Assessing Heart Rate Variability As a Surrogate Measure of Cardiac Autonomic Function in Chronic Traumatic Spinal Cord Injury

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Background: Although cardiac autonomic dysfunction is a contributing factor for cardiovascular disease development in individuals with a spinal cord injury (SCI), it remains poorly understood. Heart rate variability (HRV) analysis has the potential to non-invasively assess the cardiac autonomic nervous system. The study objectives are (a) to determine if there are differences in HRV measures across neurological level of impairment (NLI) and American Spinal Cord Injury Association Impairment Scale (AIS) subgroups, and (b) to determine if there is a relationship between HRV frequency measures (low frequency [LF] and high frequency [HF]) at rest. **Methods:** We conducted a secondary data analysis of a primary data set from a published cross-sectional study of electrocardiogram recordings of 56 subjects (44 men and 12 women, mean age ± *SD* = 46.75 ± 12.44 years) with a chronic traumatic SCI (C1-T12, AIS A-D, ≥2 years post injury). HRV was analyzed using time and frequency domain measures. **Results:** There were no significant HRV differences across NLI and AIS subgroups. The LF and HF indices were positively correlated in the entire sample ($r = 0.708$, $p < .0001$) and among impairment subgroups. **Conclusion:** No differences were observed in the HRV time and frequency measures when compared across NLI and AIS subgroups. The results were considered inconclusive, since possible explanations include inadequate sample size as well as other physiological considerations. A positive correlation was found between LF and HF when assessed at rest. The relationship between LF and HF may not necessarily represent a rebalanced autonomic nervous system, but it does question the utility of solely measuring LF:HF at rest in persons with chronic SCI.
Key words: *autonomic nervous system, cardiovascular disease, electrocardiography, heart rate, spinal cord injuries*

The odds of developing cardiovascular disease (eg, arrhythmias, heart failure, and myocardial infarction) is approximately 4 times greater in individuals with a spinal cord injury (SCI) than their age-matched peers without SCI.[1] A possible reason for the observed increase in cardiovascular disease (CVD) could be the disruption of the cardiovascular autonomic nervous system (ANS) below the neurological level of the injury.[1-5] The underlying physiological mechanisms responsible for ANS disruptions in SCI are not fully determined.[6] Cardiovascular autonomic disruption has been reported to increase the risk of developing CVD in individuals with SCI.[2-5]

Heart rate variability (HRV) has the potential to non-invasively measure modulation of the cardiac ANS.[7] Standardized guidelines for evaluating HRV, regardless of clinical population, were developed in 1996 by an International Task Force.[7] These guidelines recommend the routine use of time and frequency domain measures,[7]

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and each measure is hypothesized to reflect unique physiological phenomenon. However, not all HRV measures are well established, so only HRV measures with a suggested physiological interpretation were included in this study. For example, the recommended time domain measures consist of the square root of the mean squared differences (RMSSD) of the consecutive normal R peak to normal R peak (NN) intervals in an electrocardiogram (ECG), and the proportion of the number of interval differences of the consecutive NN intervals greater than 50 ms (pNN50) are reported to be reflective of cardiac parasympathetic modulation.[8-10] In contrast, among the frequency domain measures, the high frequency (HF) component has been reported to reflect cardiac parasympathetic modulation,[9,11-14] while interpretation of the physiological consequences of the low frequency (LF) component is still very controversial. Some claim that LF is both a marker of parasympathetic and sympathetic modulation,[9,11-14] whereas others claim that it is mainly indicative of sympathetic modulation.[2,15,16] Despite the fact that LF is considered a controversial measure, an LF:HF ratio is commonly used as a measure of the sympatho-vagal balance of the cardiac ANS.[2,15,17-21] To our knowledge, there are no universally accepted HRV measures that reflect sympathetic function. Consequently in this study, HRV was assessed to describe the overall disruption of the cardiac ANS at rest in individuals with a chronic traumatic SCI.

It is unclear whether an association exists between the neurological level of injury (NLI), severity of injury (American Spinal Injury Association [ASIA] Impairment Scale [AIS]), and HRV. To date, men with motor complete paraplegia are overrepresented in the literature, with less written about those with tetraplegia or incomplete AIS CD impairment. Nonetheless, given that ANS dysfunction, blood pressure dysregulation, and CVD have been reported to be linked with NLI and AIS,[4-6,22] we hypothesized that HRV will differ across impairment cohorts.[4-6,22] Because the sympathetic autonomic control will be altered below the level of injury potentially unbalancing the overall cardiac ANS, HRV is expected to be disrupted in individuals with an SCI above the level of T1 and the degree of disruption is expected to be greater in those with complete (classified in this article as AIS AB) versus incomplete (classified as AIS CD) injuries. Overall, the objectives of this study were 2-fold: (1) to determine whether there is a difference in HRV indices based on NLI and AIS (above T1 AIS AB, above T1 AIS CD, equal to/below T1 AIS AB, equal to/below T1 AIS CD) and (2) to determine whether there is a relationship between LF and HF indices within the chronic traumatic SCI population. Given the proposed physiological interpretation of the LF and HF indices, an examination of the relationship between the 2 indices may explain how the sympathetic and parasympathetic systems adapt over time in individuals with chronic SCI.

Methods

Study design

This study was a secondary data analysis of a primary data set from a published cross-sectional study that explored the associations between arterial stiffness and spinal cord impairment.[23] The primary study inclusion criteria were English-speaking subjects between 18 and 80 years of age living in Canada (Toronto, Ontario) with a chronic SCI (C1-T12, AIS A-D, ≥2 years post injury) of traumatic or nontraumatic etiology.[23] The primary study exclusion criteria were subjects with a previous or current history of angina, myocardial infarction, atypical chest pain, coronary artery bypass or revascularization, aortic stenosis, uncontrolled arrhythmia or left bundle branch block, hypertrophic cardiomyopathy, severe chronic obstructive pulmonary disease requiring oral steroids or home oxygen, diaphragmatic pacer, and stroke.[23] Subjects underwent medical screening, electrocardiogram, and chart review to ensure that they met the inclusion and exclusion criteria.[23] Overall, 125 potential subjects were screened, 100 consented to participate, 10 withdrew consent, and 3 did not meet the inclusion criteria; thus the final sample consisted of 87 subjects.[23] For the secondary data analysis, ECG data were required to analyze HRV; 75 out of the 87 subjects had ECG data collected. The secondary data analysis study

was approved by the University Health Network Research Ethics Board and by the University of Toronto, Office of Research Ethics. We certify that all applicable institutional regulations concerning the ethical use of human volunteers were followed during the course of this research.

Data collection

Prior to ECG data collection, subjects refrained from caffeine, nicotine, and food for at least 8 hours and refrained from exercise for 24 hours. Subjects were transferred to a supine position onto a bed in a quiet and temperature controlled (24°C) room and rested for 20 minutes before the collection from a continuous 3-lead ECG (lead II system) for 10 minutes, at a sampling rate of 1000 Hz (PowerLab/16SP; AD instruments, Inc., Bella Vista, Australia).[23] ECG was collected and analyzed in accordance with the task force guidelines for HRV analysis. The time domain measures (RMSSD and pNN50) were derived directly from the NN intervals on the ECG, while the frequency domain measures (LF, HF, LF:HF) were derived using a nonparametric mathematical algorithm known as fast Fourier transform. All of the ECG recordings were visually reviewed, with the assistance of an internist with expertise in cardiovascular stress testing and ECG monitoring (Dr. P. Oh), for rate and rhythm (normal sinus rhythm, bradycardia, or tachycardia), presence of premature atrial and/or ventricular contractions, electrical artifact, and visible variability in the RR intervals. If the subject displayed frequent premature contractions (greater than 10 per minute), arrhythmias, or excessive artifact that prevented accurate analysis of the RR intervals, he or she was excluded from the dataset for detailed analysis. Also, subjects taking medications that could influence HRV (beta-blockers, calcium channel blockers, and any other cardiac anti-arrhythmic drugs) were excluded from the study.

Participants

The primary data set consisted of 75 people living with an SCI of nontraumatic or traumatic etiology. Persons living with a nontraumatic SCI (n = 13) were excluded from the data analysis in the current study due to differences in injury etiology, age of injury onset, and comorbidities. Three subjects were excluded after the review of ECG recordings: 2 subjects displayed frequent premature ventricular contractions (PVCs ≥10/min), and one was excluded due to ECG technical difficulties. Based on medication intake, 3 subjects were excluded: 2 subjects were on beta-blockers, and one subject was taking both a beta-blocker and a calcium channel blocker. The final sample size of 56 subjects was divided into 4 subgroups (**Figure 1**) based on NLI and AIS derived from the International Standards for Neurological Classification of Spinal Cord Injury.[24] The NLI was identified as either above T1 or equal to/below T1. The severity of injury (AIS) indicated whether there was complete or incomplete disruption of the sensory or motor function in the lowest sacral segments (S4-S5).[24] The total sample consisted of 79% male; for the subgroups, AIS AB and NLI above T1 was 88% male, AIS AB and NLI equal to/below T1 was 76% male, AIS CD and NLI above T1 was 78% male, and AIS CD and NLI equal to/below T1 was 67% male. Additional demographic and clinical characteristics of the sample are summarized in **Table 1** as per the guidelines for reporting SCI data.[25]

Data analysis

Five HRV indices were calculated: LF:HF, LF, HF, RMSSD, and pNN50. HRV analyses were conducted using LabChart v.7 (AD Instruments,

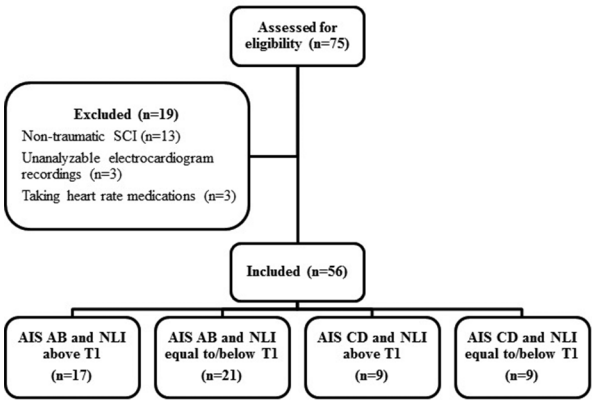

Figure 1. CONSORT flowchart reflecting the inclusion and exclusion of the final data study sample. AIS = American Spinal Injury Association Impairment Scale; NLI = neurological level of impairment; SCI = spinal cord injury.

Table 1. Demographics and clinical characteristics of the study sample

	Cohort	AIS A/B and NLI above T1	AIS A/B and NLI equal to/below T1	AIS C/D and NLI above T1	AIS C/D and NLI equal to/below T1
Sample size, *n*	56	17	21	9	9
Age, years	47 ± 12	47 ± 10	45 ± 13	54 ± 12	43 ± 13
Time post injury, years	14.2 ± 9.9	16.8 ± 9.8	17.2 ± 10.1	9.7 ± 7.6	6.9 ± 6.2
BMI, kg/m^2	26.1 ± 4.8	26.3 ± 4.6	24.8 ± 4.3	28.6 ± 7.3	26.5 ± 2.9
WC, cm	95.7 ± 14.6	99.2 ± 12.5	92.0 ± 15.7	97.9 ± 19.2	95.5 ± 9.4
HR, bpm	61.7 ± 9.0	58.4 ± 9.6	63.0 ± 8.8	63.3 ± 9.1	63.0 ± 7.9
SBP, mm Hg	109.8 ± 16.9	95.9 ± 9.0	112.6 ± 14.6	112.4 ± 17.3	126.8 ± 14.0
DBP, mm Hg	71.4 ± 13.1	62.0 ± 9.7	73.8 ± 12.3	73.1 ± 11.1	81.8 ± 12.6

Note: All the variables are reported as mean ± *SD*. None of the indices were significantly different across the cohorts ($p > .05$). AIS = American Spinal Injury Association Impairment Scale; BMI = body mass index; DBP = diastolic blood pressure; HR = heart rate; NLI = neurological level of impairment; SBP = systolic blood pressure; WC = waist circumference.

Inc., Bella Vista, Australia). Ten minutes of ECG data were divided into 3 overlapping segments of 5 continuous minutes: first 5 minutes (t = 0 – t = 300 seconds), middle 5 minutes (t = 150 – t = 450 seconds), and last 5 minutes (t = 300 – t = 600 seconds). Each 5-minute ECG recording was reviewed to confirm that all and only the R peaks were marked. The Poincaré Plots, a LabChart software feature, were checked to examine the normal and ectopic RR interval ranges and to detect any ectopic islands. The program detected ectopic islands in 26.8% of the subjects due to technical error or unknown causes. To omit ectopic islands, the data were filtered with a 45 Hz low pass filter. After analyzing all 3 ECG segments for each subject, the segment with the highest percentage of normal (ie, lowest percentage of ectopic beats) was included in the statistical analysis. If the percentages of normal and ectopic beats were equal in all 3 segments for a particular subject, then a segment was chosen randomly using a computer-based randomizer (http://www.random.org/).

Statistical analysis was conducted using IBM SPSS Statistics v.22. $P < .05$ was considered statistically significant.

Results

Relevant demographic and clinical characteristics such as age, time post injury, body mass index, waist circumference, resting heart rate, systolic and diastolic blood pressure were not significantly different across the NLI and AIS subgroups ($p > .05$) (**Table 1**). Therefore, any differences observed between groups are not likely due to demographic differences. The HRV data were not normally distributed, therefore the Kruskal-Wallis test was used to compare the HRV indices based on level (above T1 or equal to/below T1) and severity (complete [AIS AB] or incomplete [AIS CD]) of injury. Contrary to our hypothesis, the frequency and time domain measures were not significantly different across the NLI and AIS subgroups (**Table 2**). As for the relationship between the LF and HF indices, it was examined using Spearman's rho correlation coefficient in the entire sample and based on level and severity of injury. A high positive correlation was observed between LF and HF in the total sample ($r = 0.708$, $p < .0001$) (**Figure 2**) and in each of the impairment subgroups (**Table 3**).

Discussion

To our knowledge, this is the first study to describe frequency and time domain measures of HRV at rest, while adhering to the task force guidelines, in a sample of individuals with chronic (range, 5-22 years post injury) SCI and with no major cardiovascular conditions. Concerted efforts were made to exclude individuals who had medical conditions or were taking medications that would interfere with the accuracy of the HRV outcomes. Further, extensive data cleaning including removal of ectopic beats was completed. The results showed that in the entire sample and

Table 2. Comparison of HRV indices based on NLI and AIS

		Sample size (*n*)	LF:HF	LF, ms^2	HF, ms^2	RMSSD, ms	pNN50, %
NLI above T1	AIS AB	17	1.2 (0.8, 2.7)	453.7 (159.2, 771.2)	370.4 (117.1, 880.6)	36.0 (18.8, 58.8)	3.8 (0.8, 30.8)
	AIS CD	9	1.3 (0.7, 2.3)	227.4 (98.0, 1980.1)	215.0 (107.2, 1060.2)	39.2 (19.3, 81.7)	5.3 (1.4, 22.8)
NLI equal to/ below T1	AIS AB	21	1.7 (0.6, 3.5)	465.0 (245.8, 1365.0)	354.3 (182.4, 797.0)	34.5 (22.0, 47.9)	7.9 (1.7, 20.1)
	AIS CD	9	0.8 (0.4, 1.7)	717.9 (114.7, 3416.3)	1153.5 (208.7, 3481.2)	54.9 (23.8, 97.2)	15.4 (1.4, 28.2)
p value[a]	—	—	.391	.740	.551	.588	.859

Note: All the values are reported as median (lower, upper quartile). AIS = American Spinal Injury Association Impairment Scale; HF = high frequency; HRV = heart rate variability; LF:HF = low frequency to high frequency ratio; LF = low frequency; NLI = neurological level of impairment; NN = normal R peak to normal R peak; pNN50 = proportion of the number of interval differences of the consecutive NN intervals greater than 50 ms; RMSSD = square root of the mean squared differences of the consecutive NN intervals.

[a]Kruskal-Wallis test.

*$p \leq .05$.

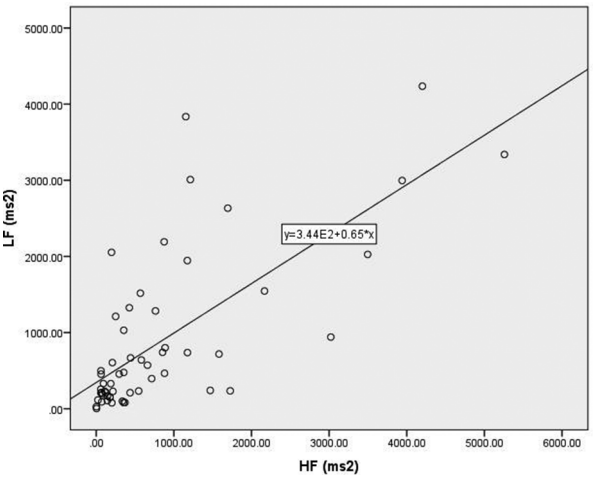

Figure 2. The total sample showed a high positive correlation was found between low frequency (LF) and high frequency (HF) ($r = 0.708$, $r^2 = 0.508$, $p < .0001$).

Table 3. Relationship of the LF and HF indices based on NLI and AIS

		Sample size (*n*)	Spearman's rho	*p* value[a]
NLI above T1	AIS AB	17	0.733	.001*
	AIS CD	9	0.850	.004*
NLI equal to/ below T1	AIS AB	21	0.432	.050*
	AIS CD	9	0.883	.002*

Note: AIS = American Spinal Injury Association Impairment Scale; HF = high frequency; LF = low frequency; NLI = neurological level of impairment.

[a]Spearman's correlation test.

*$p \leq .05$

across the impairment subgroups, a strong positive linear relationship was found between LF and HF at rest. As a result, the LF:HF will likely remain unchanged and solely reporting the ratio does not accurately describe the true picture of what is happening in this population.

Comparison across impairment subgroups

It is commonly reported in the anatomical literature that the sympathetic preganglionic neurons at T1-T5 innervate the heart and the blood vessels of the upper body, while T6-L2 innervate the blood vessels of the lower body.[4-5,21,26-28] In contrast, the parasympathetic innervation of the heart arises from the vagal nuclei of the brainstem.[4-5,21,26-28] Therefore, cardiac sympathetic function is thought to be disrupted due to sympathetic damage following a cervical SCI (C1-C8) motor complete injury and partially disrupted following a high thoracic (T1-T5) motor complete injury. The disruption of the cardiac sympathetic function was in turn thought to offset the overall cardiac ANS. However, in our study, the HRV indices measured at rest were not significantly different when compared across NLI and AIS subgroups despite both ANS dysfunction and CVD being reported to be linked with level and severity of SCI (NLI and AIS).[4-6,22] Cardiac autonomic regulation involves the integrative function of 2 complex systems, cardiovascular and autonomic, and thus differences in the pathophysiology of cardiac

autonomic function may not be ideal to examine in isolation without considering other cardiovascular or autonomic abnormalities. For instance, the cardiovascular component includes peripheral circulation, often altered in SCI, influencing cardiac function.[29] In addition, heart rate is not only modulated by the ANS, but is also affected by the intrinsic cardiac system, respiratory, and humoral factors.[29] Although HRV differences have been reported in the literature, some investigators have highlighted that HRV is not a direct measure of the parasympathetic and sympathetic nerve activity, but instead quantifies cardiac autonomic responsiveness.[7,16] Houtman and colleagues[30] examined cardiovascular responses to head-up tilt in able-bodied individuals and persons with an SCI and reported that both able-bodied subjects and only persons with paraplegia had an expected cardiovascular response since the activity of the sympathetic nervous system increased. Overall, our data, obtained from resting ECGs, support that there may be little biological merit for single measures of HRV at rest.

Relationship between LF and HF

There is equipoise and debate in the literature regarding the relationship between LF and HF in individuals with SCI. We demonstrated a positive linear relationship between LF and HF when assessed in both the entire sample and impairment subgroups. After a cervical SCI, Claydon et al[2] observed lower LF and higher HF, whereas Grimm et al[15] and Wang et al[21] reported both lower LF and HF. As for the thoracic group, Claydon et al[2] reported no change in LF and a reduced HF while Bunten et al[18] and Castiglioni et al[31] reported reduced LF but no change in the HF values. All of these studies included individuals with chronic SCI, but the cohorts were not necessarily similar. For instance, the studies used different cut-off levels for NLI and AIS classifications. The inconsistency in the reporting of chronicity of injury, NLI thresholds, and AIS makes comparisons of the current data with prior published HRV data challenging. Although our study did not show any significant differences in the HRV values across the impairment subgroups at rest, it did show that, in the total sample, 50.8% of the variation in LF was shared by HF. Therefore, a decrease or even an increase in both components of the ratio could result in a similar LF:HF ratio. Lower resting sympathetic tone in individuals with an SCI above the level of T6 have been reported[26]; thus most investigators suggest that a change in HF is required to align with the low levels of LF as the ANS rebalances to maintain homeostasis.[2,15,21,32]

Serra-Añó and colleagues[33] examined HRV in individuals with chronic complete thoracic SCI and reported that "the sympathetic and parasympathetic systems do not always act reciprocally, but may also act synergistically and complementarily."(p61) Further, a recent study by Malmqvist and colleagues[34] examined individuals with acute SCI and found that ANS homeostasis did not occur, as the LF:HF ratio was lower in the individuals with a C1-T5 injury when compared to subjects with a T6-T12 injury. Regardless of whether the SCI is chronic or acute, the physiological mechanisms underlying how the ANS rebalances itself remains unclear and may represent alternate or poorly understood physiological mechanisms. Other investigators argue that sympathetic function is preserved in individuals with an SCI above the level of T6.[35,36] However, Billman[37] has challenged the presumption of "ANS re-balancing" and has argued that the LF index is not more indicative of sympathetic function, but is rather a complex combination between the 2 ANS branches, along with other unidentified factors. In addition, Rahman and colleagues[38] contributed to the controversy regarding the interpretation of LF by arguing that it is unrelated to the cardiac sympathetic innervation, but instead is an index of baroreflex function. Furthermore, the correlation between the LF and HF indices does not meet the 9 Bradford-Hill criteria[39] for causation, thus increasing uncertainty that a change in LF produces a change in HF. Therefore, the positive correlation between the LF and HF indices in our study may or may not represent a rebalanced ANS system. Despite the indeterminate physiological reasons for the positive correlation observed between the LF and HF indices, overall our findings question whether the LF:HF, the most common measure of HRV used, is an appropriate marker to independently assess cardiac sympatho-vagal balance in individuals

with chronic traumatic SCI at rest. If the LF and HF values change simultaneously in the same direction, then the ratio would be an ineffective measure to detect clinically important changes in sympatho-vagal status.

Study limitations

Despite the study strengths, several limitations must be considered; for example, the sampling process was not ideal and may hinder generalizability. As described in the Methods section, the strict study eligibility criteria and the thorough data-cleaning processes may have limited the generalizability of findings to only the chronic SCI population who meet the study inclusion criteria. Furthermore, contrary to existing literature,[2,15-16,29,31] no differences were observed in HRV when compared based on NLI and AIS subgroups. Although this is the largest sample ($N = 56$) in which HRV has been examined in chronic SCI (prior sample sizes for chronic SCI have included 5-39 subjects), the subgroups may have been too small to detect significant differences thus increasing the likelihood of a type II error. A post hoc power analysis, based on the cohort mean LF:HF ratio, revealed that a total sample size of 72 subjects was required to detect a significant difference. In addition, neurological impairment severity (NLI and AIS) does not accurately represent severity of autonomic dysfunction or autonomic completeness. Clinical assessment of autonomic completeness using sympathetic skin response may have added value to the interpretation of the results along with measures that include physical activity level and the breathing pattern adopted during the ECG data collection. There is robust evidence indicating that regular physical activity can increase HRV indices within the healthy and clinical populations.[40-42]

Future directions

Agreement on criteria for selecting impairment subgroups must be established and employed in future HRV studies examining individuals with an SCI. West et al[5] have reported that autonomic completeness of the SCI, which can be estimated via catecholamine concentrations as well as blood pressure variability, is more closely related to the function of the cardiovascular ANS than the neurological completeness of the SCI. We suggest that it is crucial to assess the autonomic completeness of injury and additional autonomic dysfunction including, but not limited to, orthostatic hypotension and autonomic dysreflexia concomitantly prior to any future subgroup stratification. This study also places an emphasis on the recent revisions to the International Standards to Document Remaining Autonomic Function after Spinal Cord Injury[6] to include the definitions and measurement of "autonomic completeness." Provided that HRV represents modulation of the cardiac ANS, it could be a valuable tool to test the responsiveness of the cardiac ANS by evaluating intra-individual responses to different forms of ANS stress (eg, drug or exercise) making HRV an important clinical measure.

Conclusion

Despite cardiac autonomic dysfunction having been reported to be related to NLI and AIS, no measurable differences in HRV indices were evident across NLI and AIS subgroups. The observed disparity might have been due to several factors: inadequate sample size, the fact that HRV is not solely linked to NLI and AIS, and the fact that cardiac autonomic function has multiple biological complexities that cannot be measured exclusively by HRV parameters alone at rest. Given that the LF:HF ratio is the most commonly used HRV measure of cardiac sympatho-vagal balance, and the physiological interpretation of the positive relationship between LF and HF remains undetermined, the LF:HF ratio is inappropriate for the assessment of cardiac autonomic status at rest in individuals with chronic traumatic SCI. Autonomic subgrouping (autonomic complete or incomplete as defined by presence or absence of a sympathetic skin response) may be a more suitable approach to investigate the differences in cardiac autonomic responses, given the diversity in impairment. Further, an understanding of the biological interpretation of the HRV indices is paramount before routine use of HRV data can be used to monitor and/or manage cardiac ANS dysfunction in individuals with chronic SCI.

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