel wall\textsuperscript{175-177}.

**Local factors**

There are two important local factors that influence the vascular tone: 1) endothelium-derived substances, and 2) myogenic tone. The endothelium is capable of producing various forms of vasorelaxants and vasodilators; some of the major ones are as follows: NO, (vasodilator), prostacyclin (PGI\textsubscript{2}, vasodilator), endothelin-1 (ET-1, vasoconstrictor), and endothelium-derived hyperpolarizing factor (EDHF, vasodilator)\textsuperscript{178}.

The most important stimulus for NO release in endothelial cells is an increase in shear stress; NO diffuses from the endothelial cell layer into SMCs, where it stimulates the production of guanosine monophosphate and consequently leads to relaxation of the SMCs\textsuperscript{179}. Studies show that in the human brachial artery, inhibition of NOS will reduce blood flow by 30-50\%\textsuperscript{180}. This finding greatly highlights the importance of NO in maintaining baseline vascular tone. Further, cyclooxygenase-1 (COX-1) is also expressed in vascular endothelial cells, responsible for production of PGI\textsubscript{2}\textsuperscript{181}. On the other hand, EDHF mediates its effects either through myoendothelial junctions or through substances released from the endothelium, such as hydrogen peroxide or potassium\textsuperscript{182}. ET-1 binds to two types of receptors on SMCs (ET\textsubscript{A} and ET\textsubscript{B} receptors) and leads to vasoconstriction; ET\textsubscript{B} is also present on endothelial cells and facilitates the release of NO\textsuperscript{183-185}.

Bayliss in 1902 for the first time proposed the importance of myogenic tone in regulation of basal vascular tone and blood flow\textsuperscript{186}. Arterioles respond to an increase in transmural pressure by contracting through depolarization of SMCs and the subsequent entrance of Ca\textsuperscript{2+} into the vicinity of SMCs\textsuperscript{187,188}. \textit{In vivo} studies demonstrated that through modulation of \(\alpha\)-adrenoceptors, the myogenic response in the vessels could be modified\textsuperscript{189,190}. In hypertension, it has been
reported that myogenic response increases; it is now speculated that the enhanced myogenic tone is responsible for the chronic vascular adaptations\textsuperscript{191}.

**Systemic factors**

Both neural and humoral factors play a critical role in the systemic regulation of vascular tone. A large part of cardiovascular homeostasis depends on the well-being of the sympathetic nervous system (SNS); the origin and the pathways of sympathetic innervation were discussed earlier. Upon activation of the SNS, NE is released and binds to postjunctional adrenoceptors, leading to an increase in vascular tone; at the same time, the release of NE is controlled through a negative feedback loop by prejunctional $\alpha_2$ adrenoceptors\textsuperscript{192,193}. Complete blocking of adrenoceptors (using non-specific blockers) has been shown to reduce significantly the vascular tone in both forearm and legs\textsuperscript{194,195}.

The humoral aspect of vascular tone regulation is in the hands of the renin-angiotensin-aldosterone system (RAAS). RAAS activates in response to stimuli such as a deficiency in intravascular sodium and water content or reduction in the blood volume; such factors could compromise the stability of BP control and affect the extracellular fluid content of the body\textsuperscript{196,197}. Under such conditions, juxtaglomerular cells secrete renin, which converts angiotensinogen into angiotensin I. Angiotensin I, through the action of angiotensin-converting enzyme (ACE), is converted to angiotensin II\textsuperscript{198}. Angiotensin II binds to AT$_1$ and AT$_2$ receptors and regulates vascular tone by modulating the SMCs activity through PKC pathway\textsuperscript{198}.

**Peripheral resistance and SCI**

Following SCI, the sympathetic control of circulation will be lost below the level of injury. In theory, it is expected that following SCI, profound vasodilation accompanied by reduction in peripheral resistance will occur. However, it must be borne in mind that below the lesion site, vascular properties are additionally influenced by lack of activity (deconditioning of muscles) and reduced metabolic demands, which facilitate the vascular atrophy in this region\textsuperscript{143}.
Generally, reported outcomes on how peripheral resistance changes following SCI are variable. For example, some studies suggest that leg arterial inflow increases in chronic SCI individuals, but the peripheral resistance reduces in the legs of SCI subjects when compared to the control group\(^\text{199,200}\). Karlsson et al (1998) looked at the changes in peripheral resistance in the arms and legs of SCI patients and compared it to AB individuals. They reported that in AB individuals the peripheral resistance was significantly lower in arms than in legs; such a difference was not observed in the SCI group\(^\text{200}\). On the other hand, in a study by Shenberger and associates, resistance in the arms of paraplegic individuals was lower when compared to the control group\(^\text{201}\). They also noted that upper extremity exercise could acutely enhance vasodilatory response in resistance vessels\(^\text{201}\). Parallel studies on human subjects who had undergone long-term sympathetic denervation showed no significant difference in peripheral resistance of arms or legs when compared to control groups\(^\text{202,203}\). However, due to the obvious differences that exist between SCI and sympathectomized cases, it is hard to correlate the results of these studies to SCI-related cases. For example, in SCI the reflex arc may or may not be completely damaged, and depending on the severity of the damage, might still take part in controlling the vascular tone; also in SCI, as mentioned earlier, muscles below the level of injury become completely paralyzed and vessels below the level of injury will eventually become atrophied\(^\text{152}\).

Aside from studies in which no change, or a decrease in peripheral resistance have been reported, there are number of studies supporting the idea that peripheral resistance increases in the vasculature below the lesion site following SCI\(^\text{152,204-206}\). The reason these studies showed outcomes differing from previous expectations of changes in peripheral resistance following an injury could be partly explained through sympathectomized animal studies. Such studies revealed that following long-term sympathectomy, the level of eNOS reduces in the vessels; as a result the NO level (vasodilator) is also attenuated\(^\text{207}\). Such a reduction is normally accompanied with an increased expression of endothelin-1, which is associated with elevated BP, and this
mechanism could partly explain the augmented peripheral resistance following SCI\textsuperscript{207}. The role of endothelin-1 in blood flow has been also confirmed in a study on SCI human subjects, which demonstrated that blockage of endothelin-1 receptors contributes to an increase in blood flow after SCI\textsuperscript{204}.

1.4.10 Vascular Remodeling

Currently, the impact of chronic elevation of BP in the small vasculature of AB individuals is understood\textsuperscript{208,209}. Research has shown that blood flow velocity and turbulence increases as a result of chronic constriction of the blood vessels; such changes are strongly evident in smaller vasculature, with some potential outcomes such as increase in shear stress, endothelial dysfunction, and more importantly the induction of structural alternation in the vasculature known as remodeling\textsuperscript{210-212}. In the next few paragraphs, morphological changes of peripheral vasculature and small arteries in response to BP variation will be discussed. The final goal of this section is to correlate these longitudinal studies with the work done on changes in blood vessels after SCI.

Approximately 80\% of resistance to blood flow takes place in the small arteries and arterioles (D <300\( \mu \)m); therefore, small arterial vessels play a fundamental role in regulation of the BP and local distribution of the blood flow\textsuperscript{92}. The vessel wall is not a static structure composed of adventitia, contractile SMCs and endothelium; instead, it possesses a degree of plasticity that allows this structure to respond to various mechanical, neurohormonal and hemodynamic factors which ultimately shape the structural adaptations in the arteries\textsuperscript{213}. On a micro-scale, the structural modifications of the vessels are achieved through alterations in cell-to-cell connections, extracellular matrix composition and subcellular cytoskeletal organization; however, identifying such changes in very small vessels is a difficult task. For this reason, to evaluate the extent of remodeling in resistance vessels, a macro-scale system was proposed by Mulvany and associates. Factors such as lumen D, media thickness, and/or the CSA of adventitia
and media are frequently used to quantitatively measure the degree of changes in the vascular structures\textsuperscript{214}. Based on this model, remodeling could be either an inward or an outward process, which translates to a decrease in luminal D, or an increase in luminal D accordingly\textsuperscript{214}. Today, the refined model used to evaluate remodeling also includes changes in WT (wall CSA); such modifications could either be eutrophic (no change), hypotrophic (decreased) or hypertrophic (increased) in nature\textsuperscript{215}.

**Microvascular adaptations in hypertension (in vivo & in vitro evidence)**

As mentioned previously, shear stress and circumferential wall tension are the two predominant forms of hemodynamic stress on the vessel wall. These forces, generated as a result of blood flow and transmural pressure, activate SMC and endothelial cells, leading to short term or long term adaptations in vasculature\textsuperscript{216}. In the literature, the effect of shear stress on the reactivity of endothelial cells has been widely studied in cell culture\textsuperscript{217,218}; however, performing *in vitro* studies on the impact of BP on the vascular wall is more difficult. Techniques such as growing cells under cyclic stretch have been used to simulate the effect of BP on the vascular wall\textsuperscript{219,220}. It seems that the modifications of vascular structure play a primary role in the development of hypertension\textsuperscript{221}. It is generally believed that the potential outcome of an increase in transmural pressure is reduction in luminal D and an increase in WT; further, the opposite of what is described above may occur as a result of an extended period of blood flow changes\textsuperscript{222,223}.

The remodeling of vasculature following hypertension has been extensively studied in both human subjects and animal models. The majority of studies on animal models has utilized vascular beds such as cremasteric arterioles\textsuperscript{224}, coronary arteries\textsuperscript{225}, and cerebral and mesenteric arteries/arterioles\textsuperscript{226-229}. On the other hand, human studies on EH have either used subcutaneous arteries or mesenteric arterioles\textsuperscript{230,231}.

The hallmark of EH is the marked increase in peripheral resistance\textsuperscript{232}. Folkow and associates have shown that peripheral resistance increases by 37% in the established phase of
hypertension; such a finding has been also confirmed in other studies\textsuperscript{233,234}. The increased peripheral resistance in the established phase of hypertension has been attributed to either reduction in the number of the blood vessels available for blood flow (rarefaction) or decrease in D of the vessels\textsuperscript{235,236}. That being said, measuring peripheral resistance is generally a difficult task to do; thus, the majority of studies on hypertension focus on correlation between an increase in BP, which is an indirect indicator of changes in peripheral resistance, and structural modifications of the arteries. Heagerty and Mulvany developed a non-invasive technique to isolate resistance arteries from gluteal subcutaneous tissue in normotensive and hypertensive individuals\textsuperscript{231}. Using this technique, studies have shown that in EH, inward eutrophic remodeling in small arteries occurs, rather than hypertrophic changes\textsuperscript{214,237-240}. This type of remodeling in resistance arteries from EH patients is characterized by an increase in thickness of media, increased media to lumen ratio, and smaller outer D and lumen; however, the CSA remains unchanged, hence the “eutrophic” designation of this particular type of remodeling (Figure 2)\textsuperscript{214,237,239}. In animal models, inward eutrophic remodeling have been identified in spontaneously hypertensive rats (SHR)\textsuperscript{228} and 2-kidney, 1-clip Goldblatt rats\textsuperscript{241}. Lee and colleagues extensively studied the structural remodeling of both large and small mesenteric arteries in SHR animal models. Their studies suggested that in superior mesenteric arteries (SMA), the structural remodeling only begins to manifest during the established phase of hypertension (\(\geq 21\) weeks), whereas in larger and smaller branches of mesenteric arteries the structural alteration is noticeable in both the prehypertensive (3-5 weeks), and developing (10-12 weeks) phases in SHR\textsuperscript{242-244}.

The wall-to-lumen ratio has been widely used in literature as the most important parameter in hypertension studies; histological analysis on samples obtained from humans with EH\textsuperscript{230} and in an SHR animal model reported an increase in the ratio\textsuperscript{90,245}. Despite the convincing data from histological studies on the ratio changes, controversy still surrounds the extent of the D
changes in SHR: some report a change, whereas others report no significant changes in D\textsuperscript{90,246}. A setup under identical conditions is required for quantitative evaluation of vascular wall remodeling in normotensive and hypertensive samples \textit{in vitro}. This is easily achieved through measuring the parameters when samples are maximally relaxed and under the same intravascular pressure, for example by using wire myography. \textit{In vitro} studies on SHR support the findings of an increased wall-to-lumen ratio, a reduction in D and an increase in media thickness in small mesenteric arteries using wire myography\textsuperscript{246,247}.

Although hypertrophic remodeling (increase in CSA) does not appear to be the case in vascular remodeling of patients with EH, this particular type of remodeling has been identified in secondary forms of hypertension in humans\textsuperscript{248-250}, and also in animal models of hypertension such as deoxycorticosterone acetate (DOCA)-salt rats, 1-kidney, 1-clip Goldblatt\textsuperscript{251,252} and Dahl-salt sensitive rats\textsuperscript{253}. The question of whether the increase in media thickness that has been associated with hypertension is actually related to hypertrophy of SMCs or hyperplasia of these cells has been answered in older studies on hypertension. These studies are indicative of different mechanisms involved in media thickness enhancement in various arterial beds following hypertension\textsuperscript{254}; for example, in the aorta\textsuperscript{255,256} and SMA\textsuperscript{257}, hypertrophy of SMCs seem to be the predominant triggering mechanism. In smaller arteries and arterioles, hyperplasia of SMCs is the main triggering mechanism for an increase in media thickness\textsuperscript{257,258}.

Vascular remodeling could develop in animal models as early as 2 days following the initiation of the stimulus (mainly persistent changes to blood flow)\textsuperscript{259,260}. As discussed previously SCI, especially at thoracic segment 6 or above, disrupts cardiovascular control of the body. For this reason, people with high SCI frequently struggle with abnormal BP control and conditions such as AD. Consequently, SCI provides a great human model for the evaluation of vascular adaptations that occur in the body (below the level of injury) in response to extreme exposure to inactivity and disrupted sympathetic control of blood BP. Unfortunately, the long-
term impact of episodic rise of BP and the subsequent outcomes of such extreme variations in BP on vascular structures following SCI is not yet well understood. In general, research shows that following SCI, femoral arteries (below the level of injury) experience a 30% reduction in D, lower blood flow and an increase in hemodynamic stresses\textsuperscript{82,146,150,155}. One recent study has also shown that following SCI, carotid intima-media thickness increases\textsuperscript{261}. Future studies will determine whether or not a specific type of remodeling similar to what is observed in hypertension does occur in vasculature following SCI and AD. Further, it still remains to be established whether remodeling of resistance arteries following SCI triggers the development of AD, or whether these potential changes are as a consequence of AD. The altered structure of vasculature may explain the initial manifestation of end organ damage and perhaps partly explain the increased probability of CVD in patients with SCI\textsuperscript{261}. The goal of the present study is to address whether or not AD is associated with morphological changes in peripheral resistance arteries following SCI.
Figure 1. Diagram illustrating how autonomic dysreflexia (AD) is triggered in people with spinal cord injury (SCI) at T6 or above. At the level of SCI, the descending inhibitory signals that normally counteract the increase in blood pressure are blocked. For this reason, an afferent stimuli (noxious or non-noxious) below the level of injury e.g. full bladder could trigger a sympathetic response that is unopposed by descending inhibitory pathways leading to vasoconstriction of vasculature below the level of the injury and the development of AD.

Figure 2. Different patterns of vascular remodeling. Vasculature remodeling occurs in response to chronic changes of blood pressure. Outward hypertrophic remodeling is characterized by larger lumen diameter (D) and an increase in CSA. Inward eutrophic remodeling is characterized by reduction in D, but no change in CSA. Inward hypotrophic and inward hypertrophic remodeling are both characterized by reduction in D; however the CSA reduces in the first one and increases in the second one. This figure modified from Makino et al.\(^1\)
Chapter 2: Vascular Changes in Spinal Cord Injured Animals With Repetitive Episodes of Autonomic Dysreflexia

The aim of the study is to evaluate the extent of morphological alterations (media thickness, D, media thickness-to-lumen ratio and CSA) in resistance arteries of high SCI rats following the induction of repetitive AD. The experimental design is demonstrated in (Figure 3).

2.1 Materials and Methods

2.1.1 In vivo Model

All animal procedures were performed in accordance with the guidelines of Canadian Council for Animal Care and approved by the University of British Columbia Animal Care Committee. Fifteen adult male Wistar rats (250-350g, Harlan™, CA, United States) were used in this experiment.

AD induced in Wistar rats with high thoracic injury using a relatively easy and non-invasive technique known as colorectal distension (CRD). This technique is well documented in rodents as previously cited in the literature.

Animal care upon arrival

Rats were kept in a temperature and light-controlled facility with 12-hour light-dark cycle. They were caged in groups of maximum 4 and a minimum 2; thus preventing the effect of isolation (housing alone) on the level of foraging and stress. During this period, they received enriched diet of 40 g kibble (LabDiet, Rodent Diet 5001) ad libitum on the cagetop hopper.

Animal care prior and post Surgery

Animals were acclimated to post surgical diet by receiving standardized enriched diet of meal replacement shake, cereals and fruits (oranges and apples) once a day for three days prior to the surgery. Prophylactic enrofloxacin diet was carried on daily with the administration of Baytril (10mg/Kg, s.c, Associated Veterinary Purchasing [AVP], Langley, BC) for three days prior to the surgical procedure.
Post surgery, special cages were prepared for the lesioned animals by placing rubber grid flooring to facilitate the movement of the injured rats. Underneath the rubber grid an absorbant liner labmat was placed to enhance moisture absorption. On the top, the rubber grid was covered with wood chip bedding. Cages were changed once a day post operation. Rats were fed three times a day with enriched diet, containing strawberry shake, 40g nutritive transport gel (Charles River Laboratories International, Inc., Wilmington, MA), fruit (apple and oranges), cereal (cheerios and fruit loops), commercially available rat treats (e.g. bacon bits and nuts), and kibbles. To ensure easy access to water, low-reaching bottles along with small metal dishes filled with water were located in the cage floor and cagetop hopper accordingly. 14 days post surgery, the transport gel and meal replacement milkshake were removed from the cages, but animals continued receiving fruits, cereals and rat treats. Animals were monitored and scored daily based on the criteria such as body weight, activity level, social behavior, lesion healing state and clinical signs of morbidity (first two weeks); the monitoring continued to once every two days for the remaining period. Analgesics such as buprenorphine (0.02 mg/Kg, s.c, Temgesic) and ketoprofen [Anafen] (5mg/kg, s.c.) were administered daily for three days post surgery. To prevent bladder infection, endrofloxacin (Baytril, 10mg/Kg, s.c.) was also administered daily for three days post operation. In addition, rats received warmed Lactated Ringers administered below the level of injury, twice a day (5ml, s.c.) for the first three days following the injury. Skin sutures (Prolene; 4-0) were removed a week after the operation to prevent the occurrence of skin irritation in lesioned rats.

The urinary bladder was manually expressed three times a day for the first 7-10 days following the surgery; after approximately 10 days, the normal bladder function returned; but to ensure the absence of urethral obstruction/infection, the urinary bladder was checked twice per day (10 days post surgery) and once per day until the experimental end point is reached (day 30). All these procedures have been previously described in Inskip et al.²⁶⁵
2.1.2 Surgical Procedure

The surgery performed on 10-week-old rats. General anesthesia was induced with ketamine hydrochloride (70mg/Kg, i.p., Vetalar®; AVP) and medetomidine hydrochloride (Domitor®, 0.5mg/Kg, i.p., AVP) in the experimental animals (n=10). Immediately prior to the surgery, buprenophrine (0.02mg/Kg, s.c. Temgesic®, AVP), ketoprofen (Anafen®; 5mg/Kg, s.c., AVP) and enrofloxacin (Baytril, 10 mg/Kg, s.c.) were also administered to the animals. Following the injections, the surgical site was shaved, scrubbed and treated with iodine. For complete transections at thoracic segment three (T3), rats were positioned ventrally on top of 5 ml syringe to elevate the spine curvature. Midline skin incision was made and superficial muscles overlying C8-T2 vertebrae were cut through. The longitudinal muscles and fat pads were blunt dissected; T2 dorsal process got exposed and removed by rongeurs. The muscles between T2 and T3 were cleared out and dura was opened with microscissors; the cord was cut using extra-fine scissors. Under surgical microscope, the clear separation of the cut ends of the cord was visually confirmed. To minimize the bleeding, gel foam was placed in the cavity. Once homeostasis reached, the muscle layer continuously sutured with Vicryl (4-0), and skin closed with interrupted sutures using Prolene (4-0). Animals also received Lactated Ringers (5ml, s.c) and placed in a warmed chamber for recovery (Animal Intensive Care Unit, HotSpot for Birds, Los Angeles, CA). Anesthesia was reversed with atipamezole hydrochloride (Antisedan, 1mg/Kg, s.c. Novarits, Mississauga, ON, Canada).

2.1.3 Cardiovascular Assessment

This part of the procedure was not conducted on the experimental animals used in the current study. The beat-to-beat BP recordings have been previously conducted in T3 SCI-only and T3 SCI+CRD animal groups.

At the experimental end point day 30, a PU-30 cannula inserted into left carotid artery through small incision at the base of skull while rats were kept under isoflurane anesthesia. The
subcutaneously tunneled cannula exits the skin dorsally and secured to the back with sutures. Heparin (1:10, Hepalean®, AVP) along with 5% dextrose in Lactated Ringer’s used to fill the cannula. Following the cannulation, the fluid filled pressure inducers (SP844, MEMScAP, Norway) were connected to cannula and beat-to-beat arterial pressure was monitored using PowerLab and Chart™ 5 for windows (ADInstruments, Colorado Springs, USA) typically 5 minutes after connecting the cannula to transducers (to allow animals rest after handling). The recordings of baseline BP carried on for a period of five minutes. During recordings, animals were conscious and were able to move freely in the cage. For T3 SCI+CRD animal group, the AD induced using CRD technique and for each animal BP fluctuations were recorded during two episodes of AD with a minimum of 10 minutes of recovery intervening. All these procedures have been previously described in Alan et al176.

**Cardiovascular data assessment**

The cardiovascular data analysis was performed using GraphPad Prism 5 (version 5.0; GraphPad Software Inc., La Jolla, CA). The raw pressure were averaged over one second. The average of three minutes of recording time was used in animals to represent baseline BP changes. In CRD-evoked animal group, the systolic BP represents the average of two consecutive episodes of induced AD. Mean arterial pressure was calculated using Ave 1/3max+2/3min.

**2.1.4 Repetitive Colorectal Distension**

CRD is a non-invasive technique used to evoke AD. Two weeks post surgery, animals in one group (n=5) received daily bouts of CRD for 30 min daily. Colon distended in SCI rats by inserting a deflated balloon tip of a paediatric silicone Foley catheter (Fr10, Coloplast, Denmark) into the descending colon through anus; the catheter secured to the tail with a tape. Using a 5ml syringe connected to the catheter via a one-way valve, the 10mm latex balloon on the tip of the
catheter was infused with 2ml of air over a 10 second period; the inflated balloon remained in
the colon for 30 minutes.

2.1.5 Perfusion

At experimental end point (day 30), animals from uninjured (n=5), T3 SCI (n=5) and T3
SCI+CRD (n=5) groups euthanized with an overdose of chloral hydrate (trichloroacetaldehyde
hydrate, 1g/Kg, i.p.). Perfusion fixation through heart was conducted by making an incision
through the abdominal length of the diaphragm. By making horizontal cuts through the rib cage,
thoracic cavity opened up. While holding the heart steady, a needle inserted into protrusion of
the left ventricle. An incision was made in the right atrium for venous effluent. The systemic
vasculature was perfused with room temperature 0.1M phosphate-buffered saline (PBS)
followed by cold 4% paraformaldehyde (PF). At the end of the perfusion, gut tissue excised from
the animals. Superior mesenteric arteries (SMA) and first-order branches (PMA) isolated from
the gut; postfixied in 4% PF overnight. The obtained samples transferred into cryoprotected 20%
sucrose in 0.1 M phosphate buffer 24 hrs later.

2.1.6 Histology and Morphology

Sample preparation

From the excised gut tissue, segments of SMA got isolated from the proximal end (closer to
small intestine and abdominal aorta), medial portion and distal end (away from small intestine
and abdominal aorta) of the gut tissue. Further, for each animal, three PMAs were isolated and
used for the subsequent morphological analysis (Figure 4).

Under dissecting microscope, the fat surrounding the arteries were gently removed from
the cryoprotected PMA and SMA samples; subsequently, they were cut into 1cm fragments by
sharp scalpel. The small segments of SMA and PMA embedded vertically into blocks using
cryomatrix-embedding resin (Thermo Scientific “Shandon Cryomatrix”, Fisher Scientific) under
the dissecting microscope. Liquid nitrogen was used to freeze the blocks; serial 10µm cross-
sections were obtained from frozen blocks using cryomicrtome (Technoterm Integrated Service, Vancouver, BC, Canada). Cryosections were placed on Superfrost/plus microscope slides and air dried.

**Hematoxylin and Eosin Staining (H&E)**

Cross-sections were H&E stained and used for morphological analysis. Briefly, sections on each slide washed in distilled water (dH₂O, 2 times) and 99% ethanol (3 times) to remove tissue-embedding compounds. Then the slides were emerged into hematoxylin solution (5min, Sigma-Aldrich, CA). Subsequently, slides were differentiated in 0.5% hydrochloric acid (HCl, Sigma-Aldrich, CA) and blued using 1.5% sodium carbonate (Na₂CO₃, Sigma-Aldrich, CA). Slides were dehydrated in 95% ethanol and dipped into eosin (Sigma-Aldrich, CA) for 20 seconds. The slides proceeded through three more changes of 95% ethanol, followed by two changes of xylene and then cover slipped.

**Light microscopy and morphological measurements**

The morphological aspects of both SMA and PMA (media thickness, D and media-to-lumen ratio) were analyzed using the H&E stained cross-sections under the light microscope (Leica, DM5000B) equipped with Leica Application Suite (LAS, V3.1). The media thickness was measured by tracing the inner and outer elastic laminae. The thickness was measured in four parts of the artery on each cross section (top, right, bottom and left), the values averaged from each cross-section and used for the analysis. For both SMA and PMA, media thickness measurements were carried on using 20x magnification on the light microscope (Figure 5). The D was calculated by the mean value of two radial measurements perpendicular to the vessel wall (Figure 5). The D measurements were evaluated under 10x magnification for both SMA and PMA. The media thickness:lumen ratio was calculated as MT/D×100, where MT is media thickness and D is lumen D. Wall CSA for both SMA and PMA calculated as follow: CSA=
\pi(D+2WT/2)^2 - \pi(D/2)^2. All analysis performed blind folded and five cross-sections were used per arterial segment.

2.1.7 Statistical Analysis

All data are expressed as mean ± SEM. and in all cases a p-value of <0.05 was considered significant. All analysis were performed by GraphPad Prism 5 (version 5.0; GraphPad Software Inc., La Jolla, CA). One-way Analysis of Variance (ANOVA) with repeated measures were used with Bonferroni’s Multiple Comparison test for the statistical analysis. For cardiovascular assessments Student’s t-test (non parametric, Mann-Whitney test) were performed.
Figure 3. Schematic representation of the experimental design. SCI performed on Wistar rats (n=10). On day 15 after the injury, rats were undergone daily bouts of CRD for 30 minutes (n=5). At the experimental end point, animals from uninjured (n=5), T3 SCI (n=5) and T3 SCI+ CRD (n=5) perfused, SMA and PMA were isolated and used for the subsequent analysis.
Figure 4. Schematic view of superior mesenteric artery and its branches in a gut tissue. 4a is a gut tissue obtained from rat. Black circle shows SMA and its associated PMAs that are branching off from the major branch. 4b is a schematic of superior mesenteric artery (SMA) arises from the anterior surface of abdominal aorta and along with its smaller branches provides blood to intestine, transverse colon and pancreas. The width of the artery varies across the gut tissue; therefore, for the purpose of the analysis, arterial samples selected from three different regions of the vessel, based on the proximity to small intestine. Above, pink shaded areas show distal segment of SMA (A), medial segment of SMA (B), and proximal segment of SMA (C) accordingly. The blue shaded area demonstrates a primary branch of the mesentery artery (PMA).
Figure 5. A depiction of an arterial cross-section and the methods used for analysis of media thickness and diameter in SMA and PMA. In both SMA and PMA, the media thickness was measured by evaluating the thickness changes in 4 different regions of an arterial cross section (top, right, bottom and left) represented by the gray shaded boxes. The mean value of these measurements calculated and used in the analysis. The diameter was evaluated based on the mean value of two radial measurements perpendicular to the wall of the arterial cross-section, represented by dotted arrow.
Chapter 3: Results

3.1.1 H&E Cross-Sections of Superior Mesenteric Artery

Figure 6. Representative cross-sections of H&E stained superior mesenteric artery (SMA) from uninjured and T3 SCI+CRD. A: uninjured animal and B: T3 SCI+CRD animal; both A and B are taken at 10X, scale bar 50µm. C and D are representative figures from bottom segment of SMAs. C: uninjured animal and D: T3 SCI+CRD animal. Both C and D are taken at 20X, scale bar 50µm.

Above are representative H&E stained cross-sections obtained from superior mesenteric artery (SMA) of both control-uninjured and T3 SCI+CRD animal groups. Under 10X magnification, D changes were evaluated in SMAs. The thickness measurements for SMAs were performed under 20X magnification. As an example, the bottom segments of the arteries have been shown under 20X magnification.
3.1.2 H&E Cross-Sections of Primary Mesenteric Artery

Figure 7. Representative cross-sections of H&E stained primary mesenteric artery (PMA) from uninjured and T3 SCI+CRD. A: uninjured animal and B: T3 SCI+CRD animal; both A and B are taken at 10X, scale bar 30µm. C and D are representative figures from bottom segment of PMAs. C: uninjured animal and D: T3 SCI+CRD animal. Both C and D are taken at 20X, scale bar 30µm.

Above are representative H&E stained cross-sections obtained from PMA of both control-uninjured and T3 SCI+CRD animal groups. Under 10X magnification, D changes were evaluated in PMAs. The thickness measurements for PMAs were performed under 20x magnification. As an example, the bottom segments of the arteries have been shown under 20X magnification.
3.1.3 Cardiovascular Assessment of T3 SCI and T3 SCI+CRD

Beat-to-beat BP recordings of T3 SCI and T3 SCI+CRD (weeks 3 and 4 after injury) was performed a month after injury using left carotid cannulation. When CRD animal group compared with T3 SCI, the baseline cardiovascular parameters showed no significant change. In other words, CRD did not alter baseline arterial pressure and HR. For T3 SCI+CRD animal group SAP (121.1± 4.8), DAP (106.8± 6.8), MAP (111.6± 6.0), and HR (468.4± 31.7). For T3 SCI animal group SAP (132.2± 3.5), DAP (102.9± 10.0), MAP (112.7± 7.8), and HR (498.9± 26.0).
3.1.4 Media Thickness Measurements

Figure 9. Media thickness measurements obtained from H&E stained cross-sections of superior mesenteric artery (SMA) on left and primary branches of mesentery artery (PMA) on right. Values are mean ± SEM. n=5 segments from proximal, medial and distal portions of SMA and PMA from each animal group; *p < 0.05 compared to uninjured control, one way ANOVA and Bonferroni test.

Vascular remodeling evaluated by media thickness measurements in H&E stained cross-sections obtained from uninjured, T3 SCI, and T3 SCI+CRD animals. The data shows no significant difference in media thickness measurements of superior mesenteric artery (SMA) obtained from these three animal groups. In primary branches of mesentery arteries (PMA) obtained from each of these animal groups, there was no significant difference between the uninjured control and T3 SCI group. However, the media thickness of T3 SCI+CRD animal group showed a significant increase compared to control uninjured group. T3 SCI+CRD compared with T3 SCI also showed a significant increase. Mean and SEM for proximal segments from T3SCI+CRD and T3SCI are (12.9±1.6; 11.1±1.4), medial (13.3±1.3; 10.3±0.9), and for distal (12.7±1.8; 9.8±0.6). Mean and SEM between T3SCI+CRD and uninjured are as follow: proximal (12.9±1.6; 10.2±0.4), medial (13.3±1.3; 10.2±0.5), and for distal (12.7±1.8; 9.9±0.7).
3.1.5 Diameter Measurements

D changes were measured as an important parameter in evaluating the degree of morphological remodeling in the resistance arteries of control and injured animal groups. The data showed no significant difference in the $D$ values obtained from superior mesenteric arteries (SMA) in uninjured ($n=5$), T3 SCI ($n=5$), and T3 SCI+CRD ($n=5$) animal groups $p<0.05$ was considered significant. When $D$ values of primary branches of mesentery arteries (PMA) in T3 SCI+CRD are as follow: proximal (151.1±19.5), medial (161.2±15.1), and distal (144.2±18.6). For T3 SCI (proximal 188.1±19.6), (medial 168.9±8.7), and (distal 178.6±14.2). PMA from uninjured group has mean ± SEM as follow: (proximal 158.1±8.8), (medial 168.7±7.1), and (distal 155.3±5.6).

Figure 10. Diameter changes in H&E stained cross-sections obtained from superior mesenteric artery (SMA) on left and primary branches of mesentery artery (PMA) on right. Values are mean ± SEM. $n=5$ segments from proximal, medial and distal portions of arteries obtained from each animal group; *$p<0.05$ compared to uninjured control, one way ANOVA and Bonferroni test.
3.1.6 Wall-to-Lumen Ratio

![Graph showing Wall-to-Lumen ratios in superior mesenteric arteries (SMA) on the left, and primary branches of mesenteric arteries (PMA) on the right obtained from 3 different animal groups. Results are mean ± SEM; n=5 segments from proximal, medial and distal segments of arteries in each animal group. *p < 0.05 versus control group; **p < 0.01 versus T3 SCI group, one way ANOVA and Bonferroni test.](image)

In superior mesenteric arteries (SMA) the wall-to-lumen ratio did not significantly change when animals from uninjured, T3 SCI and T3 SCI+CRD were compared to one another (n=15 in each group, A). In primary branches of mesenteric artery (PMA), the wall-to-lumen ratio significantly increased in T3 SCI+CRD when compared to control-uninjured group accordingly: (proximal 0.08±0.02; 0.06±0.003), (medial 0.09±0.02; 0.06±0.004), and (distal 0.01±0.03; 0.06±0.01) *P < 0.05. Data also show that the wall-to-lumen ratio was significantly higher in T3 SCI+CRD compared to T3 SCI (proximal 0.08±0.02; 0.06±0.004), (medial 0.09±0.02; 0.06±0.01), and (distal 0.01±0.03;0.06±0.01) **p < 0.01.
3.1.7 Wall Cross Sectional Area

Figure 12. Wall cross-sectional area (CSA) for superior mesenteric artery (SMA) on the left and primary branches of mesentery artery (PMA) on the right obtained from proximal, medial and distal segments of the three different animal groups. Results are mean ± SEM. n=5 segments in each group; *p < 0.05 compared to uninjured group, one way ANOVA and Bonferroni test.

Wall CSA was measured in superior mesenteric artery (SMA), no significant difference was observed between the experimental groups and the control uninjured (n=5) used in each group. The data from CSA calculation of primary branches of mesentery artery (PMA) is showing. No significant difference was observed among groups in PMA. The proximal values for T3 SCI+CRD, T3 SCI, and uninjured are as follow accordingly (69281.52±16822.54; 101131.50±19625.39; 70114.98±7376.03). The values from medial segments are (77078.68±11706.63; 79241.36±7989.46; 78737.96±6131.49). For the distal segments the mean ± SEM are as follow for (64077.92±11764.44; 88459.55±12654.47; 67021.66±4203.10).
Chapter 4: Discussion

4.1 Synopsis

In these experiments, the effects of CRD to evoke AD on structural remodeling of resistance arteries in SCI animals (Wistar rats) were investigated. The hypothesis of the experiment was that following induction of AD in SCI animals, both the superior mesenteric artery (SMA) and the primary branches of the mesentery artery (PMA) would experience a degree of vascular remodeling similar to what has been observed in essential and spontaneous hypertension studies on humans and animal models (that is, inward eutrophic remodeling). In this experimental design, morphological changes (media thickness, D, wall-to-lumen ratio and CSA) of SMA and PMA were analyzed a month after injury in control-uninjured, T3 SCI-only and T3 SCI+CRD animal groups. The findings of the study reject the initial hypothesis that SMAs of T3 SCI+CRD will undergo structural remodeling in response to the induction of AD. The outcomes suggest that SMAs obtained from T3 SCI+CRD did not manifest any structural changes compared to T3 SCI-only and control-uninjured animal groups, when characteristics such as media thickness, luminal D, wall-to-lumen ratio and CSA were evaluated in these arteries. On the other hand, the PMAs of the T3 SCI+CRD group showed significant increase in media thickness when compared to the control-uninjured group. The wall-to-lumen ratio demonstrated a significant increase in the T3 SCI+CRD group in comparison to both control-uninjured and T3 SCI-only animals. Further, the PMAs from T3 SCI+CRD showed no changes in luminal D when compared to control-uninjured and T3 SCI-only groups. When the CSA of the PMAs of CRD group were compared with the other two groups, no significant change was observed in this measured parameter. The results from the PMAs of the experimental group suggest that a remodeling process is taking place in these smaller branches of the mesentery artery, which is to some extent different from the observed morphological changes in hypertension studies.
It is well established that after SCI, changes occur in lower limb vasculature\textsuperscript{143,144}. The remodeling process of peripheral vasculature starts soon after the injury and is most likely an adaptive response to reduced metabolic demands below the level of the lesion. Circulatory adaptations after the injury are accompanied by reduction in total blood volume (due to a more sedentary lifestyle)\textsuperscript{266} and lower resting BP, as documented in tetraplegic patients\textsuperscript{56,267}. In paraplegic individuals, a lower resting blood flow and venous capacity, compared with AB individuals, has been reported\textsuperscript{143,144}. Revisiting the reports on lower resting BP in tetraplegic SCI, a recent meta-analysis study by West et al highlighted the importance of considering both the severity of the injury and the body position (supine vs. seated) when cardiovascular parameters of the SCI population are reported and compared with one another, or with AB individuals\textsuperscript{268}. This finding is particularly important in higher levels of SCI (i.e. cervical) as their BP and HR is lower in supine position, when compared with AB, high thoracic, and low thoracic injured groups. In a seated position, the cervical injured population demonstrated significantly lower resting systolic BP when compared with the supine position\textsuperscript{268}. Also, systolic and diastolic BP in seated position was lower than both AB and low thoracic injured, whereas HR was only significantly lower in this body position when compared with the low thoracic injured group\textsuperscript{268}.

4.1.1 Diameter Changes Following CRD

The morphological changes (remodeling) of vasculature following SCI has been mostly studied with respect to changes in D of peripheral arteries above and below the level of injury, such as carotid and brachial arteries (above the lesion site), and common and superficial femoral arteries (below the lesion site). The outcomes of these studies generally suggest a reduction (~50\%) in D of the femoral artery in both paraplegic and tetraplegic patients compared to the AB population\textsuperscript{48,144,269}. In the present study, the D of SMA obtained from CRD animals showed no significant change when compared to SMAs from T3 SCI-only and control-uninjured animals; this finding was interesting as no variation in other measured morphological parameters (such as
media thickness, wall-to-lumen ratio and CSA in response to recurrent episodes of AD) was observed in this group. The question of why no changes were observed in SMAs of the CRD group will be addressed below. Further, when the D of PMAs obtained from the CRD animal group were measured, again no significant difference was observed in the CRD group when compared to the other two groups; this was surprising as the PMAs from CRD posses a significant increase in media thickness and wall-to-lumen ratio. Since in both AD and chronic forms of hypertension an elevated BP well above the baseline is observed, in the present study it was hypothesized that resistance arteries (SMA and PMA) from the animal group with recurrent episodes of AD would demonstrate a degree of remodeling similar to what had been reported in studies on chronic forms of hypertension, such as EH studies in humans and spontaneous hypertension studies on rats: the D is essentially under the control of the blood flow. However, in this study blood flow changes following an injury and CRD were not monitored. The reduced blood flow below the level of injury has been speculated to be the main contributor for the observed reduction in the luminal D of common and superficial femoral arteries below the lesion site. In a study by Krebs et al (1983), the blood flow in the splanchnic region (renal vasculature) of chronic tetraplegic SCI men was studied. The results suggested that during head-up tilt, the renal blood flow is reduced in both SCI and AB individuals. In the absence of supraspinal control, the presence of orthostatic stimulus (head-up tilt) triggers massive vasoconstriction in renal vasculature, a response that has been attributed to the recovery of vasomotor reflex in the splanchnic region. Further, an increase in the peripheral resistance of lower limb vessels has been reported in the majority of studies on SCI. Peripheral resistance and D are inversely correlated to one another, as has been postulated by Poiseuille’s law. Therefore, a reduction in D leads to an increase in peripheral resistance. The main question becomes whether mesenteric arteries following SCI in rats would experience the same degree of reduction in blood flow as what has been described by Krebs et al, and whether the reduction in blood flow following SCI
and AD is sufficient to trigger chronic changes in the D of mesenteric arteries, over the proposed experimental end point in the current study.

The majority of the available data on essential and spontaneous hypertension reported an inward eutrophic remodeling of vasculature. This form of remodeling, caused by rearrangement of the same wall material around a smaller lumen size, is essentially characterized by an increase in media thickness and wall-to-lumen ratio, along with a reduction in lumen D and no change in the wall CSA^{214,228,237,239}. It has been argued that an increase in media thickness along with a reduced lumen D in essential and spontaneous forms of hypertension is an adaptive response to minimize the circumferential tension and stress exerted on the media in response to elevated BP. SMAs of CRD animals in the current study did not undergo any morphological alternations in response to episodes of extreme hypertension. Such an observation could possibly be explained by the pathophysiological differences that exist between AD and chronic forms hypertension. Essential and spontaneous hypertensions are multifactorial disorders that may arise from abnormalities in heart, blood vessels and kidneys; they are characterized by the presence of significant and persistent elevated BP^{271}. By contrast, AD is an episodic rise of BP triggered by sensory stimulation below the level of injury^{272}. Therefore, it is likely that factors such as duration and frequency of CRD, along with the timeframe of the experimental design, may play an important role in the degree of changes that could possibly be manifested in SMAs following recurrent episodes of AD. It is also worthy to note that structural differences in the number of SMCs layers and luminal D also exist between the SMA and smaller branches of mesentery. Therefore, it could be argued that the smaller branches of mesentery, such as PMAs, are more prone to changes imposed by abnormalities in BP, as they are more closely involved with BP regulation than larger resistance arteries. Interestingly, when the severity of CRD-evoked AD was examined by Alan et al (2010), it was shown that at one month post-injury, the induced hypertension by CRD was reduced by 50% in T3 SCI+CRD group. They concluded that
although AD response was still present, the severity of the response was lower compared to animals that were not repetitively exposed to such stimuli. Such observation could somewhat explain why SMAs from the CRD group failed to show any significant differences in the measured morphological parameters. Therefore, although AD is still present in the CRD animal group, the reduced severity, and perhaps magnitude, of induced hypertension are only sufficient to promote structural changes in smaller branches of the mesentery artery. Further, although PMAs from the CRD group showed changes in media thickness and wall-to-lumen ratio, as expected by the remodeling hypothesis, they failed to show any changes in D. The differences that exist between the findings of the current study and studies on hypertension could potentially be explained by three possibilities: 1) Lee and associates showed that the structural changes of small resistance arteries varies depending on the particular phase of hypertension development. In the current study, it is plausible that changes in media thickness and wall-to-lumen ratio are the first manifestation of structural remodeling in these smaller branches of the mesentery, before changes in D occur. Further studies are required to address whether increasing both duration and frequency of CRD may play a role in the extent of remodeling in resistance vessels following recurrent episodes of AD. 2) The majority of research in SCI has mostly focused on the functional changes of endothelial cells, vascular resistance (particularly in leg and arm conduit arteries), shear stress and changes in D of muscular arteries with respect to fluctuations in blood flow following an injury. The morphological changes of resistance arteries, such as in the mesentery, have not yet been greatly investigated after SCI and more particularly following AD. Interestingly, a recent study on remodeling of small arteries in type 1 diabetes mellitus have shown a similar pattern of remodeling to what has been reported in the present study (no change in D and CSA, along with an increase in media and wall-to-lumen ratio). Although the mechanisms of triggering elevated BP are different in diabetes and AD; the inability of smaller arteries to change D in response to elevated BP, as described by Greenstein
et al (2009), may lead to transmitting the increased pressure in the arteries into smaller arterioles, capillaries and eventually to organs. The question then becomes why the incidence of end organ damage is not as high following AD compared to patients with diabetes. This question could be partly answered by the pathophysiological differences in the elevated BP between the two conditions. Perhaps the effect of intermittent hypertension on smaller arteries is not as severe as that observed in the more persistent BP elevations in diabetic patients. As mentioned previously, in SCI studies the D measurement of femoral, brachial and carotid arteries have mostly been studied. The majority of these studies suggest relatively preserved D in the upper limb vasculature, e.g. brachial arteries and common carotid arteries\textsuperscript{145,155,156}. On the other hand, the D of the vessels in inactive lower limbs, e.g. femoral artery, seems to diminish following an injury, compared to AB individuals as reported by these studies\textsuperscript{48,144,269}. It is interesting to note that when the D of the femoral artery was corrected for the changes in muscle volume and metabolic demands of lower limb following an injury, the D of the artery showed no significant difference from those of the AB population\textsuperscript{82}. It must be kept in mind that correlating changes in D of the femoral artery with those of resistance arteries is a difficult task, as these two types of arteries are quite different in terms of structure and the roles they play in the circulatory system. Unlike resistance arteries, the muscular arteries such as femoral and brachial arteries do not contribute much to vascular resistance, instead exerting their effect on total arterial compliance and acting as a conduit to transport blood to the arms and legs. This becomes particularly important when the morphological outcome of intermittent elevated BP is being evaluated in these arteries after SCI. In the literature (to my knowledge) there is only one study accounting for the effect of episodic hypertension on the mesenteric artery after high thoracic injury; this study showed that the mesenteric arteries from T3 SCI Wistar rats in which recurrent AD was induced during the first two weeks after injury develop hypersensitivity to PE (\(a_1\)-adrenoceptor agonist) without any change in endothelial function\textsuperscript{176}. More studies are required to investigate the functional and
morphological changes of resistance vessels following AD in both acute and chronic phases of SCI. For this reason, most of the outcomes of the current study will be discussed in correlation with chronic forms of hypertension in which the structural changes of resistance arteries were studied in great detail. 3) The last possibility for the observed variation in D measurements of PMAs in the CRD group of the present study compared to those with essential and spontaneous hypertension could be related to the differences that exist in the methodological approaches used in morphological analysis of these studies.

4.1.2 Media Thickness and Wall-to-Lumen Ratio Changes Following CRD

Media thickness is a common parameter usually measured in morphological studies of vasculature. This parameter is under the direct influence of changes in BP. An increase in BP could trigger an increase in media thickness as described in chronic hypertension studies. Such a change in media thickness is often associated with an increased risk of development of CVD. In the present study, the media thickness of PMAs in CRD group showed a significant increase compared to control-uninjured animals, but it did not show any significant change with T3 SCI animals. In essential and spontaneous hypertension studies, an increase in media or intima-media thickness is often reported as a part of the remodeling of vasculature along with an increase in media-to-lumen ratio. It is likely that in the present study, the episodic rise in BP triggered the changes in media thickness of the CRD group. The possibility of the presence of spontaneous or “silent” episodes of AD could not be ruled out, from either of the two injured groups (with CRD or without CRD). However, the fact that T3 SCI group showed no significant change in media thickness compared to control-uninjured and T3 CRD, shows that perhaps the magnitude of induced “silent” episodes of AD (if it is happening) is not a significant stimulus in triggering changes in media thickness, compared to the CRD technique in which intracolonic pressure of 25-35 mmHg is created to induce AD. The intima-media thickness of large superficial arteries such as the carotid artery is often measured through high-resolution B-mode ultrasonography and

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is often recognized as a surrogate marker for development of atherosclerosis\textsuperscript{275}. In one study on a SCI population, it was shown that following an injury the carotid intima-media thickness is increased\textsuperscript{261}. However, in another recent study, it was reported that intima-media thickness of brachial and carotid arteries are similar between SCI and control, but when in the same study intima-media thickness of femoral artery was measured and corrected for the D of the artery, the SCI group showed higher intima-media thickness compared to the control\textsuperscript{276}. In SCI studies there are no reports (to my knowledge) on the changes in the thickness of resistance arteries. Whether the increase in media thickness observed in the current study is as a result of hyperplasia or hypertrophy of SMCs following SCI is not yet known; however, older studies on hypertension have shown that SMAs normally go through hypertrophic changes leading to an increase in media thickness, and smaller arteries and arterioles undergo hyperplasia of SMCs\textsuperscript{257,277}.

Wall-to-lumen ratio is regarded as one of the most important parameters associated with hypertension. In the present study, the wall-to-lumen ratio significantly increased in the CRD group, compared to control-uninjured and T3 SCI-only groups. The increase in wall-to-lumen ratio observed in the present study is in accordance with the reported ratio changes of resistance arteries in essential and spontaneous forms of hypertensions. In one follow-up study of 150 individuals over a period of 10 years with various forms of BP-related conditions e.g. EH, diabetes, hyperaldosteronism etc., it was shown that an increase in wall-to-lumen ratio is associated with an increased risk of cardiovascular dysfunctions\textsuperscript{278}. The increased wall-to-lumen ratio is often associated with growth, but such growth is not necessarily as a result of adding material to either side of the vessel wall. Sometimes, rearrangement of the wall material around the smaller luminal D could trigger an increase in the ratio without changing the media CSA (eutrophic remodeling). As proposed by Short (1966), small arteries could potentially experience structural changes in 3 possible ways: 1) no change in media, reduced lumen D and an increase
in wall-to-lumen ratio; 2) increase in media, no change in luminal D and increase in wall-to-lumen ratio, or 3) increase in media thickness, increase in luminal D, and reduction in wall-to-lumen ratio. The results of the current study support the second proposed mechanism of structural changes in PMAs of the CRD group\textsuperscript{230}. The increased media-to-lumen ratio is one of the most important contributing factors to an increase in total peripheral resistance; in turn, the increased ratio is dependent upon changes in both structural (e.g. remodeling and vascular inflammation) and functional (e.g. vascular tone and endothelial dysfunction) aspects of the arteries. This study only focused on the remodeling portion of this multifactorial interacting system. The majority of studies on SCI have reported an increase in the peripheral resistance of femoral arteries\textsuperscript{204,279}. One recent study has also shown that the wall-to-lumen ratio is higher in the superficial femoral artery in the SCI control group\textsuperscript{280}. Such a finding in the femoral arteries of SCI patients may partly describe the increased peripheral resistance reported by other studies. Studying the contribution of increased wall-to-lumen ratio in resistance arteries to changes observed in the vascular resistance of conduit arteries below the level of injury may provide some insight into how vascular remodeling at the level of resistance arteries may trigger changes in vascular structure and function of arteries below the level of the injury, following an injury and AD.

4.1.3 Wall Cross-Sectional Area Following CRD

In hypertension studies, CSA has been widely used to compare arteries from hypertensive and normotensive animals, mainly due to the fact that this parameter remains unchanged regardless of the state of the artery (contracted or relaxed). Hence, it becomes a reliable standard for comparing arterial morphological changes\textsuperscript{281}. The calculated CSA of PMAs in the current study did not show any significant changes when compared to control and T3 SCI-only groups. The absence of change in CSA is indicative of eutrophic remodeling (rearrangement of wall material around the lumen). Studies on essential and spontaneous
hypertension have also shown a similar form of remodeling in the resistance arteries\textsuperscript{228-230}. The mechanism underlying eutrophic remodeling is not well understood; however, Intengan and Schiffrin (2001) have proposed that eutrophic remodeling could essentially be triggered as a result of both apoptosis of the periphery of the vessel and media growth\textsuperscript{282}. In the present study, CSA was measured only to confirm whether eutrophic remodeling is happening in smaller branches of mesentery; the mechanism triggering such a change was not evaluated.

4.1.4 Rationale for Animal Model & CRD

In this experiment, the morphological characteristics of resistance arteries in response to CRD were investigated in weeks 2-4 following high thoracic injury; therefore, a fitting animal model was required in which AD could be elicited during the chronic phase of SCI. Previous studies have shown that evoked AD in response to CRD is greater in lesioned Wistar rats when compared to Spargue-Dawley rats; based on these findings, Wistar rats were used as a chronic animal model in this study. Krassioukov and Weaver (1995) investigated the AD response at 24 hrs, 7 days, and 30 days following midthoracic (T5) injury in Wistar rats. At 24 hrs following the injury, the arterial pressure in response to CRD approximately increased by 40mmHg; however, BP response to CRD was depressed significantly on day 7 (~20mmHg lower). On day 30, the pressor response to CRD returned with a greater magnitude (~50mmHg higher)\textsuperscript{69}. They concluded that different mechanisms are responsible for triggering such variable responses to colon distension following SCI. The loss of the descending inhibitory pathway could explain the increased pressor response at 24 hrs following SCI\textsuperscript{69}. However, the return of exaggerated AD on day 30, as described by Krassioukov and Weaver (1995), could be due to the establishment of a normal dendritic arbor of preganglionic neurons; such changes, along with the formation of new synapses by local interneurons and dorsal root afferent fibres, contribute greatly to the enhanced AD response on day 30. Unfortunately, studies on pressor-response to CRD in Sprague-Dawley rats have been mostly focused on the magnitude of the response within the first week after the
injury; therefore, no chronic model of this animal model has been tested for AD response to CRD. In one study, it was shown that the pressor-response to CRD in lesioned Sprague-Dawley rats was lower than the control-uninjured group\textsuperscript{283}.

As briefly mentioned above in this experimental design three animal groups were utilized. The question may possibly arise of why in this particular study an intact+CRD group was not included as a control. To justify the answer, a previous study by Krassioukov and associates has shown that the pressor-response to CRD only increases between 5-13mmHg in control-uninjured Wistar rats\textsuperscript{69}; under clinical conditions an upsurge of 20-40mmHg in pressor-response is considered an AD response. Therefore, due to the minute response to CRD in animals with no SCI, uninjured Wistar rats with no colon distension were employed as one of the control groups. Further, in this experiment, BP was not monitored daily during CRD. Nevertheless, based on the outcomes of the previous studies, in which it was demonstrated that AD evoked by CRD is pronounced and accompanied by both hypertension and bradycardia for as long as the distended catheter remained inside the descending colon, there is very high confidence that AD was profoundly induced during daily bouts of CRD in this experimental design\textsuperscript{69,284,285}. The effect of recurrent episodes of AD on baseline BP was not monitored in the present study, because a recent local laboratory study showed that the resting BP and HR at 1 month following T3 complete transection is not significantly different between T3 SCI-only and T3 SCI+CRD animal groups\textsuperscript{176}.

Clinical AD could be induced by any noxious or non-noxious stimulus below the level of the injury. Under clinical settings, bladder distension has been reported as a major irritating factor for the development of episodic hypertension, to a greater extent than colon distension\textsuperscript{286}. Experimentally, it has been shown that both CRD and bladder distension could be used as clinically relevant and feasible techniques for induction of AD\textsuperscript{69,287}, due to the well-characterized CRD technique in chronic animal model of AD\textsuperscript{69,288}, and the non-invasive nature of this
method. In this experiment, CRD was used as a suitable means to elicit episodic hypertension following high thoracic injury, and subsequently employed to investigate the effect of recurrent AD on vascular morphology. CRD mimics a full bowel sensation and resembles some of the most common factors that initiate AD clinically, such as constipation and colon impaction.

4.1.5 Limitations

In studying the structural and functional characteristics of small arteries, the majority of hypertension studies use an ex vivo technique known as pressurized myography. This technique is specifically more advantageous than the conventional histological assessments of the arteries, as it eliminates the effect of artefacts introduced to specimen following the fixation procedure. In pressurized myography, isolated arteries are cannulated at both ends and are attached to micropipettes connected to intraluminal pressure transducers, which control for pressure. Using optic microscopy, the images of the arteries are recorded by camera and transferred to a video monitor, which is subsequently used for analysis. The luminal size of the artery is very sensitive to the methods used for preparation and measurement of the sample; in fact, it is possible that the differences existing between the measured Ds in the present study and those in essential and spontaneous hypertension studies is partly due to the differences in histological and pressurized myography techniques. Lee et al (1983) showed that histometric analysis generally underestimates the lumen size in contracted vessels. This becomes especially important in the chronic hypertension studies, as the vessels persistently remain contracted. One thing that needs to be kept in mind is that after high-level SCI, vasoconstriction of vasculature below the lesion site occurs in response to sensory stimulation below the level of the injury (AD induction). Further studies are required to determine the magnitude of difference that may exist in analyzing the D of vasculature following SCI using pressurized myography v.s. histological analysis. Also,
for complete validation of the outcomes of the current study, future work using pressurized myography is recommended.

Regardless of the vessels state (contracted or relaxed) CSA remains unchanged as reported in the study done by Lee et al (1983). This study showed that when vessels are contracted using NE or relaxed using high transmural pressure, CSA did not change. Therefore, CSA could be used as a standard parameter for evaluating remodeling in the vasculature. In the present study, CSA of the wall remained unchanged, which is an indicative of eutrophic remodeling in PMAs of CRD group. Eutrophic remodeling of the resistance vessels is associated with an increase in wall-to-lumen ratio, generally identified as the most important contributing factor to development of cardiovascular dysfunction\textsuperscript{237,291,292}.

4.1.6 Future Directions

The magnitude of vascular remodeling after SCI and more importantly AD is not well understood. The concept of vascular remodeling after SCI demands not only an improved understanding of the extent of morphological changes in large resistance arteries, but also in smaller branches such as 2\textsuperscript{nd} order and 3\textsuperscript{rd} order. To gain a better understanding of the level of structural changes in these arteries, both histological and \textit{ex vivo} studies are required to be conducted in parallel. Understanding the cellular scale organization and behavior of the individual cells in the vessel wall in response to AD will help to provide a better insight on how episodic rise in BP triggers structural changes in the arteries and subsequently effect the hemodynamic parameters such as peripheral resistance and shear rate. In this context, organization of SMCs within the vessel wall is critically important, as the plasticity of these cells play an important role in controlling vascular D both short- and long term. Aside from this, a better understanding of the effect of AD on the micro-architecture of the vessels such as collagen, elastin and collagen-to-elastin ratio is required. Integrating such knowledge with cell-
matrix and cell-cell adhesion mechanisms will give a better insight on the progression of vascular remodeling from the outset in response to AD following high level SCI.

4.1.7 Conclusive Remarks

The risk of developing CVD increases in AB individuals with unstable BP\textsuperscript{293}; similarly, in the SCI population, the cardiovascular outcomes of disordered BP are vigorous and predispose the SCI patients to development of various forms of heart diseases and stroke\textsuperscript{294}. This could become even more important in AD, which is a life-threatening condition unique to high SCI that has been characterized with episodes of extreme hypertension accompanied (usually but not always) with bradycardia\textsuperscript{295}. Since CVD have been recognized as a leading cause of mortality among chronic SCI patients\textsuperscript{296}, understanding the magnitude of the effects of labile hypertension on vascular structure becomes widely important as such changes are normally associated with an increased risk of development of arterial diseases in this population\textsuperscript{297}.

Resistance arteries play a critical role in BP regulation as they serve as a key site at which, the main drop in hydrostatic pressure occurs. Small arteries and arterioles contribute to $\sim$50\% of total peripheral resistance in the body; therefore, structural changes at the level of resistance arteries could strongly influence BP regulation and subsequently the peripheral resistance\textsuperscript{92}. As mentioned earlier, the splanchnic region is one the major blood reservoirs of the circulatory system\textsuperscript{42}. The superior mesenteric artery along with its smaller branches represents one of the major resistance arteries in the splanchnic region. This region receives major sympathetic outflow from anywhere between (T5-L2) segments in spinal cord. After SCI at T6$\geq$, a widespread vasoconstriction occurs mainly in the splanchnic vasculature through an unopposed massive sympathetic outflow initiating from the thoracolumbar region of the spinal cord in response to any noxious or non-noxious stimulus below the level of the injury. Such a mechanism is the most important triggering factor in the development of AD following high-level SCI.
Structural remodeling of the microvasculature associated with chronic forms of hypertension has been widely studied\textsuperscript{298,299}. Vascular remodeling associated with chronic oscillation of BP has been associated with an increase in WT and a reduction in luminal D. Such changes could significantly affect hemodynamic parameters such as vascular resistance, blood flow and BP regulation in the body. In fact, increased peripheral resistance is the hallmark of any forms of hypertension. The long-term impact of episodic rise in BP on the structural abnormalities of microvessels following SCI is not well understood. For this reason the main goal of this study was to investigate the structural remodeling of resistance arteries in response to recurrent episodes of AD following SCI. The outcomes of the current study suggest that smaller branches of mesentery do undergo morphological changes in response to episodic hypertension. The contribution of the observed morphological changes at the level of resistance arteries to development of AD is not yet known. Whether remodeling of the resistance arteries precede the development of AD, or the AD is the triggering factor in initiation of vascular remodeling remains to be established.
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