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ABSTRACT

Spinal cord injury (SCI) is typically a traumatic event that impacts a patient's physical, psychological, and social well-being by damaging motor and sensory neurons and altering autonomic function leading to cardiac remodeling [42]. Spinal cord injuries are based on a scale of severity, with severe, high-level lesions strongly correlating to the degree of cardiovascular dysfunction. [30][37][41]. The decline in cardiovascular function is prevalent after high thoracic SCI, with patients suffering from daily bouts of orthostatic hypotension (OH) and autonomic dysreflexia (AD), both of which prevent and delay rehabilitation efforts and lessen quality of life after injury [28][30]. Past studies have demonstrated robust cardiomyocyte remodeling and subsequent cardiovascular demise in completely transected rodent models of SCI [42][43]. This study provides more insight into the incidence of cardiomyocyte remodeling and LV atrophy following clinically-relevant thoracic vertebra, T2, contusions in rodents.

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INTRODUCTION AND SIGNIFICANCE

Spinal cord injury (SCI) is a devastating neurological condition affecting nearly 5.4 million individuals in the United States [27]. In addition to damaging motor and sensory circuitry within the spinal cord (SC), pathways of the autonomic nervous system (ANS) are also disrupted leading to the loss of cardiovascular (CV) homeostasis, or equilibrium. [14][30]. The degree of CV dysfunction is strongly correlated with both the severity and the SC level of the lesion, with higher and more severe injuries resulting in more profound impairment of metabolic, physiological, and psychological processes [30][37][41]. As such, high thoracic level (T1-T5) SCI patients have an elevated risk of developing cardiovascular disease (CVD) [37][41][42] and stroke [41]. Patients also suffer from daily bouts of orthostatic hypotension (OH) and autonomic dysreflexia (AD), both of which prevent and delay rehabilitation efforts and lessen quality of life after injury [28][30]. Reasons for the decline in CV function are due to a number of factors; and involve the increase in sedentary lifestyles [33][43] due to paralysis, morphological changes in autonomic sympathetic pre-ganglionic neurons, (SPGN) [43] and structural changes in CV end-organs (the heart and vasculature) following high-level, severe SCI [44].

Completeness of the lesion has profound ramifications on the resulting functional status of SCI individuals**.** Severe, anatomically complete injuries convey a full loss of motor and sensory functions below the lesion, whereas incomplete contusion injuries result in a partial preservation of SC circuitry and function [25]. Most published studies examining the link between CV dysfunction following high-thoracic SCI and cardiac remodeling have done so using experimental models of complete injury (complete transection of the SC) [25]. However, most clinical injuries are anatomically incomplete,

and patients experience a wide array of CV symptoms and dysfunction [25]. In a study completed by Squair et al. (2018) [37], it was determined that SCI-induced cardiomyocyte atrophy was severity-dependent, with cardiac remodeling and the resulting impairment in LV function only affecting severely-injured rodents [37]. However, experimental groups used in this study were small and it was unknown if the same findings can be replicated in other clinically-relevant injury severity models in which hindlimb locomotor function, and thus venous return to the heart, is only partially altered.

Normal function of the CV system relies on both proper cardiac activity and appropriate responses in peripheral vasculature to changes in systemic pressure [43]. The heart and vasculature are regulated by the ANS to ensure adequate perfusion during various physiological conditions [17]. Cardiac SPGNs that stimulate myocardium to increase contractility during times of stress reside in the intermediolateral gray column of the SC from thoracic vertebrae T1-T5 [43]. Sympathetic pre-ganglionic neurons are thus directly impacted by high-level SCI, and likely contribute to the CV dysfunction experienced by patients [41].

Orthostatic hypotension (OH) is a condition that is common in patients with cervical and high thoracic SCI [15]. Orthostatic hypotension can be described as an overall drop in baseline arterial blood pressure (BP) and slow heart rate (HR) following positional changes [32]. Reduced skeletal muscle pump activity and blood pooling in vasculature beds below the lesion in conjunction with reduced sympathetic modulation of vascular tone is responsible for episodes of OH and ultimately results in reduced venous return to the heart [19]. Orthostatic hypotension is a cardinal sign of autonomic dysfunction and is commonly concomitant with other heart failure (HF) conditions. Importantly, episodic

decreases in systolic BP have been shown to be associated with cardiac remodeling and cardiac decline [2].

Cardiac remodeling describes the molecular, cellular, and interstitial changes that manifest as changes in size, shape, and function of the heart after injury [20]. Cardiac remodeling is closely associated with HF [7][20][43], as the clinical manifestations of HF are the result of changes to the heart's cellular and molecular components and mediators that drive homeostatic control [43]. Cardiomyocyte atrophy progression after SCI may be due to a variety of factors, but ultimately leads to decreased left ventricle (LV) size and attenuated pumping efficiency [37].

The clinical diagnosis of cardiac remodeling involves the detection of morphological changes of the cardiomyocyte [3]. The cardiomyocyte is the most physically energetic cell in the body [32]. They are connected end-to-end by gap junctions, which allow concerted contractile activity. The contraction-relaxation cycle is controlled by the cyclic increase and decrease of calcium, initiated by depolarization of the sarcolemma, and sustained by the calcium reuptake and release by the sarcoplasmic reticulum. Cardiomyocyte health is dependent upon adequate physical activity and CV activation [3][7]. Following SCI, the paralysis-induced sedentarism does not support healthy CV function, as high-lesion SCI patients present with reduced venous return, impaired sympathetic modulation of the myocardium, hypotension, and the general lack of BP control during normal, daily activities [32][44]. Research has shown that following severe high-level SCI, in which resting BP is drastically reduced, [37] there is a decrease in cardiomyocyte length, Z-disc width, and fewer sarcomeres in the cardiomyocyte. Importantly, these changes in the morphology of the cardiomyocyte are

strongly correlated with reduced stroke volume (SV) and efficiency of the cardiac cycle, [37] and likely contribute to the decrease in cardiac function and incidence of CVD after SCI. Recent studies have shown that passive hind-limb cycling and staying active helps to prevent or completely reverse cardiomyocyte atrophy [41]. Other studies show how the use of dobutamine helps normalize sympathetic output in rodents helping to reverse the negative effects of pressure-derived complications [20][25].

While there has been a great deal of SCI research focused on improving locomotor function after injury, CVD remains the number one cause of morbidity and mortality for individuals living with chronic, high-thoracic SCI [28][33]. Understanding how cardiac remodeling contributes to the decline in CV function, and whether changes in cardiomyocyte structure and function contribute to episodes of OH and AD is of utmost importance for the SCI community [16]. Therefore, we have performed a set of experiments examining the incidence of LV atrophy and cardiomyocyte remodeling in incomplete, clinically-relevant contusion models.

HYPOTHESIS

We hypothesized that cardiomyocyte atrophy after SCI will be similar to what has been published previously [37]. We expected to find that rodents receiving severe T2 contusion injuries will have robust cardiac remodeling, including changes in cardiomyocyte shape and size and reduced presence of sarcomeres as compared to uninjured control animals. Combined with reduced locomotor function due to incomplete paralysis below the lesion and the attenuated sympathetic modulation of CV end-organs, atrophy of cardiomyocytes and the subsequent loss of LV mass will lead to greater overall CV decline and dysfunction.

MATERIALS AND METHODS

Female Sprague Dawley rats were used to complete these studies. Rats were randomly divided into two groups: Uninjured Control (CON, n=3) and Severe 35 g/cm T2 SCI (T2-SCI, n=6). Contusion injuries were delivered using the NYU Impactor (Mascis, Rutgers University). Rodents were double housed in tiny cages (7.5” x 8.5”, total floor area = 64”) to restrict locomotion. Eight weeks after injury, terminal immunohistochemistry staining and inverse microscopic techniques were performed to study the presence and extent of cardiomyocyte atrophy.

Cardiomyocyte Immunohistochemistry: To assess structural remodeling of cardiomyocytes following SCI, animals were trans-aortically perfused with phosphate buffer solution and the hearts were excised for immunohistochemical analysis. Following explanation, hearts were weighed, and submersion fixated with 10% neutral formalin solution for 48 hours. Hearts were then transversely divided into five 3mm sections, paraffin-embedded, and sectioned at 4µm intervals for staining. Actin (alpha-Sarcomeric) antibody staining was performed on the slides to help quantify the data via inverse microscopic techniques [(39)]. The slides were heated at 80°C for 30 minutes, and then placed in xylene to deparaffinize. Stepwise rehydration steps were implemented with decreasing concentrations of ethanol (100, 95, 95, 90, 80%) prior to being placed in water. The slide had all antigens exposed by immersing slides in citrate buffer (2.4g/L Sodium Citrate Tribasic Dehydrate, 0.35g/L Citric Acid, pH 6.0) overnight for 18-20 hours at room temperature. The citrate buffer is used to act as an antigen retrieval treatment step. The following day, slides were removed from citrate buffer, washed in water, and incubated in a dark hydration chamber with conjugated reagent Wheat Germ Agglutinin (WGA)-

Rhodamine (1:50 dilution, in Invitrogen Antibody Diluent Reagent) for 30 minutes to stain red fluorescence. Next, slides were washed with 1x KPBS (3 times, 3 min each) followed by incubation in a dark hydration chamber in the reagent alpha-Sarcomeric Actin (1:500 dilution, in Invitrogen Antibody Diluent Reagen) to stain for blue fluorescence. Simultaneously, the IgM negative control (1:500 dilution) of alpha-Sarcomeric Actin in Invitrogen Antibody Diluent Reagent were applied for 1 hour, and then washed with 1x KPBS (4 times, 3 min each). Finally, the reagent Alexa-Flour 488 anti-mouse IgM, staining actin, were utilized on each slide with a 1:750 dilution in Invitrogen Antibody Diluent Reagent and sudan black (200µl per slide) was applied to slides to mitigate autofluorescence signal. Slides were incubated in a dark hydration chamber for 15 minutes, washed 1x KPBS (6 times, 3 min each), rinsed in water (1 time, 3 min), and coversliped with Fluoromount G. All slides were stored at 4° C and were allowed to dry for 24 hours before imaging.

Imaging and Quantification of Cardiomyocytes: To quantify structural remodeling of cardiomyocytes after SCI, heart sections were imaged using an inverted microscope (20x). Non-biased images of the LV, right ventricle (RV), and interventricular septum (IS) (20, 10, 7 images per sample, respectively) were taken from each slide and analyzed for circular cardiomyocytes, disregarding longitudinal sections. The imaging program NIS-Elements and analysis program NIS-Elements Analysis determined whether or not the cardiomyocytes were deemed circular, or 0.895 – 1 mm. Anything below 0.895 mm was not included due to being an insufficient shape. Using NIS-Elements Analysis, the severity of the cardiomyocyte atrophy was calculated. Significance of cardiomyocyte atrophy was determined as a high number of circular cardiomyocytes present in the tissue. After the

data was collected from each sample using NIS-Elements Analysis, a mathematical mean, maximum, and minimum of cardiomyocyte area and shapefactor was calculated for each region of the heart in T2-SCI and CON animals.

Statistical Analysis: Between-group differences for working heart assessments were analyzed using an independent repeated measures ANOVA. A standard deviation outlier calculation was implemented using two criteria. 1) data was considered to be an outlier if it was ≥ 3 standard deviations outside from the calculated mean. 2) data was considered to be an outlier after the second outlier calculation if it remained ≥ 2.5 standard deviations from the calculated mean. A Post Hoc test was implemented using Tukey HSD. Correlations between cardiomyocyte area and shapefactor in LV, RV, and IS have been statistically analyzed. The mathematical mean was calculated in each sample, LV, RV, and IS, of both groups, T2-SCI and CON, and XY graphs were made to help convey correlations. Data was presented as a mean +/- standard deviation (SD), unless otherwise stated. Significance was set at $*p \leq 0.05$.

RESULTS

Cardiac tissue was stained, imaged, and analyzed to compare cardiomyocyte morphology T2-SCI and CON. Myocardial tissue was stained with antibodies such as Wheat Germ Agglutinin (WGA)-Rhodamine (ECM; red), alpha-Sarcomeric actin (cardiomyocytes; green), and counterstained with DAPI (nuclei; blue). All channels were merged to convey the best visual representation and convey an example of cardiac tissue that was analyzed. Representative images of cardiomyocyte morphology in the LV, RV, and IS are shown for T2-SCI and CON in Figure 1A and 1B, respectively. The cross-sectional area of cardiomyocytes for T2-SCI and CON were determined for the LV, RV, and IS (Figure 1C). The graphs represent mean area, maximum area, and minimum area of each sample (1Ci, 1Cii, 1Ciii, respectively). There were no significant differences between T2-SCI and CON groups for any of the variables measured. A magnified image of the cross-sectional area in myocardial tissue conveys a visual representation of various sizes and shapes of cardiomyocytes (Figure 1D). The arrow drawn provides an example of a cardiomyocyte that fit the criteria, and therefore was a data point of sample LV and group T2-SCI throughout analysis.

Cardiomyocyte morphology, was also analyzed in terms of shapefactor, as shown in Figure 2. The graphs represent mean shapefactor, maximum shapefactor, and minimum shapefactor. There were no significant differences between T2-SCI and CON groups. However, in Figure 2, image 1Ai, cardiomyocyte shapefactor RV mean approached significance, $p = 0.053$, between groups, T2-SCI and CON.

Although no significant differences between T2-SCI and CON groups were found, inter and intra correlations provided comparisons that were more representative of past

studies [34]. Some of the correlations found were that all samples, LV, IS, and RV, in groups, T2-SCI and CON, represented direct relationships in mean shape. The correlations involving area RV mean in CON, indicated inverse relationships with both shape RV mean and area IS mean. The last correlation found in CON, indicated an inverse relationship between area and shape LV mean. Other correlations found in group T2-SCI, indicated an inverse relationship between area IS mean and shape RV mean.

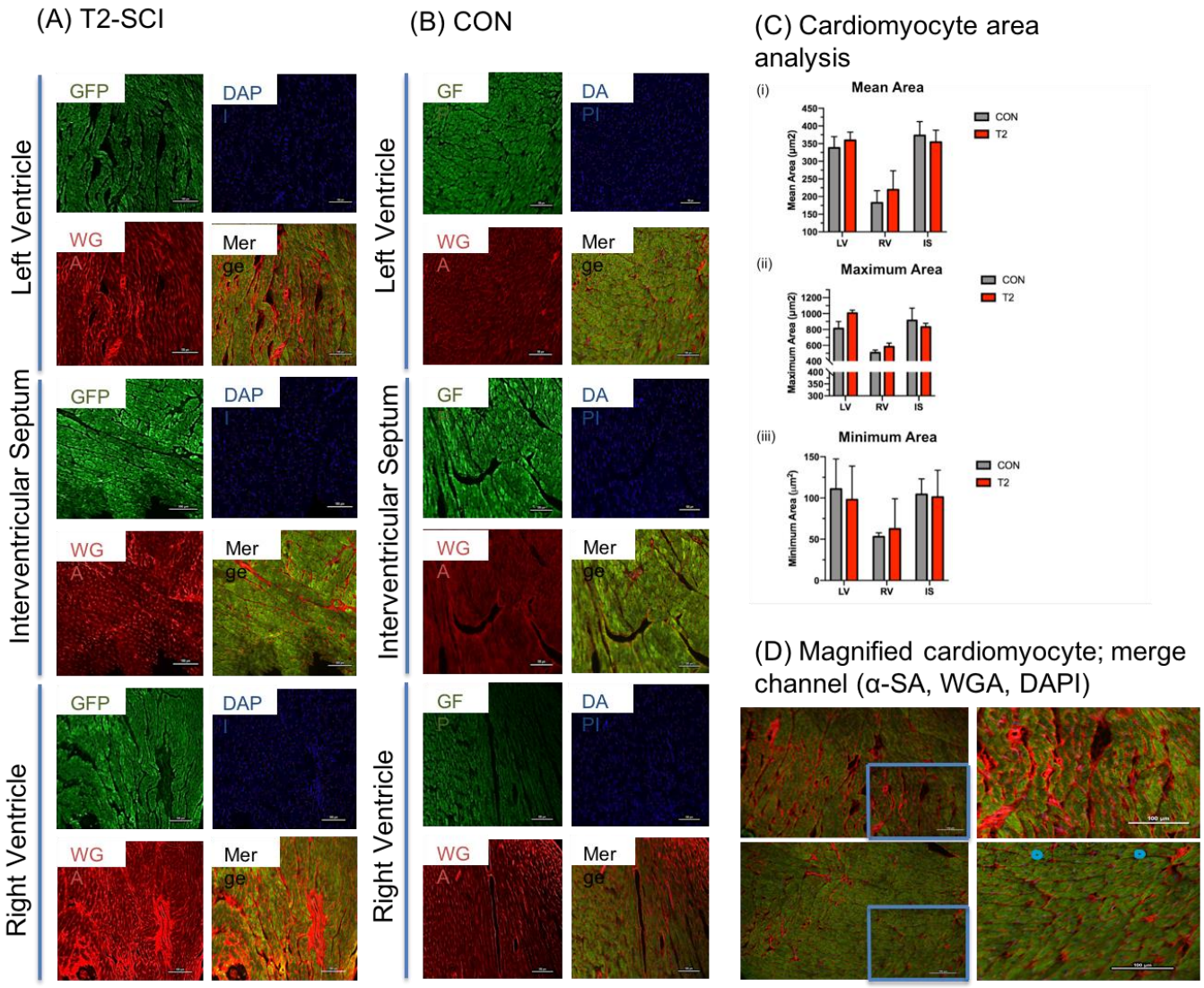
(A) T2-SCI
(B) CON
(C) Cardiomyocyte area analysis
(D) Magnified cardiomyocyte; merge channel (α-SA, WGA, DAPI)
Left Ventricle
Interventricular Septum
Right Ventricle
GFP
DAP
WG
Merge
GF
DA
PI
A
Mean Area
Maximum Area
Minimum Area
CON
T2
LV
RV
IS
Mean Area (μm2)
Maximum Area (μm2)
Minimum Area (μm²)

Figure 1. Cardiomyocyte morphology in T2 SCI and CON in LV, RV, and IS. Image A and B depicts representative images of cardiomyocyte morphology after T2-SCI and CON, respectively. Cardiomyocyte area was calculated and reported for T2-SCI vs CON in the LV, RV, and IS. Data is reported as mean, maximum, and minimum cross-sectional myocyte areas (Image 1Ci, 1Cii, and 1Ciii respectively). Image D conveys a representative myocardial tissue section that is stained with antibodies such as Wheat Germ Agglutinin (WGA)-Rhodamine (ECM; red), alpha-Sarcomeric actin (cardiomyocytes; green), and counterstained with DAPI (nuclei; blue).

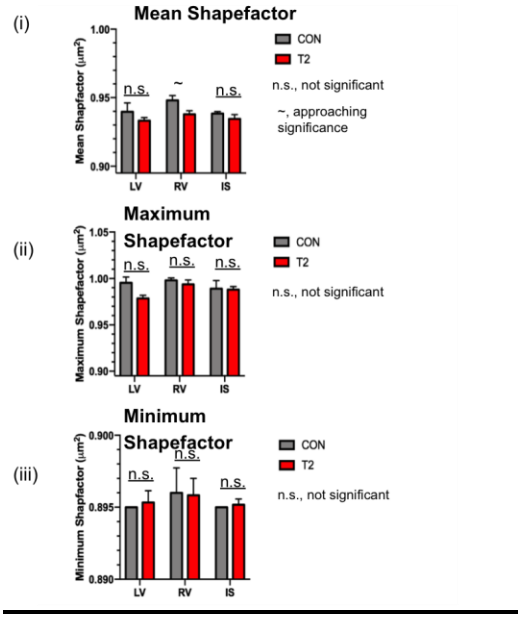

Figure 2. Shapefactor morphology after T2 contusion vs uninjured control in LV, RV, IS. The shapefactor graphs conveys the correlations between cardiomyocyte shape, including the minimum and maximum of each group, T2 SCI and CON, in LV, RV, and IS.

DISCUSSION

While there has been a great deal of SCI research focused on improving locomotor function after injury, there is limited data on cardiomyocyte morphology after SCI [28][33]. This study mimics clinically-relevant thoracic vertebra, T2, contusions to expand the data on CV morphology after SCI. The majority of previous studies utilized completely transected SC models, which do not anatomically match the majority of clinical SCI [37][42]. More than 65% of all clinical SCIs are anatomically incomplete and patients present with a functional gradient of locomotion, sensory, and autonomic phenotypes [17][39]. Luckily, SCI treatment and management have improved over the last few decades [35]. But, as patients are living longer, the incidence of life-threatening secondary complications is increasing. As such, CVD has become one of the leading causes of morbidity and mortality in chronic, high-level SCI patients [37][41]. Despite this, studies examining the link between SCI, loss of locomotor function, and the development of CVD are fairly recent. Further, the majority of published work has focused on the severe clinical manifestations of CV decline following complete, high-level lesions, such as OH and AD [28][30]. Little research has been performed to understand the morphological and molecular changes in the heart tissue itself after injury.

This study hypothesized that rodents receiving severe T2-SCI will have robust cardiac remodeling, including changes in cardiomyocyte shape and size and reduced presence of sarcomeres, or functional unit of striated muscle, as compared to CON animals. Although we did not find significant differences in cardiomyocyte morphology between T2-SCI and CON, sample sizes were small which may have prevented

extrapolation of important findings. The CV system is, in part, regulated physiologically by the two divisions of the ANS: the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). Working in tandem, the SNS and PNS utilize effector molecules such as norepinephrine (NE) and acetylcholine to regulate and maintain CV homeostasis [23]. The SPGNs that modulate cardiac function are located in the intermediolateral cell column of the SC gray matter from T1-T5 [23][41]; parasympathetic preganglionic neurons are located in the brainstem [43]. Following high-thoracic SCI, portions of the SC circuitry responsible for sympathetic modulation of CV function are isolated resulting in decentralization of the SNS [39]. The decrease in sympathetic modulation leads to changes in homeostatic maintenance of the CV system. The cardiac vagal fibers are spared following SCI, and their stimulation of cardiac structures is consequently unopposed [39], and prolonged periods of hypotension are common [39]**.** In addition, medullary controls centers of the SNS are unable to maintain efferent control over vasomotor tone and function of the heart [39]. The direct disruption of sympathetic pathways and the supraspinal control of those pathways ultimately lead to the dysregulation of HR, systolic and diastolic blood pressure, and vasoregulatory responses in daily routine situations [13][22].

After cervical and high thoracic SCI, patients experience daily bouts of hypertensive crisis due to the disruption of supraspinal control of SPGNs in the heart and surrounding vasculature [39][40]. The development of AD is most likely multifactorial, and involves the loss of bulbospinal regulation on SPGNs and aberrant reorganization and plasticity within lumbosacral circuitry [39]. Typically, episodes of AD are triggered via non-noxious visceral stimuli below the level of lesion resulting in vasoconstriction and

hypertensive episodes, accompanied by peripheral reflex-mediated bradycardia [39]. An increase in BP is accompanied by reflex-mediated bradycardia which activates the PNS, releasing acetylcholine and decreasing HR. Importantly, if left untreated, AD may result in life-threatening consequences such as stroke or cardiomyocyte infarction [9][41].

The systolic blood pressure peaks common during bouts of AD in SCI patients are similar to what is noted in patients with chronic hypertension [41]. Clinical hypertension diseases include concentric cardiac remodeling with preserved ejection fraction (EF) to dilated cardiac remodeling with reduced EF [20][41]. It has been suggested that chronic SCI patients experience concentric cardiac remodeling with reduced EF [29][37]. It is hypothesized that changes at the microscopic level, that is changes in cardiomyocytes, leads to changes at the macroscopic level. Spinal cord injury patients undergoing concentric remodeling, microscopically experience various kinase-signaling pathways that are characterized by contractile-proteins congregating into parallel units, leading to a decrease in sarcomere unit and in the length and width of individual cardiomyocytes [29][38]. As a result of cardiomyocyte stress, there is also an increase in LV wall thickness and decrease in LV diameter size during diastole and systole in hypotensive patients [37][38]. Concentric cardiac remodeling is also associated with hemodynamic cardiovascular changes, such as, decreased cardiac load from reduced blood volume, reduced sympathetic tone, and lack of skeletal-muscle pumps [29]. Although the associated macroscopic and hemodynamic cardiovascular changes after SCI have been heavily studied, there is limited data on cardiomyocyte morphology. Therefore, the exact mechanisms of concentric cardiomyocyte remodeling are unknown post SCI.

After SCI, the stimulation via SNS is decreased due to decentralization, therefore cardiac mass as well as arterial and venule tone is less maintained [37]. Post spinal cord injury the body is in a hypotensive state, meaning systemic hypotension. In addition, there is OH and periods of AD that impact the cardiac cycle. The reduced sympathetic modulation leads to an upregulation of beta-adrenergic receptors or cardiomyocyte recruitment to combat systemic and orthostatic hypotension [37][38]. There are also intact spinal reflex mechanisms that trigger a life-threatening episodic hypertension crisis that disrupts the cardiac cycle. It is the balance between prolonged hypotension and episodic hypertension coupled with cardiac unloading that drives cardiomyocyte remodeling [42].

Physiologically, the body has negative feedback loops that combat cardiovascular dysfunction. An example of this is the beta-adrenergic stimulation. In all cardiomyocytes, B1-adrenergic receptors are present, being stimulated by norepinephrine [10]. However, after SCI there is a decrease in norepinephrine due to SNS decentralization. Due to the decrease in norepinephrine, there is an upregulation of beta-adrenergic receptors or cardiomyocyte recruitment which leads to hyper-responsive beta-adrenergic receptors that increase heart rate, stroke volume, and cardiac output [37][38]. After SCI, studies have demonstrated that B-adrenergic agonists and stimulators, dobutamine and norepinephrine, remain functional, bypassing disrupted sympathetic circuitry and acting directly on cardiac beta receptors [42]. Dobutamine administration indicated that systolic function can be normalized with increased sympathetic stimulation due to an increase in HR, SV, and CO [42]. Although unknown, perhaps it is the beta-adrenergic stimulation that is responsible for

cardiomyocyte health. Other major contributors to effects on cardiomyocyte remodeling post SCI is the level of the lesion.

The level of the lesion impacts the number of intact descending sympatho-excitatory axons which maintain sympathetic cardiovascular control [29]. Severe SCI patients experienced greater reduction in LV diameter in systole and diastole, radial strain, SV, and in cardiomyocyte length and width [29]. Moderate SCI, typically below T6, experience similar afterload as severe SCI due to both injuries having similarly reduced BP. Therefore, it is hypothesized that afterload contributes less to the observed cardiac structural changes [29]. Moderate SCI experience greater preservation of descending sympathetic pathways leading to more preserved contractile function, SV, and radial strain [29] than severe SCI. To further SCI treatment, a better understanding of moderate vs severe SCI, as well as, improving data on incomplete contusion models rather than completely transected models must take place. An area that has been heavily studied to improve SCI treatment is locomotion.

Current SCI treatment focuses on further injury prevention. Patients are acutely bed ridden and wheelchair bound, as well as undergo robust cardiac decline and experience CVD more quickly [5][42]. The sudden physical inactivity of patients leads to chronic unloading associated with reduced skeletal muscle pumps, venous pooling, and overall cardiac decline. Therefore, movement maintains pre-load via muscle pumps, reduces the severity of autonomic dysreflexia, and positively impacts cardiac health [41][42]. Moving forward, researchers are expanding on the idea that movement can reduce cardiac decline and give a better blueprint to spinal cord injury treatment. For example, a study from West in 2014 implemented passive hind-limb cycling and

conveyed less reduction in systolic blood pressure and mean arterial pressure [42]. Therefore, less cardiac decline, less impact on skeletal muscle pumps, less venous pooling, and lower overall cardiac decline were all observed in the study. In addition, passive hind limb cycling prevented myocardial fibrosis and improved blood lipid profiles [39][41].

There are still questions regarding the proper timing and intensity of exercise [41][42]. Everything in life is a balance, including spinal cord injury treatment and recovery. Finding that balance is one of the most difficult challenges. Although there have been hemodynamic improvements after implementation of passive-hind limb cycling [29][38], the question of whether exercise will positively benefit cardiomyocyte morphology is still unknown. The processes associated with cardiac remodeling and the subsequent decline in CV health are complex; however, any new insights into how changes in cardiomyocyte morphology leads to cardiac demise in SCI patients help further SCI treatment and improve overall quality of life.

In this study, female rats were used to quantify the incidence of cardiac remodeling after T2-SCI contusion. Although we did not find significant differences in cardiomyocyte morphology between T2-SCI and CON, Figure 1, (images A and B) conveyed visual representations of LV, IS, and RV similarly to past studies [37]. In previous studies, T2-SCI, LV images seemed to represent less circular, wider cardiomyocytes that reduce the surface area to volume ratio making the cells less efficient. An increased collagen deposition and myocardial fibrosis indicate cardiomyocyte atrophy and reduction in contractility of the heart. Normally as shown in CON, the collagen scaffolding proteins are helping to maintain morphological and

physiological components of healthy cardiac tissue. Therefore, the gaps in cardiac tissue after severe SCI is representative of the decrease of these collagen scaffolding proteins throughout cardiomyocyte remodeling. The decrease in the amount of sarcomeres due to cardiac unloading is hard to visibly see, yet relate to the decrease in strength of contraction in the heart. In comparison, the CON, LV, image seemed to represent more circular cardiomyocytes, less collagen deposition and myocardial fibrosis, more collagen scaffolding proteins, and more sarcomeres [37]. Despite the lack of significant differences in this study, the images quantified seemed to trend toward agreeing with past studies that indicated T2-SCI undergoing concentric remodeling resulting in the most impacted sample, LV, demonstrating significant differences between T2-SCI and CON [37].

The mean, maximum, and minimum area of T2-SCI and CON cardiomyocytes along with corresponding standard deviations are conveyed in Figure 1, (images 1Ci, 1Cii, 1Ciii). Similarly, past data conveys an increase in overall cardiomyocyte area in T2-SCI [37]. The T2-SCI, LV graph conveys no significant difference in mean, maximum, and minimum area. The increase in cardiomyocyte volume without chamber increase, accompanied by increased ventricular walls in LV after T2-SCI was expected. Past studies have demonstrated that atrophied hearts may have larger volumes, yet inversely proportional weight. Therefore, atrophied hearts have weaker contractions, increased stress, increased end-systolic and end-diastolic volumes, and increased pre-load dependent oxygen demand leading to cardiovascular decline. More notable, the significantly different ($p = .013$) intra correlation conveys IS area larger than LV area in CON. Perhaps incomplete contusion models experience less robust cardiomyocyte

remodeling and mimic a more moderate-severe injury. The larger IS in CON may be due to the inactivity of the rodents. The inability to rear hindlimbs and get full myocardial contractions may reduce skeletal muscle pumps, reduce venous return, and lead to overall cardiovascular decline. As studies demonstrate that hindlimb exercise can reduce cardiac remodeling, prevent myocardial fibrosis, and improve blood lipid profiles [39], it may be plausible that a lack of movement, in post SCI and CON, leads to a reduction in LV cardiomyocyte function.

The mean, maximum, and minimum shapefactor of T2-SCI and CON cardiomyocytes along with corresponding standard deviations are conveyed in Figure 2, (images 2Ai, 2Aii, 2Aiii). Similarly, past data conveys a decrease in overall cardiomyocyte shapefactor in T2-SCI [37]. The T2-SCI, LV graph conveys no significant difference in mean, maximum, and minimum shapefactor. However, image 1Ai, conveys cardiomyocyte shapefactor RV mean approached significance, $p = 0.053$, between groups, T2-SCI and CON. The RV shapefactor significance is less notable than a significance between LV groups, T2-SCI and CON, due to physiological roles of the ventricles. After T2-SCI, cardiomyocyte morphology includes decreases in cardiomyocyte length and width which significantly correlate with decreased SV. The chronic unloading of cardiomyocytes after T2-SCI is correlated with the expected morphological changes in hypotensive patients [38].

CONCLUSION

Although the mechanisms linking SCI, cardiomyocyte remodeling, and the decline of CV health are still being elucidated, the presence of cardiomyocyte atrophy in rodents after severe SCI noted in conjunction with CV dysfunction is becoming universally accepted. The clinically-relevant contusion experiments designed facilitated an understanding of the relationships between LV, RV, and IS in relation to cardiomyocyte morphology. These correlations are important in understanding what is happening to the heart itself, in addition to orthostatic hypotension and autonomic dysreflexia. Future studies should examine multi-severity injuries to examine the correlations between groups in thoracic vertebra regions.

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