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Cardiac parasympathetic activity and ventricular diastolic interactions in individuals with spinal cord injury

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Abstract

Study design Cohort cross-sectional study.

Objective To investigate the relationship between cardiac vagal activity and left ventricular filling at rest and during vagal stimulation, via the cold face test (CFT), in individuals with spinal cord injury (SCI).

Setting University-based laboratory at Brock University, St. Catharines, ON, Canada.

Methods A total of 12 able-bodied (age: 40 ± 8.5 years) and 13 SCI individuals (age: 41 ± 8.5 years; C4-T6; AIS: A–D) were recruited. Cardiac parasympathetic activity was assessed via heart rate variability (HRV) while LV filling was assessed by conventional echocardiography. All indices of HRV and diastolic function were obtained at rest and during cardiac vagal stimulation via the CFT.

Results At baseline, the able-bodied group demonstrated strong positive correlations between HRV and early diastolic filling; however, such correlations were absent in the SCI group. The CFT resulted in elevated HRV with concomitant bradycardia in the able-bodied group, while the SCI group experienced no change in HRV or heart rate during the CFT. Able-bodied individuals showed a positive correlation between the change in HRV and the change in LV diastole during the CFT, which was attributed to increased cardiac vagal tone and not the change in heart rate, however, no relationships were observed in the SCI group.

Conclusion In able-bodied individuals, cardiac parasympathetic activity is associated with LV filling at rest and during elevated cardiac vagal tone. After SCI, there is a discord between vagal and LV diastolic activity, where changes in autonomic function do not influence LV filling, suggesting a disconnect between parasympathetic and cardiac function.

Introduction

Spinal cord injury (SCI) is a neurological disorder that results in a myriad of cardiovascular-related disorders. More recently, there has been an increased focus on the cardiac activity following SCI, both systolic and diastolic. Although

there seems to be convincing enough evidence for the presence of systolic dysfunction following refs. [1, 2], the findings regarding impaired diastolic activity remains elusive [3], as the purported attenuation in left ventricular (LV) filling is likely a result of reduced volume loading, and not a cardiac issue per se [4]. As such, the purpose of this study was not to delineate the state of diastolic function in individuals with SCI, but rather to examine the relationship between cardiac vagal tone and LV filling in able-bodied and SCI-injured persons. Correlational studies have demonstrated a relationship between cardiac parasympathetic tone, via heart rate variability (HRV), and the state diastolic function in healthy and clinical population. In healthy individuals, those with greater diastolic efficiency have higher values of HRV [5]; similarly, in clinical populations, HRV gradually decreases parallel to the deteriorating stage of diastolic dysfunction [6, 7]. These relationships are also independent of traditional predictors for cardiovascular disease [7]. More direct experimental studies

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also show that impairment of LV diastolic function and the onset of autonomic deterioration occur at approximately the same time in models of heart failure [8], while direct vagal nerve stimulation augments early LV filling with no effects on LV pressure decay [9]. As such, increasing vagal tone could serve as a potential strategy to improve LV filling efficiency.

Although the vagus nerve is anatomically dissociated from the spinal cord, individuals with SCI demonstrate impaired cardiac parasympathetic regulation [10, 11]. Thus, one could speculate that such reduced autonomic regulation could contribute to the putative alterations in diastolic function reported after SCI [3]. It is therefore reasonable to speculate that augmenting vagal outflow would improve diastolic filling. It is noteworthy to mention that the data for the current study were derived from the same participants in our previous study who did not show any altered diastolic activity [4], and therefore the purpose of this study is not to investigate the state of LV diastolic function after SCI, but rather to (1) explore the relationship between diastolic function and cardiac vagal activity at rest following SCI, and (2) determine if augmenting cardiac vagal activity would improve LV filling, which could serve as a potential strategy to improve diastolic function in those with impaired LV filling. We hypothesize that both able-bodied and SCI individuals will have a positive correlation between all indices of HRV (analogous for cardiac parasympathetic activity) and early diastolic filling, and that early diastolic filling will be enhanced during cardiac vagal stimulation. We employed the cold face test (CFT) to non-invasively stimulate cardiac vagal activity, which is an established, non-invasive strategy for increasing and assessing trigeminal-brainstem-vagal activity [12]. Although the normal systemic response to the CFT is a rise in blood pressure due to increased vascular sympathetic outflow, the typical *cardiac* response is pronounced bradycardia due to augmented cardiac vagal tone [12, 13]. Such bradycardia is independent of the rise in blood pressure and/or the adrenergic phase of the CFT [14, 15], making it a suitable test to assess cardiac vagal regulation in individuals with disordered cardiovascular sympathetic control.

Methods

Participants

A total of 12 able-bodied (AB) and 13 individuals with traumatic SCI (C4-T6; ASIA (American Spinal Injury Association) Impairment Scale (AIS) A–D; 16.5 ± 9.7 years post injury) participated in this study. Individuals from both groups were recruited by means of posted advertisement

and word of mouth. Both groups were matched for age and body mass, none had a history of cardiovascular disease, with normal resting and exercise electrocardiographs (ECGs). Participants from both groups included a range from sedentary to physically active but none were highly trained or competitive athletes. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

Study protocol

All participants were required to void their bladder and bowel prior to testing. All medications for individuals with SCI were taken as prescribed during the day of testing. Medications that the participants were taking included baclophen ($n = 11$) for spasms as well as anti-diuretics ($n = 7$) and alpha agonists ($n = 11$) for hypotension. Participants laid in the left lateral decubitus position during the pre-test resting period as well as throughout the testing, as changes in body position can alter cardiac autonomic activity [16]. After a 10-min resting period, 5 min of baseline ECG data were collected for later analysis of baseline cardiac parasympathetic activity (HRV). Following baseline ECG collection, LV diastolic function was assessed via conventional echocardiography. Continuous ECGs were then recorded immediately before (Pre-CFT) and during the CFT in order to assess changes in cardiac parasympathetic activity. To obtain optimal 4-chamber view during the CFT, the cardiac probe was continuously placed on the apical window in order to keep the 4-chamber apical view in sight for concomitant assessment diastolic function. Blood pressure was measured from the brachial artery before and directly after the CFT using an automated pressure cuff.

Cardiac autonomic activity

A one-lead (lead II) ECG was used for the collection of continuous heart rate (HR) and later analysis of cardiac parasympathetic activity (Power Lab, Lab Chart 7, AD Instruments). Electrocardiographic data were collected at a sampling frequency of 1000 Hz and ECGs were amplified using a special ECG signal amplifier from the manufacturer (AD Instruments Bioamp). Cardiac parasympathetic activity was estimated from these ECG data by analyzing the signals for HRV which was done by RR interval peak detection (HRV add-on software; Lab Chart 7, AD Instruments). Baseline HRV was evaluated from a 5-min ECG epoch collected at rest, indices included the natural log of the high-frequency power (HFln), the standard deviation of continuous RR intervals (SDNN), the root mean square of differences between RR intervals (RMSSD) and coefficient of variance (CV).

Echocardiography

All echocardiographic images were obtained using a commercially available ultrasound system (Vivid Q; GE Vingmed Ultrasound AS, Horten, Norway) with a 1.5-MHz phased-array transducer. All images were acquired by a single experienced sonographer and were stored for later offline analysis using a commercially available software (EchoPac version 6.0; GE Vingmed Ultrasound AS). Images were acquired while participants were in the left lateral decubitus position at end expiration and 3 consecutive cycles were stored for later analysis. Participants were instructed not to move during the CFT, as this could influence image quality. When an optimal image was obtained, the CFT was initiated for concomitant measures of diastole. Measures of diastolic function were calculated from the average of the three consecutive cycles. Pulsed-wave Doppler was used to assess diastolic function according to the recommendation of the American Society of Echocardiography [17]. For mitral inflow velocity, from an apical 4-chamber view, a 4 mm sample volume was placed between the mitral valve leaflet tips during diastole. Diastolic measures included peak early (E) and late (A) transmitral inflow velocities as well as their ratio (E:A).

Cold face test

Packs of ice-water slush (1.2 °C) were placed on the forehead and bilateral maxillary areas for 1 min. Care was taken to avoid the eyes in order to prevent stimulation of the oculo-cardiac vagal reflex. Participants were verbally instructed to maintain their normal breathing rate during the test, as breath holding may augment the bradycardia during facial cooling [14]. In order to account for the potential influence of autonomic excitement immediately before the onset of the CFT, the Pre-CFT data were obtained from a 1-min ECG recording collected 2 min prior to the start of facial cooling. The CFT data were collected for 1 min while the cold packs were in contact with the participant's face. Although HRV measures are typically analyzed from 5 min epochs, a 1 min epoch is sufficient to reflect vagal changes during the CFT, as maximal bradycardia is reached between 40 and 60 s [18]. In addition, the test cannot be prolonged due to discomfort. The change in HRV indices from the Pre-CFT to the CFT periods was used to assess changes in cardiac parasympathetic activity.

Statistical analysis

A Shapiro–Wilks test was performed to test for data normality, which warranted for parametric statistics. Unpaired *t*-tests were used to test for between-group differences in participant characteristics, baseline autonomic and

ventricular parameters. Pearson's correlations were used (for each group separately) to assess relationships between baseline cardiac parasympathetic activity and diastolic. A stepwise backwards elimination multiple regression was performed in order to determine if changes in LV filling during the CFT were a function of change in Heart rate or HRV. Levels of *F* to enter and *F* to remove were set to correspond to *p* levels of 0.05 and 0.10, respectively. Analysis was run for all participants, as well as able-bodied alone and SCI only. A 2-way repeated measures analysis of variance was used to detect any group by condition interactions for autonomic and diastolic function, and Tukey's post-hoc analysis was performed if interactions were found. Data are presented as means \pm standard deviation, and all statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software. Statistical significance was set at $p \leq 0.05$.

Results

Participants

All participant characteristics are provided in Table 1. There were no between-group differences in age, body mass or body mass index (BMI).

Baseline autonomic and diastolic function

There were no between-group differences in resting HR (AB: 57 ± 9 . vs. SCI: 62 ± 9.0 ; $p = 0.21$), Hfln (AB: 5.9 ± 1.4 vs. SCI: 6.2 ± 1.3 ; $p = 0.53$), SDNN (AB: 55.3 ± 22.4 vs. SCI: 60.6 ± 23.1 ; $p = 0.56$), RMSSD (AB: 42.1 ± 22.7 vs. SCI: 48.2 ± 27.1 ; $p = 0.55$) or CV (AB: 7.1 ± 2.2 vs. SCI: 6.4 ± 2.6 ; $p = 0.31$). Similarly, resting diastolic function was not different between groups, as shown by similar E (AB: 0.74 ± 0.1 vs SCI: 0.75 ± 0.1 ; $p = 0.51$), A (AB: 0.38 ± 0.1 vs SCI: 0.44 ± 0.1 ; $p = 0.76$) and E:A (AB: 2.1 ± 0.6 vs. SCI: 1.8 ± 0.6 ; $p = 0.72$). Further, as expected, able-bodied individuals demonstrated positive correlations between measures of HRV and early diastolic filling (Table 2), while the SCI group failed to show any correlations between autonomic and diastolic activity (Table 2).

Autonomic and diastolic response to the CFT

The able-bodied group exhibited a significant increase in systolic blood pressure during the CFT (105 ± 6 mm Hg to 114 ± 16 mm Hg; $p = 0.04$), while those with SCI experienced an increase that did not reach statistical significance (101 ± 12 mm Hg to 112 ± 10 mm Hg; $p = 0.07$). During the CFT, the able-bodied group had an immediate and significant reduction in HR (Fig. 1a), with a concomitant

Table 1 Participant characteristics

Participant	Age (years)	Sex	Body mass (kg)	BMI (kg/m ²)	HR (bpm)	SBP	Level	AIS	YPI
SCI									
1	55	M	80.7	26.8	73	101	C4	A	8
2	42	M	68.2	20.8	53	84	C4	C	18
3	31	F	64.0	22.7	61	96	C5	B	6
4	46	M	83.0	28.7	65	112	T6	A	19
5	23	M	83.2	27.8	57	115	C8	B	5
6	43	M	81.0	27.1	43	119	C4	A	6
7	46	F	61.0	22.7	73	109	C7	A	9
8	37	M	100.0	31.6	70	100	C5	B	18
9	38	M	96.4	25.9	54	95	C5	C	38
10	54	M	95.0	29.3	55	93	C5	D	27
11	38	F	56.0	21.1	64	82	T3	A	20
12	41	M	66.0	24.2	77	100	T5	A	16
13	44	M	91.0	27.2	67	111	T4	A	24
AVG	41.38		78.9	25.8	62	101.3	—	—	16.5
SD	8		14.6	3.3	9	12.2	—	—	9.7
AB									
1	44	M	94.3	26.3	50	115	—	—	—
2	44	M	70.1	24.8	47	106	—	—	—
3	35	F	57.2	21.2	75	104	—	—	—
4	35	M	76.0	24.5	62	103	—	—	—
5	41	M	102.0	30.5	51	117	—	—	—
6	40	M	67.6	23.7	89	110	—	—	—
7	30	F	57.4	20.9	63	102	—	—	—
8	33	M	91.1	27.8	51	95	—	—	—
9	54	M	102.1	27.4	62	103	—	—	—
10	51	M	97.1	28.9	54	109	—	—	—
11	46	M	80.3	24.7	71	107	—	—	—
12	24	M	80.0	26.1	60	93	—	—	—
AVG	39.75		81.08	25.6	61.25	105.4	—	—	—
SD	8		16.25	2.9	9	6.7	—	—	—
<i>P</i> value	0.64	—	0.72	0.84	0.22	0.31	—	—	—

AB able-bodied, *AIS* ASIA (American Spinal Injury Association) impairment scale, *BMI* body mass index, *HR* heart rate, *SCI* spinal cord injury, *YPI* years post injury

increase in HRV (Fig. 2a, c, e). In contrast, the SCI group demonstrated a blunted autonomic response during the CFT, with no change in HR (Fig. 1b) or HRV (Fig. 2b, d, f).

Both groups showed no changes in mean diastolic values during the CFT (Table 3). Furthermore, as hypothesized, the able-bodied group showed positive correlations between the change in vagal tone (HFln and SDNN) and the change in E during the CFT (Table 4). In comparison, the SCI group showed no correlations between the change in HRV and the change in diastole during the CFT (Table 4). Results from the regression show that for the able-bodied group, the change in E was a function of the change in SDNN ($R = 0.648$, $R^2 = 0.420$). The overall *F*-statistic for the model was 7.249, $d = 1, 11$, $p = 0.02$ and standardized beta weight

was 0.648 for change in SDNN. This demonstrates that approximately 42% of the change in diastolic function could be explained by the change in SDNN. However, the model for the SCI group was not significant, indicating no relationship between HRV and LV filling. When all participants were pooled, SDNN accounted for approximately 25% of the change in diastole, where a one unit increase in SDNN was related to a 0.589 unit increase in E.

Discussion

The main findings of the current investigation are that (1) at rest, able-bodied individuals demonstrate positive

correlations between HRV and early diastolic filling velocity, but these correlations are absent in persons with SCI; (2) the able-bodied group experienced pronounced bradycardia and elevated HRV during the CFT, while the SCI group had a blunted autonomic response; and (3) an increase in cardiac vagal tone during the CFT is associated with an increase in early LV filling in able-bodied individuals, but this relationship is also absent in SCI persons. These findings present evidence for discordant communication between the vagus nerve and cardiac function (both chronotropy and lusitropy) after SCI. Cardiovascular

autonomic dysfunction after SCI is conventionally thought of as a sympathetic issue, due to the blunted transmission of excitatory signals from sympathetic pre-ganglionic neurons onto their target organs; however, this research sheds light on impairments in the *parasympathetic* limb of cardiovascular control after SCI.

Baseline autonomic–diastolic interactions

At baseline, able-bodied individuals demonstrated a positive correlation between early diastolic filling and measures of HRV, suggesting that individuals with greater cardiac vagal tone activity also display superior diastolic filling. This is consistent with previous reports from healthy [5] and clinical populations [6, 7] as well as experimental models [9]. Although we hypothesized that such a relationship would be observed in the SCI group, this was not the case, as correlations between HRV and diastolic function were completely absent following SCI, suggesting that LV diastole may be operating completely independent from parasympathetic modulation.

Table 2 Correlations between baseline cardiac parasympathetic activity and diastolic function

	AB	SCI
	E	E
HFln	$r = 0.54; p = 0.06$	$r = 0.21; p = 0.50$
SDNN	$r = 0.61; p = 0.03^*$	$r = 0.18; p = 0.51$
RMSSD	$r = 0.75; p = 0.003^*$	$r = 0.28; p = 0.36$
CV	$r = 0.61; p = 0.03^*$	$r = 0.12; p = 0.69$
	A	A
HFln	$r = -0.02; p = 0.96$	$r = 0.03; p = 0.92$
SDNN	$r = -1.55; p = 0.63$	$r = 0.24; p = 0.43$
RMSSD	$r = -0.23; p = 0.47$	$r = 0.08; p = 0.79$
CV	$r = 0.17; p = 0.59$	$r = -0.21; p = 0.48$
	E:A	E:A
HFln	$r = 0.15; p = 0.62$	$r = 0.13; p = 0.66$
SDNN	$r = 0.36; p = 0.23$	$r = -0.05; p = 0.86$
RMSSD	$r = 0.70; p = 0.01^*$	$r = 0.11; p = 0.72$
CV	$r = -0.31; p = 0.31$	$r = 0.31; p = 0.31$

AB able-bodied, SCI spinal cord injury, HFln natural log of the high-frequency power spectrum, SDNN standard deviation of continuous RR intervals, RMSSD root mean square of differences between RR intervals, E early transmitral velocity, A late transmitral velocity, E:A ratio of early to late transmitral velocity, CV correlation coefficient

*Significant correlation ($p \leq 0.05$)

Autonomic and diastolic responses to the CFT

Regarding the second major finding of the study, the able-bodied group demonstrated the typical cardiac response to the CFT, pronounced bradycardia and elevation in HRV [12–15]. In contrast, these autonomic responses to the CFT were blunted in the SCI group, with no change in average HR or HRV, indicating impaired trigeminal-brainstem-vagal function. This is in agreement with observations from Wecht et al. [10], who showed no change in HR response to the CFT after SCI; however, some of their participants also experienced tachycardia, which was demonstrated by two of our SCI participants. The SCI group contained individuals with different injury levels and completeness, suggesting varying degrees of cardiovascular sympathetic regulation.

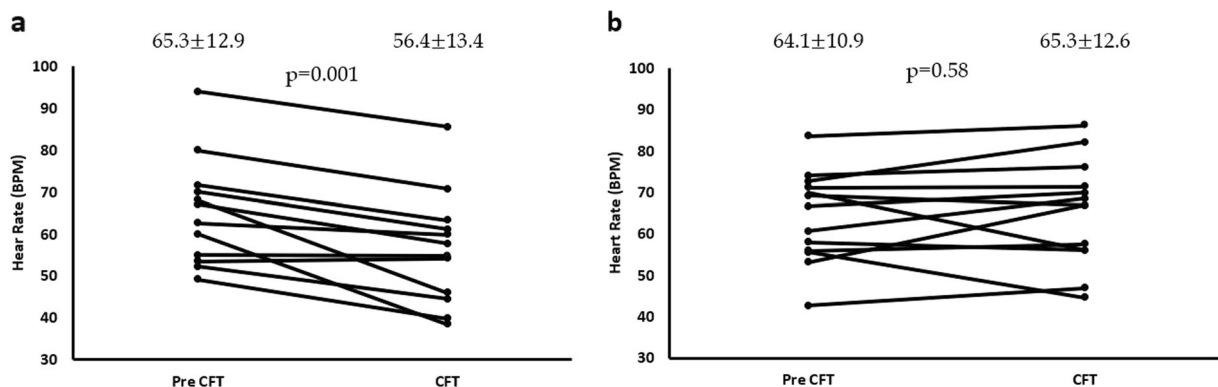


Fig. 1 **a** Heart rate during CFT in able-bodied individuals. CFT cold face test. **b** Heart rate during CFT in spinal cord injured individuals. CFT cold face test

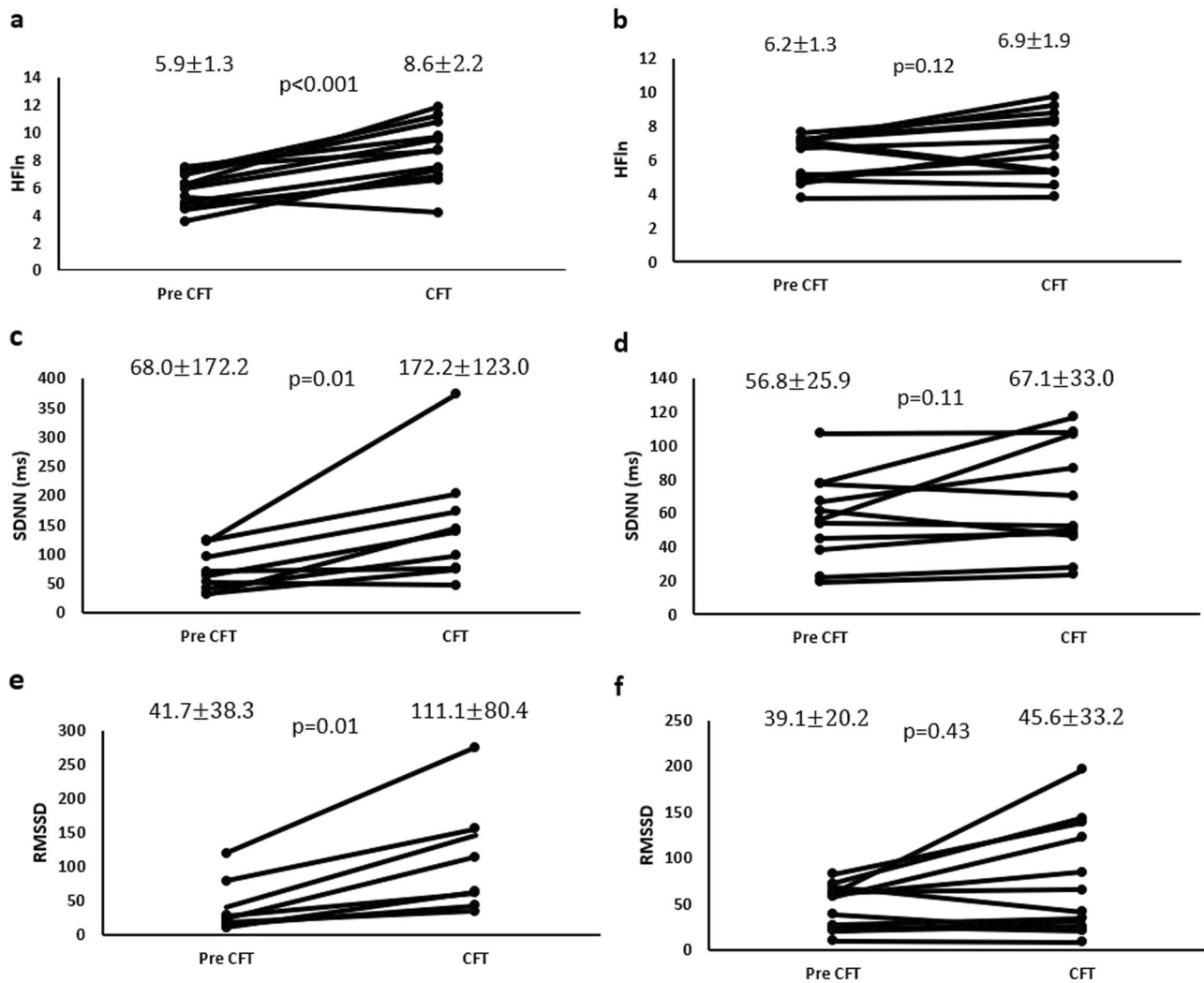


Fig. 2 a, c, e Heart rate variability during the CFT in able-bodied individuals. CFT cold face test, HFIn natural log of high-frequency domain, SDNN standard deviation of consecutive RR intervals, RMSSD root mean square of differences between RR intervals. **b, d, f**

Heart rate variability during the CFT in spinal cord injured individuals. CFT cold face test, HFIn natural log of high-frequency domain, SDNN standard deviation of consecutive RR intervals, RMSSD root mean square of differences between RR intervals

Table 3 Left ventricular diastolic function in response to the CFT

	Pre-CFT	CFT	<i>P</i> value (interaction)
E(m/s)			
AB	0.74 ± 0.1	0.71 ± 0.1	0.51
SCI	0.75 ± 0.1	0.70 ± 0.1	
A(m/s)			
AB	0.38 ± 0.1	0.41 ± 0.1	0.76
SCI	0.44 ± 0.1	0.44 ± 0.1	
E:A			
AB	2.1 ± 0.6	1.8 ± 0.6	0.72
SCI	1.8 ± 0.6	1.7 ± 0.5	

AB able-bodied, SCI spinal cord injury, E early transmitral velocity, A late transmitral velocity, E:A ratio of early to late transmitral velocity, DT deceleration time

Despite these purported difference in sympathetic regulation, we do not believe it influenced the cardiac response to the CFT, as the cholinergic phase of the CFT operates independently from any rise in sympathetic activity or blood pressure [14, 15]. It is also noteworthy to mention that baseline HR and HRV were similar between groups, but impaired cardiac vagal regulation was evident only when the autonomic system was challenged. According to these observations, we posit that after SCI, cardiac vagal tone is sufficient enough to maintain normal HR oscillations only at rest, but once the autonomic system is challenged or provoked, it is unable to modulate HR accordingly. Therefore, “normal” baseline autonomic values in persons with SCI should be interpreted with caution, as they do not necessarily suggest absence of pathology.

Table 4 Correlations between change in cardiac vagal activity and change in diastole during the CFT

	AB	SCI
	ΔE	ΔE
$\Delta HFln$	$r = 0.60; p = 0.05^*$	$r = -0.52; p = 0.08$
$\Delta SDNN$	$r = 0.65; p = 0.02^*$	$r = -0.10; p = 0.97$
$\Delta RMSSD$	$r = 0.46; p = 0.14$	$r = -0.13; p = 0.66$
ΔCV	$r = 0.30; p = 0.37$	$r = 0.01; p = 0.97$
	ΔA	ΔA
$\Delta HFln$	$r = 0.20; p = 0.54$	$r = -0.53; p = 0.06$
$\Delta SDNN$	$r = -0.02; p = 0.95$	$r = -0.18; p = 0.55$
$\Delta RMSSD$	$r = -0.03; p = 0.92$	$r = -0.18; p = 0.55$
ΔCV	$r = -0.25; p = 0.47$	$r = -0.26; p = 0.40$
	$\Delta E:A$	$\Delta E:A$
$\Delta HFln$	$r = 0.16; p = 0.62$	$r = 0.02; p = 0.95$
$\Delta SDNN$	$r = 0.43; p = 0.16$	$r = 0.11; p = 0.73$
$\Delta RMSSD$	$r = 0.42; p = 0.17$	$r = -0.02; p = 0.94$
ΔCV	$r = 0.61; p = 0.04^*$	$r = 0.22; p = 0.47$

AB able-bodied, SCI spinal cord injury, Δ change, E early transmitral velocity, $HFln$ natural log of the high-frequency spectrum, $SDNN$ standard deviation of continuous RR intervals, $E:A$ ratio of early to late transmitral velocity, DT deceleration time, $RMSSD$ root mean square of differences between RR interval, CV coefficient of variance

*Significant correlation ($p \leq 0.05$)

Regarding the third finding of the study, the CFT was used in concert with assessing diastolic filling in order to understand diastolic–vagal interactions. We hypothesized that augmentation of cardiac parasympathetic activity during the CFT would enhance early diastolic filling [9]. Indeed, this was evident in able-bodied individuals, although it was a group trend and not an individual-based response, which explains the no change in average diastolic function during the CFT. The regression analysis demonstrates that the change in cardiac vagal activity ($SDNN$) was accountable for the changes in diastolic activity during the CFT. Although the exact mechanisms of how cardiac vagal tone increases LV filling are unknown, an earlier study in dogs showed that increasing vagal activity augments left atrial pressure which enhances ventricular filling [9]. In contrast, the SCI group did not show any changes in diastolic filling during the CFT, which was not surprising, as HRV was not altered during the challenge; in addition, the regression model (as well as unreported Pearson's correlations) showed no relationship between HRV and diastolic activity. Notwithstanding cardiac vagal tone's influence on diastole as the main focus of this study, another possible mechanism for the discrepancy in diastolic response to the CFT between groups is the between-group differences in vascular sympathetic activation. The CFT caused a significant increase in systolic blood pressure in the able-bodied individuals, which was likely accompanied by

augment venous return, which would enhance LV filling. The SCI group demonstrated a non-significant increase in blood pressure, likely due to impaired, or partially impaired, vascular sympathetic outflow, likely causing less venous return, and therefore did not enhance early diastolic filling. Accordingly, the reported autonomic–diastolic responses are likely due to a combination of differences in cardiac vagal and vascular sympathetic activity. An unanswered question that would be interesting to study is how would diastolic function respond if parasympathetic activity was blocked? Based on previous studies, able-bodied individuals are expected to experience a reduction in diastolic filling [19, 20]; however, would those with SCI not respond? This can provide additive evidence for a dissociation between parasympathetic and cardiac function following SCI, especially that our regression analysis showed no relationship between the change in HRV and the change in diastole. The long-term goal of this experiment was essentially to determine if cardiac vagal stimulation can be used as a form of therapy to enhance diastolic function after SCI, but this may not be efficacious as parasympathetic activity appears to fail at modulating cardiac function in this population. However, the idea of using vagal stimulation as a potential form of therapy should not be discounted on the basis of these results alone, since the CFT is a non-invasive and superficial method of doing so. Future studies could employ direct vagal stimulation or M1-muscarinic agonists to conduct a more direct investigation of the relationship between parasympathetic and cardiac function. Indeed, it is difficult to ascertain the cause for such vagal–diastolic alterations in this SCI cohort from the current ECG measures, as HRV is a downstream measure that reflects the collective action of supraspinal activity, vagus nerve function, cardiac cholinergic receptor activity and cardiac electrical activity. As such, the observed diastolic–vagal impairment can be a result of abnormal activity in any of these stages (which may have also resulted in the paradoxical HR responses), and further investigation is warranted on elucidating the source of such impairments.

Limitations

The first limitation of the study was the relatively small number of participants for a correlational study; thus, a greater number of participants would have better substantiated our results. Second, although the CFT results in pronounced bradycardia and increases cardiac vagal outflow, it is an indirect strategy for vagal stimulation, and has similar concomitant effects on vascular sympathetic activity. Therefore, a better method to understand isolated vagal–diastolic dynamics would be direct vagal stimulation to circumvent the potential influence of sympathetic activity. Third, we did not perform any tests for autonomic

completeness, and therefore we do not know if varying degrees of autonomic control influenced vagal–diastolic responses; however, individual data suggest it did not. Additionally, differences in sympathetic regulation may have not affected HR or HRV during the CFT [14, 15]; however, differences in vascular activation may have impacted venous return and LV filling indirectly.

Conclusion

Resting cardiac vagal outflow is associated with early LV filling in able-bodied individuals, but this relationship is lost after SCI. In addition, individuals with SCI demonstrated impaired trigeminal-brainstem-vagal activity, as they did not experience a change in HR or HRV during the CFT. Able-bodied individuals exhibited augmented diastolic function during the CFT, while those with SCI had no change in diastole, indicating impaired vagal modulation over cardiac activity after SCI.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Squair JW, DeVeau KM, Harman KA, Poormasjedi-Meibod MS, Hayes B, Liu J, et al. Spinal cord injury causes systolic dysfunction and cardiomyocyte atrophy. *J Neurotrauma*. 2017;35:424–34.
2. Squair JW, Liu J, Tetzlaff W, Krassioukov AV, West CR. Spinal cord injury-induced cardiomyocyte atrophy and impaired cardiac function are severity dependent. *Exp Physiol*. 2018;103:179–89.
3. Driussi C, Ius A, Bizzarini E, Anonini-Canterin F, d'Andrea A, Bossone E, et al. Structural and function left ventricular impairment in subjects with chronic spinal cord injury and no overt cardiovascular disease. *J Spinal Cord Med*. 2014;37:85–92.
4. Sharif H, Wainman L, O'Leary D, Ditor D. The effect of blood volume and volume loading on left ventricular diastolic function in individuals with spinal cord injury. *Spinal Cord*. 2017;55:753–8.
5. Antelmi I, Yamada AT, Hsin CN, TsuTsui JM, Grupi CJ, Mansu AJ. Influence of parasympathetic modulation in Doppler mitral inflow velocity in individuals without heart disease. *J Am Soc Echocardiogr*. 2010;23:762–5.
6. Poanta L, Porojan M, Dumitrascu DL. Heart rate variability and diastolic dysfunction in patients with type 2 diabetes mellitus. *Acta Diabetol*. 2011;48:191–6.
7. Poulsen SH, Jensen SE, Møller JE, Egstrup K. Prognostic value of left ventricular diastolic function and association with heart rate variability after a first acute myocardial infarction. *Heart*. 2001;86:376–80.
8. Ishise H, Asanoi H, Ishizaka S, Joho S, Kameyama T, Umeno K, et al. Time course of sympathovagal imbalance and left ventricular dysfunction in conscious dogs with heart failure. *J Appl Phys*. 1998;84:1234–41.
9. Xenopoulos NP, Applegate RJ. The effect of vagal stimulation on left ventricular systolic and diastolic performance. *Am J Physiol*. 1994;266:H2167–73.
10. Wecht JM, Weir JP, DeMeersman RE, Schilero GJ, Handrakis JP, LaFontaine MF, et al. Cold face test in persons with spinal cord injury: age versus inactivity. *Clin Auton Res*. 2009;19:221–9.
11. Wecht JP, Weir JP, Bauman WA. Blunted heart rate response to vagal withdrawal in persons with tetraplegia. *Clin Auton Res*. 2006;16:378–83.
12. Khurana RK, Watabiki S, Hebel JR, Toro R, Nelson E. Cold face test in the assessment of trigeminal-brainstem-vagal function in humans. *Ann Neurol*. 1980;7:144–9.
13. Stemper B, Hilz MJ, Rauhut U, Neundorfer B. Evaluation of cold face test bradycardia by means of spectral analysis. *Clin Auton Res*. 2002;12:78–83.
14. Khurana RK, Wu R. The cold face test: a non-baroreflex mediated test of cardiac vagal function. *Clin Auton Res*. 2006;16:202–7.
15. Khurana RK. Cold face test: adrenergic phase. *Clin Auton Res*. 2007;17:211–6.
16. Chen GY, Kuo CD. The effect of the lateral decubitus position on vagal tone. *Anaesthesia*. 2009;52:653–7.
17. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Kokanish H, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur Heart J Cardiovasc Imag*. 2016;17:1321–60.
18. Heath ME, Downey JA. The cold face test (diving reflex) in clinical autonomic assessment: methodological considerations and repeatability of responses. *Clin Sci*. 1990;78:139–47.
19. Johannessen KA, Cerqueira M, Veith RC, Stratton JR. Influence of sympathetic stimulation and parasympathetic withdrawal on Doppler echocardiographic left ventricular diastolic filling velocities in young normal subjects. *Am J Cardiol*. 1991;67:520–6.
20. Stratton JR, Levy WC, Caldwell JH, Jacobson A, May J, Matsuoka D, et al. Effects of aging on cardiovascular responses to parasympathetic withdrawal. *J Am Coll Cardiol*. 2003;41:2077–83.