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Orthostatic Blood Pressure and Arterial Stiffness in Persons with Spinal Cord Injury: The Effect of the Renin Angiotensin Aldosterone System

Caitlyn G. Katzelnick
caitlyn.katzelnick@student.shu.edu

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By

Caitlyn G. Katzelnick

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Abstract

With advances in acute medical care, longevity has increased in persons with SCI; however, morbidity due to cardiovascular disease (CVD) occurs at an earlier age compared to the general population. Arterial stiffness (AS) is recognized as an independent risk factor for CVD and, specifically, pulse wave velocity (PWV) has been proven to be a valid tool to predict and track structural arterial changes that reflect arteriosclerosis. Evidence has shown that persons with SCI have increased AS compared to uninjured able-bodied controls; however, possible contributors to this increase are not yet fully understood. After a SCI, sympathetic control in the regions below the lesion level are severely disrupted; however, parasympathetic function is preserved. Due to the dissociation between the two systems, those with lesions above T6 experience low resting blood pressure (BP) and further decreases in BP when changing postures (orthostatic hypotension (OH)). As a consequence, individuals with high-level injuries have a heightened reliance on the renin-angiotensin-aldosterone system (RAAS) to maintain and stabilize BP. A mechanism for increased AS in the uninjured population is over activation of the RAAS. In this study, individuals with high-level SCI (injured above T1) and low-level SCI (injured between T6-T12) had increased AS compared to age-matched controls. The change in renin from supine rest to 60-degree head-up tilt (HUT) was a significant predictor to the change in systolic BP; however, the group predictor was not significant. Additionally, group and change in renin were significant predictors to AS. The data indicate that individuals with high-level injuries rely heavily on RAAS to maintain and normalize BP during OH, which is correlated to increased AS. A better way to treat asymptomatic OH in the SCI population is needed to decrease CVD.

Keywords: spinal cord injury, orthostatic hypotension, renin-angiotensin-aldosterone system, cardiovascular disease, arterial stiffness

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Chapter I

INTRODUCTION

Approximately 17,000 new spinal cord injuries (SCI) occur each year in the United States (National Spinal Cord Injury Statistical Center). The spinal cord is a critical component to send sensory information to the brain and for regulation of motor and autonomic functions (Kirshblum & Campagnolo, 2011, p. 6). The loss of the supraspinal control leads to reduced control of overall sympathetic activity below the level of lesion (LOI) and unopposed parasympathetic outflow through the intact vagal nerve. Disruption of the autonomic nervous system (ANS) leads to altered cardiovascular function including heart rate (HR), blood pressure (BP) and vasomotor tone (Kirshblum & Campagnolo, 2011, p. 136). Due to segmental differences in sympathetic innervation, cardiovascular dysfunction is directly related to the specific level and severity of the SCI, including resting hypotension (low BP) and large fluctuations in BP (Kirshblum & Campagnolo, 2011, p. 136; Krassioukov, Warburton, Teasell, Eng, & Team, 2009; Lee, Phillips, & Krassioukov, 2017). Early recognition and management of cardiovascular dysfunction is important in the SCI population.

Cardiovascular disease (CVD) has emerged as the leading cause of mortality in individuals with SCI (Cragg, Noonan, Krassioukov, & Borisoff, 2013; Hagen, Lie, Rekand, Gilhus, &

Gronning, 2010). In fact, it has been speculated that premature cardiovascular aging in persons with SCI may be due to chronic inflammation (Wang et al., 2007), sedentary lifestyle (Stoner, Credeur, Dolbow, & Gater, 2015), and dysfunction in cardiovascular autonomic control (Myers, Lee, & Kiratli, 2007; West, Mills, & Krassioukov, 2012). Matos–Souza and colleagues (2009) demonstrated that individuals with SCI had higher common carotid artery (CCA) intima-media thickness (measure of vascular stiffness) than able-bodied controls even after controlling for traditional cardiovascular risk factors (Matos-Souza et al., 2009). Thus, other non-traditional risk factors may play a role in increased atherosclerosis (hardening of arterial walls).

Individuals with injuries above T6 are more prone to experience orthostatic hypotension (OH) which is a decrease in BP within 3-minutes of moving from supine to the upright position, with or without symptoms (Neurology, 1996). Evidence has suggested that there is an association between markedly reduced plasma norepinephrine (NE) and orthostatic hypotension (OH) (Claydon, Steeves, & Krassioukov, 2006; Wecht & Bauman, 2018; Wecht et al., 2008). Decreased NE reflects impaired sympathetically mediated vasoconstriction and inability to stimulate the adrenal medulla (Claydon et al., 2006). However, these individuals have reduced symptoms associated with pre-syncope which is believed to be due to increased dependency on the renin-angiotensin-aldosterone system (RAAS), which has been shown to occur independently of the SNS p. 142 (Kirshblum & Campagnolo, 2011; Wecht & Bauman, 2018). Individuals with a diminished plasma NE response to OH may demonstrate increased reliance on the RAAS for BP maintenance.

A possible link between increased RAAS and arterial stiffness (AS) may explain the increased incidence of CVD in the SCI population (Katzelnick et al., 2017; Wecht & Bauman, 2018). In the general population, inappropriate chronic activation of RAAS activates signal

transduction pathways that promote cell growth, inflammation and fibrosis (Duprez, 2006). Thus, individuals with high cord lesions may be more susceptible to increased AS; however, the association between RAAS and AS in the SCI population has not been established. Increased AS has been reported in high-level SCI (C3-T5) compared to low-level SCI (T7-T12) (Katzelnick 2017); while other prior work has documented increased AS in low-level SCI (T1-T12) compared to high-level SCI (C2-C8) (Miyatani et al., 2014). Further investigation is needed to ascertain the relationship between lesion level and AS.

Non-invasive testing modalities have been developed to more easily and accurately screen AS and track atherosclerosis (Stoner et al., 2015). Specifically, pulse wave velocity (PWV) measured at the carotid and femoral arteries is considered the gold standard measurement of AS (Stoner et al., 2015; Van Bortel et al., 2011) because of its high reliability (Currie, Hubli, & Krassioukov, 2014; Miyatani et al., 2012) and validity to track CVD (Van Bortel et al., 2011; Vlachopoulos et al., 2010). PWV is calculated by measuring the time taken for the arterial waveform to pass between two points (carotid and femoral) of a measured distance apart (Stoner et al., 2015; Van Bortel et al., 2011).

The primary purpose of this research study will be to investigate the influence of orthostatic change of BP and NE on the RAAS responses to orthostasis. Our first hypothesis is there will be a linear relationship between the change in orthostatic BP and change in plasma NE concentration from supine rest to 60º HUT. Second hypothesis is plasma renin activity will be inversely related to plasma NE concentrations during HUT in persons with SCI. Additionally, our secondary aim will be to determine if increased reliance on the RAAS for orthostatic BP regulation contributes to differences in supine AS in individuals with SCI. Our hypotheses are the change of renin from supine rest to orthostatic tilt will be associated with supine PWV in

high-level SCI and there will be no relationship among the change of renin from the supine rest to orthostatic tilt with supine PWV in low-level SCI and controls.

Chapter II

LITERATURE REVIEW

A traumatic spinal cord injury (SCI) is a sudden devastating condition that affects a person's everyday activities and it has been estimated that there are approximately 17,730 new cases of SCI each year in the United States (White & Black, 2016). An injury to the spine interrupts neural transmission from cortical centers to destinations below the level of injury (LOI), which may adversely impact regulation of the cardiovascular system by the autonomic nervous system (ANS), which may be dependent on the LOI and the degree of motor and sensory dysfunction (Garstang & Miller-Smith, 2007; Kirshblum & Campagnolo, 2011; West, Mills, & Krassioukov, 2012). Improvements in acute and sub-acute care post injury have resulted in increased life expectancy for individuals with SCI, contributing to increased risk of age-associated disorders including cardiovascular disease (CVD), which is a leading cause of mortality and morbidity in individuals with chronic SCI (Myers, Lee & Kiratli, 2007). Arterial stiffness (AS) is one of the earliest detectable indicators of changes in vascular remodeling which may be a predictor of CVD progression (Balta et al., 2014; Cavalcante, Lima, Redheuil, & Al-Mallah, 2011; Stéphane Laurent & Boutouyrie, 2007; Vlachopoulos, Aznaouridis, & Stefanadis, 2010). Understanding the underpinnings of CVD in this population by measuring AS can help

clinicians more effectively screen and detect individuals with SCI at increased risk thereby preserving cardiovascular health and longevity.

An intact spinal cord is responsible for transmitting neuronal signals from the periphery to the brain and back to properly regulate sensory, motor and autonomic function (Nógrádi & Vrbová, 2006). This multifaceted network of neurons is critically dependent on connection to the brain and cannot function appropriately under circumstances of partial or complete cortical disconnection (Nógrádi & Vrbová, 2006). The nervous system is divided into central and peripheral nervous systems. The central nervous system (CNS) includes the brain and spinal cord (Robertson, Low, & Polinsky, 2011). The peripheral nervous system is comprised of the somatic, enteric, and ANS, each controlling specific aspects of organ function. The somatic nervous system regulates voluntary movement in the skeletal muscles and responds to sensory feedback from afferent peripheral nerve transmission (Brodal, 2004). The enteric nervous system controls function of the gastrointestinal tract (esophagus, stomach and intestines) and is thought to coordinate and direct the functions of the digestive system (Furness, 2006).

The ANS has two branches, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), which regulate the cardiovascular system controlling hemodyanmic function including blood pressure (BP), heart rate (HR) (Kirshblum & Campagnolo, 2011), coronary blood flow and peripheral vasomotor tone (Garstang & Miller-Smith, 2007; West et al., 2012) on a beat-by-beat basis. The ANS regulates these functions in response to afferent feedback from the periphery by altering neural efferent transmission to alter cardiac chronotropy (HR) and inotropy (myocardial contractility) as well as peripheral vasomotor tone (Garstang & Miller-Smith, 2007). While both the SNS and PNS are actively involved in cardiovascular regulation, the PNS is primarily involved in modulating cardiac

chronotropy through direct innervation of the sinoatrial node (SA-node), whereas the SNS controls cardiac rate and contractility as well as vascular tone (Kirshblum & Campagnolo, 2011).

SNS control originates in the rostroventrolateral medulla (RVLM) and modulates the cardiovascular system through sympathetic preganglionic neurons, which stem from the 1st thoracic vertebra (T1) to the 2nd lumbar vertebra (L2) (Kirshblum & Campagnolo, 2011). Pre-ganglionic sympathetic nerve fibers travel on to the postganglionic neurons which directly innervate target organs resulting in an increase in peripheral SNS activity (Phillips & Krassioukov, 2015). Origin of SNS innervation to the heart and the upper extremity vasculature is from T1-T4, whereas, SNS innervation to the blood vessels of the splanchnic bed and lower extremity originates at spinal segments T6-T12 (Kirshblum & Campagnolo, 2011). Control over the blood volume in the vascular bed regulate stroke volume and cardiac output, which controls arterial BP (Kirshblum & Campagnolo, 2011).Thus, the LOI may play a direct role in altering SNS cardiovascular regulation. On the contrary, origin of PNS cardiovascular innervation is through the vagus nerve and cranial nerve X which exits the brain stem above the cervical spine. Therefore, SCI does not anatomically alter PNS cardiac innervation although functional impairment may be evident (Krassioukov et al., 2012).

The spinal cord receives sensory information from somatic and visceral receptors through afferent nerves and transmits this information to supraspinal regulatory centers through ascending tracts. The brain structures then transmit these signals to muscle and organ targets sites through the efferent nerves (Campagnolo, Kirshblum, Nash, Heary, & Gorman, 2011). SCI affects the somatic nervous system because the conduction of sensory and motor signals cannot transmit across the site of lesion appropriately (Kirshblum et al., 2011); thus, it is important to classify these individuals into a sensory and motor level by assessing dermatome, area of skin

innervated by sensory axons, and myotomes, muscle fibers innervated by the motor axons (Kirshblum et al., 2011). The American Spinal Injury Association (ASIA) provides a more precise approach to grade the degree of motor and sensory impairment (Kirshblum et al., 2011). Complete motor and sensory injury are classified as ASIA impairment scale (AIS) A and is defined as having no sensory or motor function below the neurological LOI. AIS B, C and D are considered incomplete injuries, which have some partial preservation of sensory (AIS B) and/or motor function (AIS C & D) below the lesion level (Kirshblum et al., 2011). Physiologic changes are directly related to the level and completeness of motor and sensory injury with greater impairments in individuals with higher level and more complete lesions (Kirshblum & Campagnolo, 2011); however, the level of ANS impairment may not relate to AISA classification of motor and sensory impairment (Krassioukov et al., 2012).

After a SCI, descending sympathetic preganglionlic neurons are disrupted causing loss of supraspinal regulation of cardiovascular function (Kirshblum & Campagnolo, 2011; Phillips, Krassioukov, Ainslie, & Warburton, 2012), resulting in a reduction or absence of autonomic cardiovascular control that may be directly related to the LOI and severity of trauma to the cord (Garstang & Miller-Smith, 2007; S. Kirshblum & Campagnolo, 2011; West et al., 2012). After a high-level injury (T1 and above), there is interruption of the descending signals from the medulla to the thoracic spinal cord thereby altering SNS, but preserving PNS cardiovascular control (Garstang & Miller-Smith, 2007; Krassioukov, Warburton, Teasell, Eng, & Team, 2009; Partida, Mironets, Hou, & Tom, 2016; Phillips & Krassioukov, 2015; West, Alyahya, Laher, & Krassioukov, 2013), which results in resting hypotension, orthostatic hypotension (OH) and episodes of extreme high and low BP (Kirshblum & Campagnolo, 2011; Krassioukov et al., 2009; Krassioukov, Karlsson, Wecht, & Wuermser, 2007; Phillips & Krassioukov, 2015; Wecht

& Bauman, 2013; West et al., 2013). Another name for individuals with high cord injuries is tetraplegic, which refers to an injury to the cervical spine and results in motor, sensory and ANS impairment of the upper and lower extremity, the truck and pelvic organs (Kirshblum et al., 2011). Although there is interruption between the sympatho-excitatory pathways from the brainstem to the sympathetic preganglionic neurons, hypotensive individuals with tetraplegia remain clinically asymptomatic (no symptoms of dizziness, blurred vision, light-headedness, etc.) (Handrakis et al., 2009; Wecht et al., 2011; Wecht, Rosado-Rivera, Handrakis, Radulovic, & Bauman, 2010). Support of the renin-angiotensin-aldosterone system (RAAS) is believed to be upregulated and adequately compensates for the lack of SNS vascular control in this population (Handrakis et al., 2009; Popa et al., 2010; Wecht & Bauman, 2018; Wecht et al., 2011; Wecht et al., 2010).

The RAAS plays an essential role in maintaining long-term BP and has been detected in tissues such as heart, kidney, peripheral vasculature and the brain (Aroor et al., 2014; DeMello & Frohlich, 2009). In uninjured controls, when the baroreceptors sense a decrease in BP and blood volume, the kidneys release renin, primarily by the juxtaglomerular cells, to activate the RAAS (DeMello & Frohlich, 2009). This system is an enzymatic cascade in which renin is released into the blood and acts on angiotensinogen to form angiotensin I, which forms angiotensin II (ANG II) by the angiotensin converting enzyme (ACE) (Bandeira, Gharib, Golbert, Griz, & Faria, 2013; DeMello & Frohlich, 2009; Gordan, Gwathmey, & Xie, 2015). ANG II is a potent vasoconstrictor primarily by increasing BP and secondarily by increasing retention of sodium and water and by stimulating aldosterone release from adrenal glands (DeMello & Frohlich, 2009). Recent evidence has suggested that ANG II not only plays a role in cardiovascular

physiology, but also may contribute to cardiovascular pathophysiology (Husain, Hernandez, Ansari, & Ferder, 2015; Mehta & Griendling, 2007; Zhong et al., 2010).

Despite the important benefits of the RAAS in regulating BP homeostasis, there is recent evidence that chronic overproduction of ANG II may result in remodeling and restructuring of peripheral and coronary blood vessels, heart and the kidneys by augmenting the generation of reactive oxygen species, a main molecule responsible for inflammation (Duprez, 2006; Weir & Rolfe, 2010). ANG II has been shown to create oxidative stress in vessel walls, and enhance production of inflammation, thrombosis and fibrosis specifically on vascular smooth muscle cells and endothelial cells (Duprez, 2006). Additionally, ANG II is a major contributor in profibrotic mechanisms including increasing the production of matrix metalloproteinases, which modulate collagen and elastin turnover in the vascular wall (Duprez, 2006). All of these adverse effects may result in chronic inflammation of vessel walls, which is a major facet of atherosclerosis (El Bekay et al., 2003). Recent evidence has demonstrated that the use of ACE inhibitors or ANG II receptor blockers (ARBs) reduce vascular remodeling (Mahmud & Feely, 2000; Shahin, Khan, & Chetter, 2012; Yusuf et al., 2000), and moreover, the use of ARBs have been shown to decrease vasculature stiffness independent of BP (Karalliedde et al., 2008; Azra Mahmud & Feely, 2004; Shahin et al., 2012). Using ARBs has been shown to reduce collagen production and increase the elastin to collagen ratio (Shahin et al., 2012). A meta-analysis demonstrated that the use of ARBs and ACE inhibitors reduced the risk of myocardial infarction, stroke, cardiovascular mortality and total mortality in uninjured subjects (Reboldi et al., 2008), suggesting that the excessive ANG II production may lead to an increased risk of CVD.

CVD has developed as a leading cause of mortality in individuals with chronic SCI and; furthermore, morbidity due to CVD occurs at an earlier age in the SCI compared to the uninjured

population (Jonathan Myers, Matthew Lee, & Jenny Kiratli, 2007). In the general population, CVD risk is attributed to age, hypertension, physical (in)activity level, body composition and dyslipidemia (Cragg, Noonan, Krassioukov, & Borisoff, 2013; Sowers, Epstein, & Frohlich, 2001); however, there is evidence to suggest that these risk factors do not fully explain the increased incidence of CVD in the SCI population (Cragg et al., 2013). A possible, previously not considered, risk factor for this population could be the overuse of ANG II, since individuals with SCI have a heightened reliance on the RAAS to maintain BP homeostasis (Mathias, Christensen, Frankel, & Peart, 1980; Mathias, Frankel, Davies, James, & Peart, 1981; Wecht et al., 2005). Renin was shown to be higher during resting conditions in individuals with SCI compared to uninjured controls, indicating that upregulation of the RAAS is not dependent on the integrity of the SNS (Johnson, Park, & Frankel, 1971; Mathias et al., 1975; Mathias et al., 1980; Mathias et al., 1981). Goothius and colleagues (2010) demonstrated an increase in ANG II during baseline in SCI compared to uninjured controls, however, ANG II levels were not excessively high (Groothuis et al., 2010). Additionally, their results showed that ANG II explained increased leg vascular resistance, but not forearm resistance, which may be because AT_1 receptors have increased sensitivity or density in the legs compared to the arms (Groothuis et al., 2010). This concept was further studied in rats with a chronic spinal transection at T11 demonstrating increased reactivity of vascular muscle to ANG II (Al Dera & Brock, 2014). Due to dysfunction of supraspinal SNS regulation to the peripheral vasculature, the RAAS may play a greater role in regulating vascular resistance (Al Dera & Brock, 2014) for maintenance of BP homeostasis.

The ANS is responsible for postural homeostasis; therefore, with impairment to the system, BP will fall after moving to an upright position (Fedorowski et al., 2009). According to a

Consensus statement from the American Autonomic Society and American Academy of Neurology, OH is defined as a decrease in systolic BP (SBP) of 20+ mmHg and/or a fall in diastolic BP (DBP) of 10+ mmHg within 3-minutes of moving from supine to the upright position, with or without symptoms (Neurology, 1996). In an uninjured person, movement to the upright position reduces BP due to pooling of blood in the lower extremities. This is sensed by the baroreceptors which elicit withdrawal of tonic vagal (PNS) tone to the SA-node and activation of SNS vasomotor tone via descending spinal tracts, causing vasoconstriction and increase in cardiac rate and contractility resulting in a rise in BP (Benvenuto & Krakoff, 2011; Claydon, Steeves, & Krassioukov, 2006). In the uninjured population this quick feedback loop results in non-significant changes in SBP, slight increases in DBP and a corresponding increase in HR during an orthostatic challenge (Freeman et al., 2011).

After a SCI, the SNS arm of the baroreceptor reflex, is interrupted or lost, resulting in alteration in orthostatic BP control (Claydon & Krassioukov, 2008; Phillips, Cote, Bredin, Krassioukov, & Warburton, 2012). Generally, lesions above T6 experience orthostatic instability due to SNS dysfunction, which leads to the inability to adequately adjust vasomotor tone and maintain BP (Claydon et al., 2006). Individuals with injuries above T6 would be expected to have some degree of impaired vascular innervation to the splanchnic bed and the lower extremities, which holds a majority of blood volume (Garstang & Miller-Smith, 2007; Sahota, Ravensbergen, McGrath, & Claydon, 2012). Additionally, the lack of skeletal muscle pump may contribute to increased blood pooling in the lower extremities venous vasculature resulting in lower venous return thereby restricting stroke volume (Claydon et al., 2006; Krassioukov & Claydon, 2006). With higher LOI already demonstrating low resting BP (West et al., 2012), a further decline in BP during orthostatic re-positioning can result in symptoms of cerebral

hypoperfusion including: dizziness, weakness, light-headedness, blurred vision, pre-syncope and syncope, which can adversely impact the activities of daily living (Claydon et al., 2006; Garstang & Miller-Smith, 2007; Phillips, Krassioukov, Ainslie, Cote, & Warburton, 2014).

Another facet of the underlying pathophysiology of OH following SCI is decreased resting catecholamine levels. The SNS regulates the response of releasing circulating hormones from the adrenal medulla (S. Kirshblum & Campagnolo, 2011, p. 138). Measuring free plasma catecholamines correlates with SNS integrity due to their short half-life (Claydon, Hol, Eng, & Krassioukov, 2006; Kirshblum & Campagnolo, 2011). Orthostatic BP responses are related to circulating levels of norepinephrine (NE), and lower levels of NE are associated with an increased incidence of OH in persons with SCI (Claydon & Krassioukov, 2008). Relevant literature has shown decreased plasma epinephrine and NE concentration in individuals with cervical lesions compared to individuals with thoracic injuries and uninjured controls (Krassioukov et al., 2009; Mathias et al., 1975; Schmid, Huonker, Barturen, et al., 1998; Schmid, Huonker, Stahl, et al., 1998). Specifically, after 10 minutes of an orthostatic challenge, NE increased 14% in individuals with high cord lesions, compared to 115% increase in uninjured controls (Mathias et al., 1975). Additionally, the investigators reported that epinephrine was unaffected by the change in posture in individuals with cervical lesions (Mathias et al., 1975). The small change in catecholamines after orthostatic repositioning in persons with tetraplegia is due to impaired supraspinal control of the sympathetic efferent pathways (Mathias et al., 1980) and consequently, results in the inability to increase vasomotor tone and maintain BP (V. Claydon et al., 2006)

With a greater fall in BP during OH in individuals with tetraplegia, the use of the RAAS is critically important to maintain BP; however, the pathological effects of ANG II may supplant

the benefits. Renin was shown to increase in individuals with tetraplegia during head-up tilt (HUT), an orthostatic challenge, at a much quicker and higher rise compared to uninjured controls (Mathias et al., 1975; Mathias et al., 1981; Popa et al., 2010); conversely, one study demonstrated that renin was not increased with HUT in individuals with tetraplegia, which the investigator explained may be due to large inter-subject variability (5 of the 7 subjects were motor and sensory incomplete) (Wecht et al., 2005). In individuals with cervical injuries, the use of anti-hypotensive agents (midodrine and L-NAME) to normalize BP may reduce orthostatic dependency on the RAAS because plasma renin and aldosterone levels after HUT were comparable with supine levels during HUT and there was a significant inverse association between mean arterial pressure and renin responses to HUT (Wecht et al., 2011). This finding may have significant clinical implication because the level of dependency on the RAAS may be reduced if BP is maintained during an orthostatic challenge (Katzelnick et al., 2017; Jill M Wecht et al., 2011). Additionally, Groothuis et al. (2010) demonstrated that ANG II contributed to increased supine leg vascular resistance in individuals with thoracic injuries (T4-T12) but did not explain the increased vascular tone during 30°of HUT. This disparate finding may relate to an insufficient orthostatic stress (30°) since higher degrees of HUT (45 and 60 degrees) have been shown to place greater strain on SNS (Phillips et al., 2014; Steinback et al., 2005; Jill M Wecht et al., 2009). Additional evidence is needed to demonstrate if renin and ANG II levels are increased during an orthostatic provocation in persons with SCI and if so, how this might contribute to increased vascular resistance and AS.

AS has been identified as an independent risk factor for CVD (Cavalcante et al., 2011; Azra Mahmud & Feely, 2004; Shahin et al., 2012) and is one of the earliest detectable indicators of adverse vascular changes (Cavalcante et al., 2011). The primary function of the arterial system is

to deliver blood from the heart to the peripheral tissues and to dampen intermittent left ventricle ejection pressure thereby maintaining a steady flow of nutrient rich blood to vital organs (London & Pannier, 2010). The ability of the arteries to accommodate changes in blood volume during the cardiac cycle depends on the ratio of elastic to collagen fibers (Oliver & Webb, 2003). In a more compliant arterial system, reflected waveforms return to the aorta from the periphery during diastole, increasing DBP and augmenting coronary artery blood flow, which translates into reduced AS. Conversely, in a relatively stiffer arterial system, due to increased collagen and lumen diameter thickness (Duprez, 2006; Safar, Blacher, & Jankowski, 2011), reflective waveforms return to the aorta during systole, thereby augmenting ventricular work, limiting coronary artery perfusion and increasing AS (London & Pannier, 2010; Oliver & Webb, 2003).

In the general population, AS is influenced by two principal factors: age and BP (Cecelja & Chowienczyk, 2009; Hansen, 2010; London & Pannier, 2010). Both elements cause elastic fibers to spilt, fracture and become fragmented after repetitive stress cycles, and cause elastin fibers to degenerate while collagen fibers increase. Stiffening of the vascular walls with aging is associated with increased hemodynamic load, hormonal changes, glycemic diet and overall decline in the structure and function of the vascular system (Shirwany & Zou, 2010). Augmentation of AS with age occurs gradually and this trajectory is similar in males and females (Benetos et al., 2002). A stiffening vasculature works to increase SBP by amplifying the initial pressure wave generated during left ventricular ejection, accelerating return of the reflected wave from the periphery during systole and, reducing pressure during diastole (London & Pannier, 2010; Shirwany & Zou, 2010). This adversely impacts cardiac function by increasing myocardial oxygen demand while hindering ventricular ejection and comprising coronary perfusion (Benetos et al., 2002; London & Pannier, 2010).

Vascular remodeling is largely accomplished within 3 weeks following SCI (C4-L1) and these rapid changes include a 25% reduction in femoral artery diameter and lower extremity blood volume, and doubling of shear stress rate (de Groot, Bleeker, van Kuppevelt, van der Woude, & Hopman, 2006). Additionally, Thijseen and colleagues (2012) observed gradual increases in arterial wall thickness within 24 weeks after injury which was present and similar below (femoral artery) and above (carotid artery) the spinal lesion, demonstrating that arterial wall thickness is systemic and not localized (Thijssen et al., 2012). Increased AS has been documented in individuals with SCI compared to age-matched uninjured controls (Huang, May-Kuen, et al., 2013; Katzelnick et al., 2017; Miyatani et al., 2009; Phillips et al., 2012; Thijssen et al., 2012; Wecht, Weir, DeMeersman, Spungen, & Bauman, 2004); however, there is controversy comparing stiffness in individuals with tetraplegia and paraplegia.

Increased AS was reported in individuals with thoracic cord lesions (T2-T12) compared to those with high cord lesions (C2-C8) (Miyatani et al., 2014). This finding is surprising because most of the risk factors (BP lability, deconditioning and impaired SNS) for increased central AS is reduced in those with paraplegia individuals (Popa et al., 2010; Claydon et al., 2006; West 2012), but may be contributable to categorical segregation by level of motor impairment (i.e., paraplegia versus tetraplegia) rather than by level of sympathetic cardiovascular interruption (i.e., above versus below T6). Another study reported no difference in AS between individuals with high and low cord lesions, which may be due to variability in duration of injury (3 to 518 months) (Wu et al., 2017). Recently, our group was the first to document heightened stiffness in high cord lesion (C3-T5) compared to low thoracic lesions (T7-T12) (Katzelnick et al., 2017). In this study, we measured AS by augmentation index at the radial artery, whereas in the prior report (Miyatani et al., 2014) PWV was determined at the carotid to the femoral artery.

Individuals with low cord lesions would be expected to have full sympathetic innervation of the radial artery, compared to individuals with high cord lesions who have partially to complete decentralized autonomic control. Further investigation is needed to clarify and ascertain the relationship between lesion level and AS.

There is evidence supporting a direct linear association between AS and LOI, with AS increasing with ascending LOI (Katzelnick et al., 2017). In this same study, the investigators reported that individuals with increased AS reported experiencing OH (Katzelnick et al., 2017); and furthermore, Currie et al. (2019) demonstrated that hypotensive events within 24-hour period were associated with increased arterial stiffness in individuals with injuries to T6 and above. This suggests that BP instability may play a role in causing structural vascular adaptations AS post SCI (Currie et al., 2019; Phillips et al., 2014). A recent study demonstrated increased AS during HUT in individuals with cervical injuries compared to uninjured controls (Phillips et al., 2014); conversely, Huang and colleagues (2013) showed no significant group difference in AS in a similar study design (Huang, Wong, et al., 2013). This discrepancy may be due to the differences in duration of injury (5-144 weeks vs. 3-289 months) and completeness of injury (Phillips et al. all motor complete (AIS A & B) vs. Huang et al. AIS A, B, C & D). Interestingly, our team recently demonstrated that there was significant inverse relationship between seated SBP and AS (Katzelnick et al., 2017); and furthermore, Phillips et al. (2014) demonstrated that the increase in AS during HUT in SCI was reversed by a pre-treatment of midodrine hydrochloride, an alpha-adrenergic agonist. We speculate that this may be related to a reliance on RAAS for orthostatic BP maintenance, which may have detrimental effects on AS. Although increased AS has been reported in individuals with SCI, controversy abounds and additional

evidence is needed to gain a better understanding of impact of morphologic and physiologic vascular adaptations that occur following SCI on orthostatic BP regulation and AS.

A variety of non-invasive testing modalities are used to accurately assess and track AS (Jae, Heffernan, Lee, & Fernhall, 2008). Ultrasound techniques can be used to assess vessel distention and derived stiffness indexes or flow waveforms (Townsend et al., 2015). These modalities (i.e. flow mediated dilation, carotid intima-media thickness) are generally more expensive and require a high level of technical skill (Stoner, Credeur, Dolbow, & Gater, 2015). MRI-based approaches can examine almost any vessel and provide more accurate distance and area measurements, but these approaches are very expensive and impractical (Townsend et al., 2015). Applanation tonometry has been used extensively in the literature because it is relatively inexpensive, requires a short testing time, and does not demand highly technical skill levels (Stoner et al., 2015; Townsend et al., 2015). Pulse wave velocity (PWV), which may be assessed using applanation tonometry, is calculated by measuring the distance between two vessels (i.e. carotid, brachial, radial, femoral, ankle) and the pulse wave arrival time at each vessel (R wave of ECG) (Stoner et al., 2015).

Assessment of aortic PWV (aPWV, using the carotid and femoral arteries) is considered to be the gold standard technique (Townsend et al., 2015) because of its high reliability and validity. Using the SphygmoCor CPV system (AtCor Medical Pty Ltd., West Ryde, NSW., Australia), aPWV was validated against invasive measurement of aPWV from the ascending aorta to the iliac bifurcation in 135 participants (Weber et al., 2009). The mean difference between the invasive aPWV and noninvasive aPWV was -0.2 m/s, (Spearman's $R = 0.73$, $p<0.0001$) (Weber et al., 2009). Additionally, the reproducibility of the system for within-observer variability was 0.07 ± 1.7 m/s and for between-observer difference was 0.30 ± 1.25 m/s

(Wilkinson et al., 1998). Measuring aPWV along the aortic and aorto-iliac pathway is most appropriate because the aorta is the principal cushioning artery and is responsible for the pathophysiological effects of AS (Stephane Laurent et al., 2006; London & Pannier, 2010).

Summation and Relevance

In summary, medical advances have contributed to increased life expectancy in individuals with SCI; however, CVD has become a leading cause of morbidity and mortality in individuals with chronic SCI (Cragg et al., 2013; Krause, Cao, DeVivo, & DiPiro, 2016; LaVela et al., 2012). Predominantly sedentary lifestyles (Stoner et al., 2015), ANS dysfunction (Cragg et al., 2013) and heightened inflammatory processes (LaVela et al., 2012) may contribute to the increased incidence and premature development of CVD in the SCI compared to the uninjured population (Myers, Lee, & Kiratli, 2007). It is well recognized that CVD risk increases with age, hypertension, adverse body compositional changes and physical inactivity; however, there is support to suggest that these traditional risk factors may not fully explain the early onset of CVD in the SCI population (Cragg et al., 2013; Miyatani et al., 2014). In fact, it has been demonstrated that PWV was increased in SCI participants compared to healthy uninjured controls after matching for age, body composition and activity levels (Phillips et al., 2014). Additionally, individuals with tetraplegia demonstrated heightened CCA intima-media thickness compared to individuals with paraplegia, even with similar inflammatory profile (Matos-Souza et al., 2010). Both of these studies suggests that other factors may mediate increases in AS in SCI population, and specifically, individuals with high-level injuries.

Individuals with tetraplegia rely heavily on the RAAS for both short- and long-term regulation of orthostatic BP (Wecht et al., 2011), which may play an important role in determining AS in this population (Katzelnick et al., 2017; West et al., 2013). However, the

association between ANG II levels and AS during an orthostatic provocation has not been studied in the SCI population. Understanding the contribution of activation of the RAAS and elevated levels of ANG II to the development of premature and heightened AS in the SCI population will guide clinical treatment of hypotension and OH, which may lead to improved BP regulation and preservation of cardiovascular health and longevity.

Theoretical Framework

Asymptomatic OH occurs in up to 50% of individuals with chronic tetraplegia (Krassioukov et al., 2009; Phillips et al., 2012) and may lead to pre-syncopal symptoms that include light-headedness, dizziness, blurred vision, fatigue, nausea, dyspnea, which adversely impacts their quality of life, hindering their ability to perform orthostatic maneuvers during routine daily activities and mobilization (Claydon et al., 2006; Krassioukov et al., 2009; Phillips et al., 2012). Uninjured individuals with persistent and episodic OH have been shown to be at an elevated risk of stroke (Benvenuto & Krakoff, 2011; Eigenbrodt et al., 2000), and it has been speculated that OH may explain the 2-3 fold increase of stroke in the SCI population (Cragg et al., 2013; Phillips et al., 2012). Additionally, OH is associated with cognitive deficits in the uninjured population (Novak & Hajjar, 2010; Yap, Niti, Yap, & Ng, 2008), which may contribute to, in addition to chronic hypotension, documented deficits in memory, attention, processing speed, and executive function in hypotensive individuals with SCI (Jegede et al., 2010; Wecht et al., 2011). Several large population-based studies reported increased AS may contribute to cognitive decline with aging in the uninjured population (Pase et al., 2010). Specifically, three studies have documented that association between increased AS and cognitive decline in global cognitive function (mini-mental state examination score) after co-varying for age, MAP (mean arterial pressure), education level, and CVD (Elias et al., 2009; Scuteri et al.,

2007), as well as in verbal learning, delayed recall, and memory (Waldstein et al., 2008). Thus, premature changes in vascular morphology may predispose individuals with SCI to cognitive deficits. Furthermore, increasing vascular stiffness is associated with an increased risk of CVD in the general population (Cecelja & Chowienczyk, 2012; Mitchell et al., 2010; Vlachopoulos et al., 2010). Recent literature reported that more rostral level and more complete SCI are associated with greater risk of all CVD (Groah, Weitzenkamp, Sett, Soni, & Savic, 2001). It is imperative that we identify the possible association between increased reliance on the RAAS for BP maintenance and the increased incidence of AS in persons with SCI with the goal of reducing CVD progression and improving cognitive function in this highly vulnerable population.

Gaps

Based on recent literature and our theoretical model, convincing evidence is needed to demonstrate that liberal use of anti-hypotensive medications in individuals with SCI might mitigate reliance on the RAAS and reducing levels of ANG II during orthostasis. Using this type of medication would expect to improve BP homeostasis, thereby lessening the symptoms associated with cerebral hypoperfusion and improving long-term cardiovascular and cognitive outcomes. As of today, clinical practice guidelines recommend that only individuals who experience symptomatic OH be prescribed anti-hypotensive medications; however, the homeostatic maintenance of BP, regardless of symptoms, may be critical in promoting long-term health and independence in the SCI population. Secondly, sufficient evidence is lacking to describe the association between orthostatic BP changes and the increased incidence of AS in the SCI population. Investigation into this association have consisted of heterogeneous study subjects with varying levels, severities and durations of injury, which would be expected to differentially impact the SNS response to orthostasis. Adequate plasms catecholamine responses

during HUT would improve orthostatic BP responses thereby limiting use of the RAAS. Moreover, during the acute phase of SCI (<1 years post injury), the morphology of the arterial vessels is changing rapidly; therefore, it may be more effective to measure AS after the initial paralysis and atrophy of the muscles. Lastly, there is no research demonstrating if the overuse of RAAS, specifically ANG II, contributes to the increased AS in the SCI population. Recent literature has stated that there may be additional risk factors other than the traditional ones that explain the increased CVD in this population, thus the over production of ANG II may explain the morphologic vascular mal-adaptations that occur following a SCI.

Scientific Relevance

A heightened prevalence of CVD is now the leading cause of morbidity and mortality in the SCI population, and ANS dysfunction underlines several irregularities that contribute to CVD (Myers et al., 2007). It has been speculated that persons with chronic SCI have accelerated cardiovascular aging (Myers et al., 2007; Yarar-Fisher et al., 2017) due to sedentary lifestyle (Stoner et al., 2015), increased inflammatory processes (La Favor, Hollis, Mokshagundam, & Olive, 2011; Sherri L LaVela et al., 2012), and cardiovascular autonomic dysregulation (. Claydon & Krassioukov, 2008; Cragg et al., 2013; Myers et al., 2007; Nier & Hansen, 2017). Recent literature speculates that additional risk factors are likely to contribute to premature CVD in the SCI population, and it has been shown that individuals with SCI have a 2 to 3 fold increase in the odds of developing heart disease and stroke (Cragg et al., 2013). Additionally, in the general population, a 1.0 m/s increase in aPWV translates to a 15% increase in CVD risk (Vlachopoulos et al., 2010) and consistent evidence demonstrates that individuals with SCI have an accelerated aPWV that is increased 2-3 m/s compared to uninjured individuals (Lee, Phillips, & Krassioukov, 2017; Miyatani et al., 2009; Phillips et al., 2012). Thus, it cannot be over

emphasized that health care providers are faced with significant challenges associated with prevention, diagnosis and management of older individuals with SCI at heightened risk for development of chronic diseases (Groah et al., 2001). Identifying the potential contribution of increased reliance on the RAAS, and ANG II, for orthostatic BP hemostasis in the SCI population and the effect on AS will help clinicians understand and screen for additional risk factors that contribute to CVD.

Summary

As a consequence of decentralized SNS, individuals with high level lesions are hypotensive with episodic falls in BP during orthostasis (Kirshblum & Campagnolo, 2011; A. Krassioukov, 2009). OH after SCI is thought to be related to baroreflex dysfunction, reduced skeletal muscle pump, and diminished catecholamines, which leads to an increased reliance on the RAAS for BP maintenance (S. Kirshblum & Campagnolo, 2011; Jill Maria Wecht & Bauman, 2018). The RAAS has been increasingly recognized as a major risk factor in the progression of atherosclerosis and increasing CVD risk in the uninjured population (Williams, 2001). Specifically, ANG II has been shown to modify the structure of the arteries by altering the ratio of collagen and elastic fibers in the vasculature (Shahin et al., 2012). Therefore, due to increased dependency on the RAAS to maintain orthostatic BP homeostasis (Wecht & Bauman, 2018), individuals with SCI, particularly those with high cord lesions, may be at increased risk for premature and increased AS.

Chapter III

METHODS

Participants

All subjects (N=33) were between the ages of 28-69 years with no known history of CVD, diabetes mellitus, and hypertension. All were current nonsmokers for a minimum of 1 year before investigation and were not receiving medications known to affect autonomic cardiovascular function. Subjects with high-level injury (HIGH-SCI; n=11; C4-T1) and low-thoracic injury (LOW-SCI; n=11; T6-12) were injured for at least 1 year, chronically wheelchair bound, and ASIA grade A, B or C. The uninjured control subjects (n=11) were matched for age, height, and weight to the subjects with SCI.

Individuals with SCI will be recruited from the Northern New Jersey SCI Model System database, which currently contains over 1000 people with traumatic SCI. Additionally, uninjured control subjects were recruited from the local community and hospital personnel via study solicitation flyer distribution. Participants were screened before study for inclusion and exclusion criteria. The Institutional Review Board for Human Studies of Seton Hall University, James J. Peters Veterans Affairs Medical Center and Kessler Foundation granted approval for the study, and informed consent was obtained before study initiation.

Procedure

All subjects were told to avoid caffeine, alcohol and exercise for at least 12 hours before testing and to be well hydrated. Participants were asked to remain seated in a testing chair to collect 5 minutes of seated resting hemodynamic data. After, subjects were asked to transfer to the tilt table and remained in the supine position for 10 minutes prior to baseline supine hemodynamic measurement, PWV, and first blood draw. Subjects were then progressively tilted from supine to 30 º, 45 º, and 60 º for 10 minutes at each tilt. A 3-lead ECG (Impedance Pneumograph, model RESP 1: UFI, Morro Bay, CA, USA) was used to monitor beat-to-beat HR, with ECG electrodes placed at the right and left mid-axillary lines in the 5^{th} intercostal space and at the right anterior axillary line. Before placing each electrode, each site was cleaned with alcohol pads. Continuous BP was assessed at the finger using photoplethysmograph (Finometer PRO, Finapres Medical Systems B. V., Netherlands) and at the brachial artery with manual auscultation (Series Wall Mobile Sphygmomanometers, Trimline Medical Products, Raritan, NJ, USA). Mean arterial pressure (MAP) was calculated as diastolic blood pressure (DBP) + 1/3(SBP-DBP). Continuous HR and BP were viewed and monitored in real-time and were stored on a desktop hard drive for offline analysis. HR and BP signals were collected at a sample rate of 500 Hz/channel with a 12-bit analog-to-digital converter (DAQcard-700, National Instruments, Austin, TX) and were analyzed using customized programs written with LabVIEW graphical software for instrumentation (National Instruments). In each position and tilt angle, HR and BP were measured and averaged over 5 minutes to use for analysis. At 60 º, second blood draw was collected.

Tilt Table

The tilt table was padded and motorized. Restraining straps were used on the lower extremities and trunk to ensure subject safety during higher inclinations of HUT and to avoid lower-extremity muscle contractions in the control subjects. The straps were wide and padded for subject comfort and to insure that stimulation of sympathetic spinal reflexes was not evoked. The orthostatic provocation included a progressive HUT from supine position to 30º, 45 º and 60 º for 10 minutes at each angle of tilt. Adjustment of the tilt table to each angle was accomplished in less than 5 seconds, and subjects were questioned at each angle of tilt regarding symptoms of syncope (i.e., blurry vision, dizziness, light-headedness, or nausea).

Pulse Wave Velocity

After supine hemodynamic recording, PWV was measured using the SphygmoCor CPV system (AtCor Medical Pty Ltd., West Ryde, NSW., Australia) at the carotid and femoral sites. The average 5-minute resting supine BP was entered into the software. A 3-lead ECG designed for the system measured the R-R wave with the ECG electrodes located just below the supra-sternal notch, on the chest over the sternum and just above the left hip on the chest. The carotid and femoral arteries were palpated for the strongest pulse and then measured from the participant's supra-sternal notch to each artery. The PWV measurement was taken in two steps: a tonometry reading of site A (carotid artery) with an ECG signal simultaneously recorded captured after 10 seconds of good data, followed by a 10 second reading of site B (femoral artery) with an ECG signal (Research Applications Manual). Good quality PWV measurement was accepted if the standard deviation was low, defined as the difference in HR between site A and B measurements more than 5 beats per minute.

Hormonal Assessments

Active plasma renin (surrogate measure of ANG II) and norepinephrine levels were assessed by immunoassay. Antecubital venous blood samples were collected at supine and at the 60 º. Blood samples were immediately placed in an ice bath for subsequent centrifugation. Plasma and serum were separated from blood products and frozen at -30 º for subsequent batched analysis.

Data Analysis

Statistical analyses were performed using SPSS 16.0 (SPSS, Inc., Chicago, Illinois, USA) software. All continuous variables were reported as mean plus or minus standard deviation (mean ± standard deviation), unless otherwise stated. Data were assessed for normality using Kolmogorov-Smirnov test and P-P plots. Pearson or Spearman's corrleations for normal and non-normal distributed data were used to examine if there was an influence of change of orthostatic SBP on the RAAS responses to orthostasis and relationship between PWV and change in renin. Univariate analysis of variance (ANOVA) were used to determine group differences in hemodynamics, orthostatic reliance on the RAAS hormonal assessment (renin) and supine PWV. Tukey HSD post hoc tests were used if the omnibus interaction effect was significant. The level of statistical significance was set at α less than 0.05.

Chapter IV

RESULTS & DISCUSSION

The first aim was to determine group differences in hemodynamics and hormone concentrations among the groups. We hypothesized that SBP would decrease during HUT in HIGH-SCI, while SBP would remain unchanged during HUT in LOW-SCI and able-bodied controls. In addition, we predict that plasma renin levels will be significantly greater at HUT in HIGH-SCI compared to LOW-SCI and able-bodied controls.

Our second aim was to investigate group differences for change in hemodynamics and hormones from supine rest to 60-degree HUT. We hypothesized that individuals with HIGH-SCI would have a greater change in SBP compared to LOW-SCI and able-bodied controls. Similarly, we hypothesized that HIGH-SCI group would have an increased change in renin levels, while renin levels would be unchanged in LOW-SCI and able-bodied controls.

Thirdly, we investigated the influence of the change in BP and change in plasma renin from supine rest to 60-degree HUT. We hypothesized that individuals with HIGH-SCI would have a positive relationship between the change in BP and change in renin, while there would be no relationship between the two variables in the LOW-SCI group and able-bodied controls.

Lastly, we aimed to determine if increased reliance on the RAAS for orthostatic BP regulation contributes to differences in AS. We hypothesized that there would be a positive

relationship between change in renin and AS in HIGH-SCI, while there would be no relationship between the two variables in LOW-SCI and able-bodied controls.

Participant Characteristics

The demographics of the study sample are presented by group (Table 1). The groups did not differ significantly for age, height, weight or body mass index. In addition, the SCI groups did not differ in AIS, DOI and age at time of injury.

Hemodynamics

Two-way ANOVA models were used to determine main and interaction effects for group (able-bodied controls, HIGH-SCI, LOW-SCI) and orthostatic position (supine, HUT) in hemodynamics and plasma renin.

Heart Rate

There were significant main effects for group ($F(2,120)= 5.90$, $p=.01$, $\omega^2= .05$) and position ($F(3,120)= 11.69$, $p<.001$, $\omega^2= .19$); however, the interaction effect was not significant.

Systolic Blood Pressure

While the main effect for group was significant (($F(2,120)= 6.48$, $p=.002$, $\omega^2=.07$); the main effect for position and the interaction effects were not significant

Diastolic Blood Pressure

There was a significant group main effect ($F(2,120)= 11.51$, $p<.0001$, $\omega^2=.14$); however, the main effect for position and the interaction effect were not significant.

Plasma Renin Responses

There was no significant main or interaction effects for plasma renin.

Arterial Stiffness

One-way ANOVA was used to determine a significant group main effect for the AS ($p<.0001$; eta^2 (η^2) =0.67). Pairwise comparisons (Tukey HSD procedure) indicated that AS was significantly increased in HIGH-SCI (HIGH-SCI: 8.81 ± 1.91 m/s) and LOW-SCI (LOW-SCI: 7.36 ± 1.58 m/s) groups compared to age-matched controls (5.53 ± 0.95 m/s; p=.04). AS was higher in the HIGH-SCI group compared to LOW-SCI; however, not significantly different.

Linear regression models were used to determine the effect of age and injury characteristics on PWV among the groups. Although age did not contribute significantly to PWV in the groups with SCI, age accounted for 83% of the variance in PWV in the control group (r^2=0.83; p=.002); In addition, there was no relationship between DOI, level of lesion and AS in SCI groups,

Change in Hormonal Response

There was a significant main effect for the change in renin from supine to 60 degree HUT, F(2,30)=6.31, p=.01, ω^2=.25. Pairwise comparisons (Tukey HSD procedure) indicated that individuals with HIGH-SCI had significantly higher changes in renin levels (11.54 ± 12.49 pg/ml) compared to individuals with LOW-SCI (0.47 ± 5.67 pg/ml, p=.01) and able-bodied controls (1.22 ± 3.45 pg/ml, p=.02).

Orthostatic BP and Renin

Multiple regression analysis on the change in SBP and plasma renin was performed in the total study sample (n=33). The results suggest that the model significantly improved our ability to predict SBP, F(2,30)=7.61, p=.002. Change in renin was a significant predictor to the change in SBP and contributed to the model, t(30)=-3.90, p<.001; however, the group predictor is not significant and did not contribute to the model, t(30)=0.26, p=.80. The change in plasma renin

accounted for 35% of the variance in the changes in SBP with tilt in the total study sample (r^2= 0.347; p=.002).

Arterial Stiffness and Renin

Multiple regression analysis on AS and change in renin was performed in the total study sample (n=33). Assumptions were met to perform multiple regression. The results suggest that the model significantly improved our ability to predict AS, F(2,30)=19.66, p<.0001. The group, t(30)=3.32, p=.002, and change in renin, t(30)=5.42, p<.0001 were significant predictors to AS and contributed to the model. Relationship between change in plasma renin and AS was significant in the total study sample (r^2= 0.57; p<.0001).

Separate regression analysis by group were then used. Assumptions were met to perform Pearson's correlation for all three groups. As hypothesized there was a significant relationship between AS and change in plasma renin in HIGH-SCI, r = .62, p =.04, while there was no relationship in able-bodied controls, r=.04, p= .91.

For LOW-SCI, we performed Grubb's test to identify an outlier in the group. The absolute z score of the change in renin (2.91) was significantly higher than the upper significance levels at sample size 11, so that score is an outlier (Grubbs & Beck, 1972). There was no significant relationship between the AS and change in plasma renin in individuals with LOW-SCI, r=.18, p=.62.

Discussion

As per design, the controls were age, height, weight and body mass index matched to the SCI participants. In the present study, AS was measured by PWV, and was compared in age-matched individuals with HIGH-SCI, LOW-SCI and able-bodied controls. AS was increased in those with SCI compared to the uninjured controls; however, there were no differences in AS in

the SCI groups. Secondly, the change in orthostatic BP from rest to 60-degree HUT was inversely associated to the change in plasma renin levels in the total sample. Furthermore, the change in plasma renin levels during HUT was directly related to AS in the HIGH-SCI group.

Arterial Stiffness by Group

It is well appreciated that the natural aging process increases AS, and the values of AS in the control group were similar to those previously reported for healthy individuals (Boutouyrie and Vermeersch, 2010). However, the association between age and AS was not significant in our participants with SCI, regardless of the level of lesion, which is in agreement with our previous study (Katzelnick et al., 2017). In addition, DOI, age at injury and level of lesion did not play a role in AS. Again, this is similar to our previous report (Katzelnick et al., 2017); however, this is in contrast to a recent study that reported a correlation between stiffness index and duration of injury (Wu et al., 2016). The disparity between our findings and those previously reported may be attributed to inclusion of individuals with acute and chronic injuries (3-518 months) in the prior report and differing methodology (i.e. stiffness index by digital volume pulse vs. PWV).

It has become increasingly clear that individuals with SCI have increased AS compared to age-matched able-bodied controls (Katzelnick et al., 2017; Phillips et al., 2012; Huang et al., 2013; Miyatani et al., 2014; Lee et al., 2017; Wecht et al., 2004). Conversely, the relationship between level of lesion and AS is still not certain. We previously reported that AS (measured by augmentation index at the radial artery) was increased in HIGH-SCI compared to LOW-SCI (Katzelnick et al., 2017); however in the present study, we found no difference in PWV between the SCI groups. This may be attributed to differing methodology. In the present study, PWV was measured at the carotid and femoral arteries, which is gold standard measurement for AS; however, in our previous work, the augmentation index was performed at the radial artery, where

we would expect to have full sympathetic innervation in individuals with low cord lesions, but would be partially to completely decentralized among those with high cord lesions. Although not significant, individuals with HIGH-SCI had slightly higher AS than LOW-SCI group. With a larger sample size, we may see a significant difference between the two groups. Miyatani et al. (2014) reported higher AS in individuals with paraplegia (T1-T12) compared to individuals with tetraplegia (C2-C8). This is highly surprising given that most risk factors for increased AS is increased in those with higher level injuries including BP liability, physical inactivity, and impaired SNS function (Lee et al., 2017; West et al., 2012; Popa et al., 2010; Weaver et al., 2012). Additionally, Miyatani and colleagues included all levels of thoracic injuries in their paraplegia group, including those that have control of their splanchnic bed and those who do not. It is essential to understand that within the SCI population, there is a large degree of cardiovascular variability that is directly related to the specific level of injury.

AS is recognized as an independent predictor for CVD (Vlachopoulos et al. 2010), and because CVD is the leading cause of mortality in the SCI population (Craigg et al.,), AS may serve as a vital prognostic tool (Currie et al., 2019). An individual presenting with AS of greater than 10 m/s is considered at elevated risk of a cardiovascular event (Van Bortel et al., 2011), and in our sample, we have three individuals with HIGH-SCI that have a AS above 10 m/sec. Furthermore, we compared the AS values in our study with available general population reference values (Boutouyrie and Vermeersch, 2010). As a result, highly abnormal AS was present in 63% of the HIGH-SCI group and 45% of the LOW-SCI group. There was no abnormal AS in the age-matched control group. Of note, the clinical threshold for abnormal AS values have not been established in the SCI population. These results demonstrate that

individuals with high-level SCI may have higher related cardiovascular-related risk compared to individuals with low-level SCI.

Orthostatic Blood Pressure and Renin Levels

Our first aim was to investigate the influence of the change in BP and change in plasma renin from supine rest to 60-degree HUT. Plasma renin activity has been reported to be elevated in response to HUT in persons with high-level injuries (Mathias et al., 29175; Mathias et al., 1980) in order for this population to regulate BP during an orthostatic provocation. All renin values were within normal range (supine: 3-30pg/ml; seated/standing: 7-50 pg/ml) (Bauman et al., 2017), except for two individuals in the LOW-SCI group. Both of them had higher supine plasma renin levels (Subject 1: 40.31 pg/ml and Subject 2: 78.78 pg/ml) and subject 2 had higher than normal plasma renin level during 60-degree HUT (62.76 pg/ml). This may be due to an analysis error.

The change in plasma renin levels were inversely related to the change in BP from supine to HUT at 60-degrees in the total sample. We hypothesized that we would see a positive relationship; however, this inverse relationship suggests that those with greatest plasma renin response to HUT, also have the greatest fall in BP. We might have seen an increase in SBP if we measured the blood pressure the last 5 minutes of the HUT, instead of the first 5 minutes in order to see the increased renin levels vasoconstricting the arteries and increasing BP. Handrakis and his colleagues (2009) demonstrated that there was a correlation between change in plasma renin and an increase in MAP in individuals with tetraplegia, which suggests that those who have increased RAAS activity, had an increase in BP.

Plasma Renin and Arterial Stiffness

Our second aim was to determine if increased reliance on the RAAS for orthostatic BP regulation contributes to differences in AS. Overstimulation of RAAS triggers numerous signaling pathways that are implicated in vascular remodeling, endothelial dysfunction, and other mechanisms that may contribute to AS and accelerated vascular aging (Neves et al., 2018). The RAAS system acts predominately on restricting arterioles and, has been shown to modify the structure and function by altering collagen and elastin fibers (Mahmud and Freely, 2004; Duprez, 2006; Neves et al., 2018). Although many studies have hypothesized that increases in RAAS may be related to increases in AS in the SCI population (Katzelnick et al., 2017; West et al., 2013; Wecht and Bauman, 2018), this report is the first to document the change in plasma renin during HUT is directly associated with AS in the HIGH-SCI group. The chronic contraction of blood vessels and other vascular remodeling processes of RAAS during orthostatic challenges is a significant contributor to increased AS.

Individuals with asymptomatic hypotension are often undiagnosed and untreated because it is believed that hypotension may actually have cardiovascular benefit. However, evidence has shown adverse impact of asymptomatic OH on activities of daily living (Currie et al., 2015; Barber et al., 2000), quality of life (Carlozzi et al., 2013; Craig et al., 2016; Jegede et al., 2010), cognitive function (Jegede et al., 2010; Phillips et a l., 2014) and cerebral circulation (Wech et al., 2013; Wecht et al., 2012; Phillips et al., 2017) in persons with chronic SCI, and this should raise clinical concern. Our findings lend further support that it is necessary to normalize and stabilize BP, even in asymptomatic individuals with SCI, to reduce the dependency on RAAS and improve cardiovascular health and longevity.

Chapter V

SUMMARY AND CONCLUSIONS

Conclusion

The SCI population has an increased prevalence of OH due to impairment in supraspinal regulation affecting the autonomic cardiovascular control. Adverse impact of asymptomatic OH during activities of daily living, such as transfers and rehabilitation, has not been fully appreciated, but should raise clinical concern. Our findings lend further support that the SCI population is prone to premature onset of CVD compared to age-matched uninjured able-bodies. A new risk factor contributing to increased AS in the SCI population may be the hyperactivity of RAAS in order to maintain BP and avoid syncope. Further research should examine pharmalogical and nonpharmalogical techniques to increase BP and suppress activation of RAAS in order to decrease CVD in the SCI population.

Limitations

We acknowledge that the small sample size may limit the generalizability of our findings; however, critical factors that affect AS were controlled for in our study, such as age, height, weight, and smoking status. Additionally, the sample included a large age range, and we know

that AS increases with natural aging. Although we matched our SCI population to the uninjured controls, future studies should have a smaller age range to make sure age is not contributing to AS. Lastly, we did not survey the SCI sample for autonomic dysreflexia, which is a condition that increases BP to possibly extreme levels. Autonomic dysreflexia may play a role in AS since AS is affected by BP.

Future Research

Future research should examine pharmacological (i.e., hypertensive medication) and nonpharmacological (i.e., transcutaneous stimulation) techniques to increase blood pressure and suppress activation of RAAS in order to decrease cardiovascular disease in the SCI population.

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Appendix A

Seton Hall University IRB Approval Form

PRE - IRB FORM

Pre-IRB review is mandatory for all proposals. Proposals that do not have a pre-IRB review will not be considered by the IRB and will be sent back to the investigator.

Pre-IRB form to be filled by the department/schools:

Investigator(s): Caitlyn G. Katzelnick, MS; Genevieve Pinto Zipp, EdD; Jill M. Wecht, EdD; Michael LaFountaine, EdD

Proposal Title: Orthostatic Blood Pressure and Arterial Stiffness in Persons with Spinal Cord Injury: The Effect of the Renin-Angiotensin-Aldosterone System

Required statement by pre-IRB reviewer:

I have reviewed the proposed research. I state that:

a) the question(s)/hypothesis of the research is sound and is clearly stated;
b) the study design is appropriate to answer the question(s) or prove the hypothesis of the research;
c) the research has reasonable likelihood of contributing to generalizable knowledge.

My signature (1) affirms that the proposed research is scientifically sound, and (2) represents my approval of the research.

Pre-IRB reviewer's signature

Nagle, Chair

Pre-IRB reviewer's name and title

9/5/18

Date

Appendix B

Veterans Affair Medical Center IRB Approval Form

Institutional Review Board
Bronx VA Medical Center
Research & Development Program (151)
130 West Kingsbridge Road • Bronx, NY 10468 • 718-741-4228 • Fax: 718-741-3937

IRB APPROVAL - Initial Review

Date: May 17, 2018
From: Juan C. Bandres, M.D., Ph.D., Chairperson
Investigator: Jill M. Wecht, Ed.D.
Protocol: Orthostatic Blood Pressure and Arterial Stiffness in Persons with Spinal Cord Injury: The Effect of the Renin-Angiotensin-Aldosterone System
ID: 01806 Prom#: N/A Protocol#: WEC-18-25

The following items were reviewed and approved at the 05/03/2018 meeting:

- Abstract (Protocol Summary) (04/09/2018)
- Budget Page (04/09/2018)
- Consent Form (04/09/2018)
- Financial Disclosure Form (04/09/2018)
- HIPAA Authorization (VA form 10-0493) (04/09/2018)
- HIPAA Revocation of Authorization (VA form 10-101 (04/09/2018)
- Lay Research Summary (04/09/2018)
- Personnel Record (04/09/2018)
- Research Financial Conflict of Interest Statement (04/09/2018)
- Research Protocol (04/09/2018)
- Research Protocol Safety Survey-form 10-0398 (04/09/2018)
- Research Data Inventory Form (04/09/2018)
- Signature Page(s) (04/09/2018)
- Training (04/09/2018)
- Waiver of HIPAA (04/09/2018)
- Data Management Access Plan (04/09/2018)

The following additional items were received to address stipulations and are now approved:

- Abstract (Protocol Summary) (05/11/2018)
- Consent Form (05/11/2018)
- HIPAA Authorization (VA form 10-0493) (05/11/2018)
- Research Protocol (05/11/2018)
- Research Protocol Safety Survey-form 10-0398 (05/11/2018)
- Response to Contingent Approval (05/11/2018)

Conditions of Approval are attached. These conditions are further detailed in the HHS, FDA, and VA regulations, which are available in the Research Office.

James J. Peters Veterans Administration Med Ctr (The)FWA00000898; Bronx Veterans Med Rsch Foundation, Inc. FWA00001746

Approval is granted for a period of 12 months and will expire on 05/02/2019. Your Continuing Review is scheduled for 04/04/2019, and the requirements are attached.

The protocol was determined to have the following level of risk:
Greater than minimal risk

The protocol was determined to have the following level of benefit to participants:
No direct benefit, but potential to yield generalizable knowledge about subjects' disorder/condition

The following contingencies were resolved:
1) Table of procedures not include. Blood draw-should include under 'hormonal assessments' (in protocol)
2) Application - enrollment listed as 22SCI, 11 controls. Protocol states33SCI, 111 controls. Consent states 33 SCI, no controls listed. Please correct for consistency throughout all documents.
3) Please complete list of risks of study (4)
4) Please address transfer of data to Seton Hall. DUA needed for this? Please consult with Privacy Officer.
5) This not a clinical trial. Please remove from consent.
6) consent does not match HIPAA for data banking. Consent states data will be destroyed. HIPAA states data banking. Please add to consent and submit repository application and SOPS for this.
7) Please answer yes for P. 1, blood in safety survey
8) Please define plan for control recruitment.
9) Add to ICF risks for venipuncture and tilt-table maneuver.
CPRS flaggingnot required.

The following other committee reviews are scheduled:
Research & Development Committee [06/20/2018]

Approval by each of the following is required prior to study initiation:
Institutional Review Board
Research & Development Committee

Approval for study initiation is contingent upon your compliance with the requirements of the Research Service for the conduct of studies involving human subjects.

Appendix C

Kessler Foundation IRB Approval Form

KESSLER FOUNDATION

INSTITUTIONAL REVIEW BOARD
1199 Pleasant Valley Way, Suite 2080
West Orange, NJ 07052
(973) 243-6972 Tel
(973) 243-6984 Fax

Notice of Approval
IRB Protocol Number: R-1021-18

Principal Investigator(s): **Trevor Dyson-Hudson, MD & Jill Wecht, EdD**

Title: **Orthostatic Blood Pressure and Arterial Stiffness in Persons with Spinal Cord Injury: The Effect of the Renin-Angiotensin-Aldosterone System**
Sponsor: **NJ Commission on Spinal Cord Research**

Type of Review: **Full [X]** Expedited [ ] Exempt [ ]

Type of Approval: **Initial [X]** Continuation [ ] Amendment [ ]

Approval Date: June 27, 2018 **Expiration Date:** June 26, 2019

1. **ADVERSE EVENTS:** Federal regulations require that any unanticipated complications or unexpected event(s) that occur in conjunction with this study must be reported promptly to the IRB Office. Serious Adverse Events must be reported to the IRB within 48 hours; unexpected adverse events of moderate or greater severity must be reported to the IRB within 5 business days (for details see Policy No. 5010 – March 15, 2004).

2. **RENEWAL**: Approval is valid until the expiration date on the consent form. You are required to apply to the IRB for a renewal prior to the expiration date for as long as the study is active. Renewal notifications will be sent to you three-months prior to expiration; however, it is your responsibility to ensure that the renewal is submitted in a timely fashion.

3. **CONSENT FORM:** Attached is your IRB-approved **Consent Form** that has been stamped on each page. This is the only valid Consent Form that can be presented to subjects enrolled in your study or candidates interested in participating. Please retain the original and use it to make photocopies to be signed by the research subjects. After one year, when your project is due for renewal, you will be asked to submit a clean, unstamped copy of this form so that the IRB can ascertain that you are using the correct Consent Form(s), and stamp it with a new expiration date. (All subjects must receive a copy of the consent form; the original signed copy must be kept in a secure place by the Principal Investigator.)

4. **SUBJECTS:** Number of participants approved for this study: **33** . If you wish to increase the number of subjects in this protocol, you must first obtain approval for an amendment from the IRB.

   NOTE: Studies with a Co-Principal Investigator who is a trainee (predoctoral student, medical resident, clinical fellow or postdoctoral research fellow) are provisionally approved to enroll 5 subjects. After 5 subjects are enrolled, the IRB staff should be contacted to arrange a research file audit to assess compliance with good clinical practices and the protection of the rights of human subjects. Continuation of these studies, beyond the initial 5 enrolled subjects, will depend on their receiving a passing grade in the audit process.

5. If additional procedures are being added to the protocol and you would like to apply the new procedures to subjects who have already been consented, you will need to re-consent these subjects using the revised consent form.

6. This approval applies only to the above-referenced project. It is important to secure prior approval of the IRB for any changes in your approved protocol that would affect the involvement of human subjects.

7. If the investigators submit future funding applications to obtain support for this protocol, they must provide the following information to the IRB:

   a. Name of Funding Agency
   b. Amount of Funding Requested
   c. Time Period of Funding
   d. Grant (or contract) number
   e. If the protocol submitted to the funding agency is identical to the one approved by the IRB, the PI should so certify in writing to the IRB. If the protocols are different, the PI should describe the differences in a memo to the IRB and submit an Amendment application to cover any differences.

Richard Greene, M.D., Ph.D., Chair, IRB

July 11, 2018
Date

ds