Reliability of five-minute vs. one-hour heart rate variability metrics in individuals with spinal cord injury

Siriwipa Srirubkhwa [1], **Lars Brockmann** [2], **Ratana Vichiansiri** [1], **Kenneth J. Hunt** [2], **Jittima Saengsuwan** [Corresp. 1]

[1] Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[2] The Laboratory for Rehabilitation Engineering, Institute for Human Centred Engineering, Bern University of Applied Sciences, Biel, Switzerland

Corresponding Author: Jittima Saengsuwan
Email address: sjittima@kku.ac.th

Background : A previous study showed low reliability of 1-hr HRV outcomes in participants with spinal cord injury (SCI), but it was not certain whether the low reliability was due to the unrestricted activity of participants. We aimed to investigate test-retest reliability of HRV metrics in individuals with SCI using a 1-hr measurement in a supine position.

Methods: Individuals with SCI underwent two sessions of 1-hr recording of the time between consecutive R waves (RR-intervals) in a supine position. HRV outcomes were obtained from a single 5-min data segment and for the full 1-hr recording. HRV parameters of interest were: standard deviation of all normal-to-normal R-R intervals (SDNN) and square root of the mean of the squared differences between successive R-R intervals (RMSSD) (time domain); and high frequency power (HF), low frequency power (LF), very low frequency power (VLF), ultra-low frequency power (ULF) and total power (TP) (frequency domain). Relative reliability was assessed by intraclass correlation coefficient (ICC). Absolute reliability was assessed by coefficient of variation (CV) and Bland-Altman limits of agreement (LoA).

Results: Data from 37 individuals (14 with tetraplegia and 23 with paraplegia) were included. Relative reliability was higher for the 1-hr (ICCs ranged from 0.13 - 0.71) than for the 5-min duration (ICCs ranged from 0.06 - 0.50) in the overall SCI group for all HRV metrics. Participants with tetraplegia had lower relative reliability compared to participants with paraplegia in all HRV metrics for the 5-min duration (ICCs ranged from -0.01 - 0.34 vs. 0.21 - 0.57). For the 1-hr duration, participants with paraplegia showed higher relative reliability than participants with tetraplegia in all HRV metrics (ICCs ranged from 0.18 - 0.79 vs. 0.07 - 0.54) except TP (ICC 0.69 vs. 0.82). In terms of absolute reliability, the CVs and LoAs for the 1-hr duration were better than for the 5-min duration. In general, time domain metrics showed better reliability than frequency domain metrics for both durations in participants with tetraplegia and paraplegia. The lowest CV and narrowest 95 % LoA were found for SDNN in 5-min and 1-hr durations overall and in both lesion levels.

Conclusions: The supine position did not provide better reliability compared to unrestricted activity in participants with SCI. HRV analysis using a 5-min duration is of limited value in SCI due to poor reliability. For the 1-hr analysis duration, interpretation of the reliability of HRV varies according to lesion level: it is recommended to take lesion level into account when interpreting reliability measures.

Reliability of Five-Minute vs. One-Hour Heart Rate Variability Metrics in Individuals with Spinal Cord Injury

Siriwipa Srirubkhwa[1], Lars Brockmann[2], Ratana Vichiansiri[1], Kenneth J. Hunt[2], Jittima Saengsuwan[1]

[1]Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[2]The Laboratory for Rehabilitation Engineering, Institute for Human Centred Engineering, Bern University of Applied Sciences, Biel, Switzerland

*Corresponding author:

Jittima Saengsuwan

Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

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durations overall and in both lesion levels.

Conclusions:

The supine position did not provide better reliability compared to unrestricted activity in
participants with SCI. HRV analysis using a 5-min duration is of limited value in SCI due to
poor reliability. For the 1-hr analysis duration, interpretation of the reliability of HRV varies
according to lesion level: it is recommended to take lesion level into account when interpreting
reliability measures.

Introduction

Heart rate variability (HRV) is the physiological phenomenon of variation in the time interval between consecutive heartbeats which is generated by the SA node in the right atrium of the heart (Shaffer & Ginsberg 2017). Variations in heart rate are analyzed by different methods such as time domain, frequency domain or combined time-frequency measures (Shaffer & Ginsberg 2017). HRV is usually measured with short (approx. 5 min) and long (24-hr) durations (Malik et al. 1996; Shaffer & Ginsberg 2017). Interestingly, short term and long term HRV reflect different underlying physiological processes. Long term HRV is attributed to changes in circadian rhythm, core body temperature, the renin-angiotensin system, and the sleep cycle (Malik et al. 1996; Shaffer et al. 2020). Short term HRV is thought to be generated by four sources: 1. interactions between sympathetic and parasympathetic autonomic regulation, 2. respiration, 3. baroreceptor reflex, that regulates blood pressure, and, 4. rhythmic adjustment in blood vessel diameter (Shaffer & Ginsberg 2017). Thus, long term HRV is not interchangeable with short term HRV (Shaffer et al. 2020). Long term HRV has been found to be a powerful and independent predictor of an adverse event such as morbidity or death in multiple patient populations (Fang et al. 2020; Hohnloser et al. 1997; Nunan et al. 2010; Patel et al. 2017). However, some studies demonstrated that short term or even ultra-short-term HRV recording (< 5 min) might also be used to predict adverse events (Fei et al. 1996; Karp et al. 2009; Kautzner et al. 1998; La Rovere et al. 2003).

To employ HRV in clinical practice, apart from its validity, the HRV metrics should be reliable and therefore applicable across individual patients. For reliability analysis, it is necessary to estimate outcome variability within individuals and in different populations. It was found that clinical populations showed poorer reliability than healthy subjects (La Fountaine et al. 2010; Lord et al. 2001). Additionally, reliability was poorer during interventions such as tilt or pharmacological stimulation compared to rest (Sandercock et al. 2005). Sandercock et al. stated that describing HRV in general as a reliable measurement technique is an oversimplification, because the results of reliability studies are heterogenous and depend on a number of factors, thus studies in different clinical populations are required to assess HRV reliability (Sandercock et al. 2005).

The spinal cord is a compact bundle of neural structures that facilitate the transmission of both motor and sensory information between the brain and the rest of the body. Consequently, any damage to the spinal cord can disrupt the conduction of signals associated with sensory, motor, and autonomic functions across the site of the injury (Rupp et al. 2021). Spinal cord injury (SCI) can be categorized into two primary groups, determined by the level of the lesion: tetraplegia refers to the impairment or loss of motor and/or sensory function in the cervical segments of the spinal cord, while paraplegia refers to the impairment or loss of motor and/or sensory function in the thoracic, lumbar or sacral segments (Rupp et al. 2021). Individuals with SCI also suffer from consequences of autonomic dysfunction, including issues such as sexual dysfunction as well as bowel and bladder problems (Hou & Rabchevsky 2014). Regarding the

102   autonomic control of the heart, the parasympathetic system is undamaged in individuals with
103   SCI, since its regulation is from the vagal nuclei in the brain stem (Takahashi et al. 2007). On the
104   other hand, sympathetic control of the heart relies on bulbospinal input via the cervical region
105   and spinal sympathetic preganglionic neurons located in the upper half (T1-T6) of the thoracic
106   segments. As a result, those with injuries at or above T6 have compromised sympathetic control
107   of the heart (Fossey et al. 2022; Malmqvist et al. 2015; Rodrigues et al. 2016). Research
108   indicates that individuals with SCI exhibit lesion-dependent impairment in resting cardiovascular
109   function. Among these individuals, those with the highest injuries (tetraplegia) demonstrate the
110   greatest degree of cardiovascular dysfunction compared to individuals with high thoracic (T1-
111   T6) and low thoracic (below T6) paraplegia (West et al. 2012). Notably, cardiovascular disease
112   is the leading cause of death after SCI (Sabre et al. 2013; Savic et al. 2017). Additionally,
113   individuals with SCI, particularly individuals with tetraplegia, have higher risk of cardiovascular
114   events and deaths when compared to their able-bodied counterparts (Chamberlain et al. 2019;
115   Cragg et al. 2013; Jia et al. 2023). Apart from an altered metabolism and decrease in physical
116   activity, autonomic dysfunction is one of the proposed contributing factors (Raguindin et al.
117   2021). In comparison with healthy counterparts, participants with SCI had lower HRV values for
118   both time and frequency domain metrics (Rodrigues et al. 2016; Serra-Ano et al. 2015).

119   Our previous study in 45 individuals with SCI and without any restriction on activity
120   showed that when the duration of HRV recording was shorter (i.e. 1, 3 or 6 hours), the reliability
121   was lower than long-duration recording (24 hours) (Ruangsuphaphichat et al. 2023). However,
122   24-hour recording may not be convenient in clinical practice and it has not been integrated in
123   general medical care (Shaffer et al. 2020). Additionally, the low reliability observed for 1-hour
124   recording in our previous study may also have resulted from placing no restriction on activity.
125   We thus aimed to investigate test-retest reliability of short term HRV measurement, namely 5-
126   min and 1-hour durations, when participants rest in the supine position. The 5-min duration was
127   chosen because it is a standard and generally used method that requires a short time to perform
128   (Malik et al. 1996; Sassi et al. 2015; Shaffer & Ginsberg 2017). The 1-hour duration was used in
129   order to be able to establish the test-retest reliability of the ultra-low-frequency (ULF) outcome
130   since the shortest time period for ULF to be valid is one hour (Tan & Jiang 2013).

131

Materials & Methods
Participants

134   We studied individuals with SCI who were admitted at Srinagarind Hospital, which is a
135   university hospital in the northeast region of Thailand, from September 2021 to January 2023.
136   Inclusion criteria were SCI of any cause, and age $\geq$ 18 years. Exclusion criteria were abnormal
137   breathing pattern (respiratory rate > 20 breaths/min or < 10 breaths/min), concomitant cardiac or
138   neurological disease and fever (body temperature $\geq$ 37.8 deg C). Ethical approval for this study

139 was obtained from the Khon Kaen University Committee for Ethics in Human Research (ref.
140 HE641355). Written informed consent was obtained from all participants before inclusion.

HRV measurement protocol

142   Participants underwent two test sessions. Each session was separated by at least 24 hours.
143 The recording time started at the same time for both sessions, i.e. between 9am and 10am. Prior
144 to each test, individuals were instructed to refrain from smoking, and from drinking caffeine or
145 alcohol for 24 hours before the measurement. During each test session, participants lay in a
146 supine position for one hour while they wore a chest belt sensor (Polar H10; Polar Electro Oy,
147 Kempele, Finland) and a wristwatch receiver (Polar V800) to record the raw RR intervals.
148 Participants were instructed to breathe normally during recordings.

Outcomes and data processing

150   Raw RR intervals were exported as a text file to HRV analysis software implemented in
151 Matlab (The Mathworks, Inc., USA). Some recordings were invalid because of poor signal
152 quality. The remaining data sets were preprocessed for artefact detection and removal. Artefact
153 detection was performed using two methods: (i) maximal and minimal values for plausible RR
154 values were defined (min = 400 ms; max = 1650 ms), (ii) the difference between two successive
155 RR intervals was set to be at a maximum of ±20 % of the previous value. For the removal of the
156 detected artefacts, special care was taken not to add spurious information to the original data sets
157 by removing any artificially introduced combinations of two successive RR intervals from the
158 analysis. The recording was done for 1-hr, and the 5-min segment to be used for short-term
159 analysis was obtained from minutes 5 to 10 of the recording.

160   For the time-domain analysis, the metrics used were standard deviation of all normal-to-
161 normal R-R intervals (SDNN) and square root of the mean of the squared differences between
162 successive R-R intervals (RMSSD). In the frequency-domain analysis, power in the high
163 frequency (HF, 0.15 - 0.4 Hz), low frequency (LF, 0.04 - 0.15 Hz), very low frequency (VLF,
164 0.0033 - 0.04 Hz), and ultra-low frequency (ULF, < 0.0033 Hz) bands, together with total power
165 (TP) was calculated. The Lomb-Scargle least squares spectral analysis method was used because
166 it is more appropriate for spectral analysis when the data are irregularly sampled such as in RR
167 time series (Fonseca 2013; Krafty et al. 2014; Stewart et al. 2020).

Statistical analysis

169   The outcomes are presented as median (with 25th and 75th percentiles) because the data
170 were found to be not normally distributed. Extreme outliers were identified and excluded from
171 data analysis when the changes in HRV metric values were more than 3 interquartile ranges
172 (IQR) above Q3 or below Q1 (Jones 2019). Wilcoxon signed-rank tests were used to test paired
173 differences for each participant with significance level set to α = 0.05. Relative test-retest
174 reliability was analysed using the intraclass correlation coefficient $ICC_{3,1}$ and is presented as ICC
175 and 95 % confidence interval (CI). ICC ≥ 0.75 represents excellent reliability, ICC < 0.4 is poor

176   reliability and ICC between these ranges is regarded as moderate to good reliability (Andresen
177   2000). Absolute reliability was evaluated using the coefficient of variation (CV) (Bland &
178   Altman 1996) and Bland-Altman limits of agreement (LoA) (Bland & Altman 1986). Regarding
179   Bland-Altman analysis, since all data were heteroscedastic, the data were log-transformed prior
180   to analysis. Then the data are transformed back and the results are presented in the Bland-Altman
181   plots as a linear function $\pm b\bar{x}$ (Euser et al. 2008), where $\bar{x}$ is the data mean and b is the slope of
182   the LoA. The statistical analyses were performed using SPSS (IBM SPSS Statistics for
183   Windows, Version 28.0. Armonk, NY: IBM Corp).

184

185   ## Results

186   ### Demographic data

187   Sixty-six individuals were recruited. During HRV data processing, some data sets were
188   invalid. The exclusion of data from 29 individuals was due to signal gap (58.9 %), noisy signal
189   (30.4 %) and multiple skipped heart rate measurements (16.1 %). As a result, data from 37
190   individuals were included for analysis (14 tetraplegia and 23 paraplegia). More than half of the
191   participants were male (54 %). The mean age was 49.5 years and the median duration after SCI
192   was 7.6 years (Table 1).

193

194   Regarding 5-min HRV, 7 HF values and 1 LF value could not be obtained from the
195   analysis. Additionally, the removal of outliers led to an exclusion of 1 SDNN, 4 HF, 3 LF and 1
196   TP values. Since TP represents the combination of all frequency spectra, the exclusion of the
197   above-mentioned frequency band data resulted in 25 data pairs for TP analysis. Regarding 1-hr
198   HRV, 5 HF values and 1 LF value could not be obtained from the analysis. The identification of
199   outliers led to the exclusion of an additional 4 HF, 3 LF, and 1 VLF values, resulting in 27 data
200   pairs for TP analysis.

201

202   ### Overall Reliability of HRV in SCI

203   There were no significant differences (p > 0.05) in any pairs of HRV values for both
204   durations (5 min and 1 hr) in the overall analysis (i.e. without consideration of lesion level). For
205   the 5-min duration overall, HF had moderate to good relative reliability (ICC = 0.50), but all
206   other outcomes had poor reliability (ICC 0.06 - 0.36). For the 1-hr duration, overall relative
207   reliability was moderate to good for SDNN, RMSSD, HF, ULF and TP (ICC 0.40 - 0.71), while
208   it was poor for LF and VLF (ICCs of 0.13 and 0.28, respectively). For all outcomes, relative
209   reliability was higher (higher ICC) for the 1-hr than for the 5-min duration.

210   In terms of absolute reliability, the CVs for the 1-hr duration were better than for the 5-
211   min duration. In general, time domain metrics showed better reliability than frequency domain
212   metrics for both durations. The CVs for the 5-min HRV outcomes were 47.2 % (SDNN) and
213   67.3 % (RMSSD) for the time domain metrics and in the range of 86.6 % to 158.0 % for the
214   frequency domain metrics. For the 1-hr HRV outcomes, CVs were 28.8 % (SDNN) and 51.4 %
215   (RMSSD) for the time domain metrics and in the range of 47.5 % to 103.2 % for the frequency
216   domain metrics. The lowest CV and narrowest 95 % LoA were found for SDNN. Similar to the
217   CVs, the limits of agreement were narrower for the 1-hr duration compared to the 5-min duration
218   for all outcomes (Table 2, Fig 1).

219

220   Reliability of HRV classified by lesion level

221   The 5-min HRV outcomes showed a significant difference ($p = 0.034$) in HF within the
222   group with paraplegia. However, there were no significant differences ($p > 0.05$) in any pairs of
223   HRV values in the 5-min HRV outcomes of participants with tetraplegia. Similarly, no
224   significant differences were found for the 1-hr HRV outcomes in both groups.

225   Regarding relative reliability, participants with tetraplegia generally had lower reliability
226   compared to participants with paraplegia across most HRV metrics, both in 5-min and 1-hr
227   durations. Among all HRV metrics, LF exhibited the lowest relative reliability within both the 5-
228   min and 1-hr durations in both groups. Similar to what was observed in overall SCI, relative and
229   absolute reliability improved with longer duration (1-hr). For relative reliability, the three most
230   reliable metrics were HF, RMSSD and TP for the 5-min duration and TP, HF and SDNN for the
231   1-hr duration.

232   For the 5-min HRV, the CVs of participants with tetraplegia were poorer than participants
233   with paraplegia in the frequency-domain metrics but not in the time-domain metrics. Regarding
234   the 1-hr HRV, the CVs of participants with tetraplegia were smaller than participants with
235   paraplegia except for VLF. The smallest CVs and narrowest LoAs were found for the time
236   domain metrics (SDNN and RMSSD) in both groups (Table 3).

237

238   Discussion

239   We aimed to investigate test-retest reliability of short term HRV measurement, namely 5-
240   min and 1-hr durations, when participants rest in the supine position. In general, participants
241   showed a lower degree of HRV for 5-min and 1-hr metrics compared to healthy subjects. For 5-
242   min HRV, the medians of HF, LF and TP were 151.7 ms^2, 259.1 ms^2 and 922.2 ms^2, respectively
243   (Table 2). The corresponding mean HRV values in healthy subjects were reported elsewhere to
244   be 975 ms^2, 1170 ms^2 and 3466 ms^2 (Malik et al. 1996). Regarding 1-hr HRV outcomes, mean
245   SDNN and RMSSD were 90.4 ms and 47.8 ms in healthy subjects (Evrengul et al. 2006) and the

246 medians of SDNN and RMSSD were 57.5 ms and 22.9 ms in our population (Table 2). The
247 finding of lower HRV values in participants with SCI compared to healthy participants is
248 consistent with multiple previous studies (La Fountaine et al. 2010; Rodrigues et al. 2016; Serra-
249 Ano et al. 2015; Thayer et al. 2016).

250   SDNN showed lowest CV and narrowest 95% LoA. However, the overall CV of SDNN
251 between test and retest measures of the 5-min HRV was large (47.2 %) compared to previous
252 reports in healthy participants (8.0 %) (Sinnreich et al. 1998). Additionally, populations with SCI
253 had lower relative reliability (ICC of 0.27 and 0.36 for SDNN and RMSSD, respectively)
254 compared to healthy participants measured for the same duration and in the same position (ICC
255 of 0.82 and 0.76 for SDNN and RMSSD) (Pinna et al. 2007).

256   Our study agrees with previous studies which reported that frequency domain measures
257 (HF and LF) demonstrated larger variation and higher CV compared to time domain measures
258 (Nunan et al. 2010; Pinna et al. 2007; Ponikowski et al. 1996; Salo et al. 1999). The relative
259 reliability of frequency domain measures was lower than in healthy participants. For example,
260 for 5-min HRV reliability, our ICCs for HF and LF were 0.50 and 0.06 while the respective
261 values in healthy participants were 0.63 and 0.79 (Pinna et al. 2007). Additionally, for the 1-hr
262 duration in a supine position, the ICCs of HF and LF were 0.66 and 0.13 in our study, and 0.81
263 and 0.86 in normal subjects (da Cruz et al. 2019). Our study shows much lower reliability of 1-hr
264 HRV in frequency domain measures in participants with tetraplegia (ICCs of HF and LF were
265 0.54 and 0.07) compared to a previous study: La Fountaine et al. found that the ICCs for HF and
266 LF were 0.66 and 0.44 in individuals with tetraplegia and the ICCs were 0.90 and 0.74 in healthy
267 individuals (La Fountaine et al. 2010). To consider the least reliable HRV parameters among LF
268 and HF, the results were inconsistent among studies: some studies found lower relative reliability
269 of LF and some found the opposite (Freed et al. 1994; Nunan et al. 2010; Sinnreich et al. 1998).
270 Our study showed that LF had lowest relative reliability in participants with SCI based on 1-hr
271 HRV metrics.

272   Regarding absolute reliability, the trend of higher variability in the frequency domain was
273 found in both healthy participants and in populations with diseases such as chronic heart failure.
274 Nunan et al. showed that the overall CVs for SDNN and RMSSD were 32 % and 37 % while the
275 CVs in LF and HF were 56 % and 116 % for the short term recording (5-min) of HRV in healthy
276 adults (Nunan et al. 2010). Ponikowski et al. demonstrated that the CVs of HF, LF, VLF and TP
277 were 66.4 %, 81.5 %, 56.0 % and 45.9 % while the CV of SDNN was 25.4 % in 5-min recording
278 data in patients with chronic heart failure (Ponikowski et al. 1996). Lord et al. showed that the
279 CV for LF was 45 % in healthy participants and 76 % in cardiac transplant recipients (Lord et al.
280 2001). Our study demonstrated the CV of SDNN and RMSSD of 47.2 % and 67.3% while the
281 CV of HF, LF, VLF and TP were 158.0, 151.6, 114.8 and 86.6 %. LF, HF, VLF and ULF seem
282 to have lower absolute reliability based on CV and LoA compared to TP.

283   Poor reliability of short term (5-min) HRV has been identified in our study. This is in line
284 with other studies which were done in patients with chronic heart failure, type-2 diabetes and
285 cardiac transplant recipients (Lord et al. 2001; Ponikowski et al. 1996; Sacre et al. 2012). One
286 possible explanation is the low initial HRV values reported in clinical populations, therefore,
287 slight changes in the test-retest value can result in a high CV. Lower absolute values among
288 participants may also lead to lower ICC (Sandercock et al. 2005). Regarding 5-min and 1-hr
289 reliability of HRV, we found that the supine position did not provide better reliability compared
290 to unrestricted activity in participants with SCI (Ruangsuphaphichat et al. 2023). This has to be
291 interpreted with caution. Firstly, the results not showing improvement in reliability may be
292 partially explained by differences in group proportions: the present study had a higher proportion
293 of participants with tetraplegia (37.8 %) compared to the previous study (22.2 %)
294 (Ruangsuphaphichat et al. 2023). We also had data from participants with complete tetraplegia
295 while the former study did not. We found that individuals with tetraplegia showed poorer HRV
296 reliability compared to those with paraplegia. This may be explained by the alteration in
297 cardiogenic autonomic control in participants with higher lesion level, which results in higher
298 inter-day variation of HRV (Bauman et al. 2012). Secondly, the influence of different body
299 positions might contribute to the observed differences in reliability results. In a previous study, a
300 reliability analysis of HRV in 37 non-SCI young men in different positions demonstrated
301 noteworthy differences. The ICCs of HRV in the supine position were found to be lower than in
302 the standing position. Specifically, the ICCs were as follows: log of SDNN (ICC of 0.60 vs 0.88
303 [supine vs. standing]), log of RMSSD (ICC of 0.74 vs. 0.86) and log of HF (ICC of 0.81 vs.
304 0.84). However, no such trend was observed for LF (ICC of 0.63 vs. 0.32) (da Cruz et al. 2019).
305 This might imply that distinct body postures influence the reliability of HRV, particularly in the
306 context of individuals with SCI. Given these findings, there emerges a potential need for further
307 investigation into the impact of different postures on HRV metrics and their reliability within the
308 SCI population.

309   Our participants had various medications, and some medications have been shown to alter
310 HRV, e.g. gabapentin, antihypertensive medication or tricyclic antidepressants (Ermis et al.
311 2010; Miyabara et al. 2017; Pavithran et al. 2010; van Zyl et al. 2008). However, since the
312 medications were taken at regular times, these should not have directly affected test-retest
313 differences. Our study had too few participants to conduct a robust comparison of HRV data in
314 individuals with respect to whether there was a complete or incomplete lesion, which may lead to
315 additional differences in reliability of HRV. Multiple invalid data sets due to poor signal
316 recording has to be improved for clinical application, e.g. by careful monitoring or regularly
317 checking the data recording during the test period. Our results were limited to the supine
318 position, and the single centre study may limit data generalization. It may be possible that
319 different positions, or measurements with paced breathing, may lead to different reliability
320 outcomes (Nunan et al. 2010; Pinna et al. 2007; Sinnreich et al. 1998; Tonello et al. 2015).
321 Additionally, the inclusion of age- and sex-matched able-bodied controls might have provided
322 more comparable data in the same measurement setting and allowed more insightful

323   interpretation. For future studies, emphasis on examining reliability in different body positions or

324   during paced breathing sessions might offer avenues to improve the reliability of HRV

325   measurements within this population. By including a larger number of participants, it would

326   enable the analysis of the potential impact of variables such as the severity and completeness of

327   the lesion.

328

Conclusions

330   The supine position did not provide better reliability compared to unrestricted activity in

331   participants with SCI. HRV analysis using a 5-min duration is of limited value due to poor

332   reliability in individuals with SCI. For the 1-hr analysis duration, SDNN, RMSSD, HF and TP

333   have moderate to excellent reliability overall and when grouped based on lesion level; ULF has

334   moderate to good reliability overall and for paraplegia; VLF has moderate to good reliability for

335   paraplegia only; but LF has poor reliability overall and in both groups. Interpretation of the

336   reliability of HRV varies according to lesion level: it is recommended to take lesion level into

337   account when interpreting reliability measures.

338

339

340 **Figure legends**

341

342 **Figure 1** Bland-Altman plot of mean differences and 95 % limits of agreement (LoA) among
343 1A. time domain HRV measures (5-min and 1-hr) and 1B. frequency domain HRV measures (5-
344 min and 1-hr) in a supine position in individuals with SCI. The diagonal lines represent the 95%
345 LoA. The difference between test and retest values increases as the mean values increases,
346 indicating heteroscedasticity of data, in all HRV metrics. 1-hr HRV shows narrower LoAs
347 compared to 5-min HRV. HF: high frequency, LF: low frequency, RMSSD: square root of the
348 mean of the squared differences between successive R-R intervals, SDNN: standard deviation of
349 all normal-to-normal R-R intervals, TP: total power, ULF: ultra-low frequency, VLF: very low
350 frequency.

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512

Demographic data (n = 37)

1 **Table 1:** Baseline characteristics of individuals with SCI (n = 37)

Variables	n (%)
Sex	
Male	20 (54.1)
Female	17 (45.9)
Age (years), Mean (SD)	49.5 (13.8)
Range (years)	26 - 76
SCI level	
Tetraplegia:	
Complete	4 (10.8)
Incomplete	10 (27.0)
Paraplegia:	
Complete	6 (16.2)
Incomplete	17 (45.9)
Cause of SCI	
Traumatic	20 (54.1)
Non-Traumatic	
Degenerative	3 (8.1)
Inflammatory	1 (2.7)
Neoplastic	8 (21.6)
Infection	3 (8.1)
Other	2 (5.4)
Duration of SCI (years), Median (p25, p75)	7.6 (26, 146.5)
Range (years)	0.3 – 19.2
Underlying diseases	
No underlying disease	28 (75.7)
Hypertension	4 (10.8)
Dyslipidemia	3 (8.1)
Other	5 (13.5)
Medications	
Antihypertensive	4 (10.8)
Amlodipine	3 (8.1)
Enalapril	1 (2.7)
Alpha-blockers	7 (18.9)
Anticholinergics	27 (73.0)
Oxybutynin	23 (62.2)
Trospium	7 (18.9)
Detrusitol	1 (2.7)
Medications for neuropathic pain	24 (64.9)
Gabapentin	20 (54.1)
Pregabalin	3 (8.1)
Amitriptyline	4 (10.8)
Antispastic	24 (64.9)
Baclofen	20 (54.1)
Tizanidine	3 (8.1)
Clonazepam	11 (29.7)
Diazepam	1 (2.7)

2 p25, 25th percentile; p75, 75th percentile; SD standard deviation

Test-retest reliability; 5-min and 1-hour recordings; n = 37

1   **Table 2:** RR intervals, mean heart rate, time- and frequency-domain HRV and test-retest reliability of 5-min and 1-hr HRV in all participants (n = 37)

	Day 1, Median (p25, p75)	Day 2, Median (p25, p75)	p-value	ICC (95 % CI)	CV	95 % LoA
5-min						
RR interval (ms)	840.0 (138.0)	815.0 (111.0)				
HR (bpm)	73.4 (12.6)	75.0 (10.5)				
SDNN (ms), (n = 36)	35.9 (22.2, 49.5)	38.1 (28.3, 46.5)	0.36	0.27 (-0.05 - 0.54)	47.2	± 0.95 $\bar{x}$
RMSSD (ms)	19.4 (12.1, 26.1)	21.0 (14.7, 33.2)	0.44	0.36 (0.04 - 0.61)	67.3	± 1.22 $\bar{x}$
HF (ms^2), (n = 27)	151.7 (31.9, 417.0)	224.5 (88.3, 542.8)	0.36	0.50 (0.15 − 0.74)	158.0	± 1.71 $\bar{x}$
LF (ms^2), (n = 33)	259.1 (122.2, 665.9)	298.8 (172.0, 556.0)	0.90	0.06 (-0.27 − 0.39)	151.6	± 1.68 $\bar{x}$
TP (ms^2), (n = 25)	922.2 (493.2, 2304.3)	1434.1 (761.0, 2011.5)	0.34	0.35 (-0.04 - 0.65)	86.6	± 1.33 $\bar{x}$
1-hr						
RR interval (ms)	854.0 (134.0)	843.0 (109.0)				
HR (bpm)	72.1 (12.1)	72.4 (9.9)				
SDNN (ms)	57.5 (47.8, 71.1)	61.2 (48.4, 79.4)	0.14	0.61 (0.37 - 0.78)	28.8	± 0.65 $\bar{x}$
RMSSD (ms)	22.9 (14.0, 29.7)	25.2 (16.7, 33.6)	0.46	0.46 (0.15 - 0.68)	51.4	± 1.03 $\bar{x}$
HF (ms^2), (n = 28	270.0 (153.1, 867.5)	432.7 (166.2, 646.2)	0.35	0.66 (0.40 - 0.83)	75.9	± 1.30 $\bar{x}$
LF (ms^2), (n = 33)	467.1 (182.5, 859.1)	615.0 (261.2, 880.6)	0.55	0.13 (-0.21 - 0.45)	97.4	± 1.45 $\bar{x}$
VLF (ms^2), (n = 36)	976.4 (479.5, 1880.1)	1234.3 (728.9, 2053.7)	0.31	0.28 (-0.04 - 0.55)	67.5	± 1.18 $\bar{x}$
ULF (ms^2)	1297.0 (705.1, 2620.8)	1593.2 (875.3, 3810.2)	0.22	0.40 (0.10 - 0.64)	103.2	± 1.47 $\bar{x}$
TP (ms^2), (n = 27)	3198.8 (1819.6, 6140.9)	3622.6 (1687.0, 7245.0)	0.70	0.71 (0.46 − 0.86)	47.5	± 0.97 $\bar{x}$

2   CI confidence interval; CV coefficient of variation; HF high frequency power; ICC intraclass correlation coefficient; LF low frequency power; LoA limits of
3   agreement; p25, 25th percentile; p75, 75th percentile; RMSSD root mean square of successive differences between normal heartbeats; SDNN standard deviation
4   of all normal-to-normal R-R intervals; TP total power; ULF ultra-low frequency power; VLF very low frequency power; $\bar{x}$ data mean

Test-retest reliability of 5-min and 1-hour duration HRV classified by tetraplegia and paraplegia; n = 37

Table 3: Time- and frequency-domain HRV, and test-retest reliability of 5-min and 1-hr HRV classified by tetraplegia (n = 14) and paraplegia (n = 23)

	Day 1, Median (p25, p75)	Day 2, Median (p25, p75)	p-value	ICC (95%CI)	CV	95% LoA
5-min, Tetraplegia (n = 14)						
SDNN (ms), (n = 13)	42.0 (31.4, 50.7)	42.0 (36.0, 46.1)	0.68	0.18 (-0.37 – 0.65)	45.4	± 0.94 $\bar{x}$
RMSSD (ms)	20.4 (14.6, 28.9)	24.2 (17.9, 36.5)	0.63	0.32 (-0.25 – 0.72)	53.6	± 1.07 $\bar{x}$
HF (ms^2), (n = 10)	359.4 (186.7, 721.9)	307.8 (88.4, 542.7)	0.49	0.34 (-0.35 – 0.78)	184.6	± 1.82 $\bar{x}$
LF (ms^2), (n = 12)	455.7 (226.9, 953.7)	425.6 (150.3, 544.8)	0.34	-0.01 (-0.59 – 0.56)	164.0	± 1.76 $\bar{x}$
TP (ms^2) (n = 9)	1711.6 (776.9, 2487.9)	1794.1 (524.3, 2204.2)	0.91	0.21 (-0.44 – 0.74)	77.3	± 1.29 $\bar{x}$
5-min, Paraplegia (n = 23)						
SDNN (ms)	32.9 (22.1, 48.2)	38.1 (27.6, 46.9)	0.44	0.34 (-0.08 – 0.66)	48.2	± 0.98 $\bar{x}$
RMSSD (ms)	18.5 (9.5, 24.7)	20.4 (13.2, 32.1)	0.73	0.38 (-0.04 – 0.68)	75.1	± 1.30 $\bar{x}$
HF (ms^2), (n =17)	80.7 (31.0, 228.7)	131.4 (90.2, 342.1)	0.034	0.57 (0.16 – 0.82)	142.3	± 1.60 $\bar{x}$
LF (ms^2), (n =21)	174.0 (80.5, 498.2)	293.4 (191.7, 692.5)	0.27	0.21 (-0.21 – 0.57)	144.4	± 1.62 $\bar{x}$
TP (ms^2), (n =16)	637.5 (484.9, 2085.0)	1200.2 (771.7, 1719.6)	0.32	0.50 (0.002 – 0.79)	91.7	± 1.38 $\bar{x}$
1-hr, Tetraplegia (n = 14)						
SDNN (ms)	58.5 (55.0, 71.1)	65.3 (51.0, 84.7)	0.079	0.54 (0.07 – 0.82)	26.9	± 0.58 $\bar{x}$
RMSSD (ms)	20.9 (14.0, 33.4)	26.3 (18.9, 35.0)	0.42	0.41 (-0.15 – 0.76)	43.8	± 0.92 $\bar{x}$
HF (ms^2), (n = 11)	617.5 (229.1, 978.7)	461.0 (182.3, 699.4)	0.067	0.54 (0.01 – 0.85)	58.6	± 1.15 $\bar{x}$
LF (ms^2), (n = 13)	681.5 (346.4, 859.1)	619.0 (242.1, 816.6)	0.89	0.07 (-0.53 – 0.59)	92.5	± 1.47 $\bar{x}$
VLF (ms^2), (n = 13)	1321.7 (731.0, 1820.0)	1363.7 (914.8, 2089.9)	0.38	0.19 (-0.34 – 0.64)	81.5	± 1.26 $\bar{x}$
ULF (ms^2)	1201.6 (934.9, 2620.8)	1775.4 (1004.4, 3971.7)	0.091	0.20 (-0.27 – 0.62)	98.3	± 1.33 $\bar{x}$
TP (ms^2), (n = 10)	3575.0 (1819.6, 6533.4)	4112.9 (2752.4, 6150.6)	0.32	0.82 (0.44 – 0.95)	41.5	± 0.83 $\bar{x}$
1-hr, Paraplegia (n = 23)						
SDNN (ms)	55.7 (46.0, 71.6)	57.0 (38.0, 79.4)	0.71	0.66 (0.36 – 0.84)	29.9	± 0.69 $\bar{x}$
RMSSD (ms)	23.3 (9.3, 27.6)	25.0 (15.2, 31.6)	0.85	0.49 (0.10 – 0.75)	55.7	± 1.10 $\bar{x}$
HF (ms^2), (n = 17)	263.0 (143.2, 556.9)	239.6 150.0 628.2	0.55	0.79 (0.51 – 0.92)	86.3	± 1.33 $\bar{x}$
LF (ms^2), (n = 20)	413.8 (173.0, 864.8)	550.4 (271.9, 904.5)	0.37	0.18 (-0.26 – 0.57)	100.6	± 1.41 $\bar{x}$
VLF (ms^2)	916.7 (448.5, 1940.1)	1104.9 (655.1, 2017.5)	0.80	0.64 (0.31 – 0.83)	59.2	± 1.12 $\bar{x}$
ULF (ms^2)	1297.0 (483.7, 2665.8)	1555.1 (578.6, 3810.1)	0.82	0.48 (0.08 – 0.74)	106.1	± 1.53 $\bar{x}$
TP (ms^2), (n = 17)	3163.3 (2206.4, 5612.1)	3117.4 (1412.1, 7245.0)	0.68	0.69 (0.33 – 0.88)	50.9	± 1.05 $\bar{x}$

1 CI confidence interval; CV coefficient of variation; HF high frequency power; ICC intraclass correlation coefficient; LF low frequency power; LoA limits of
2 agreement; p25, 25th percentile; p75, 75th percentile; RMSSD root mean square of successive differences between normal heartbeats; SDNN standard
3 deviation of all normal-to-normal R-R intervals; TP total power; ULF ultra-low frequency power; VLF very low frequency power; $\bar{x}$ data mean

Figure 1

Bland-Altman plot of mean differences and 95 % limits of agreement (LoA) among time domain HRV measures (5-min and 1-hr) in a supine position in individuals with SCI.
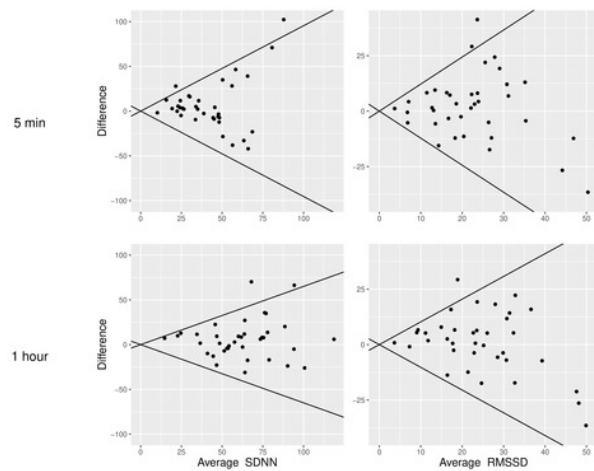
Figure 2

Bland-Altman plot of mean differences and 95 % limits of agreement (LoA) among frequency domain HRV measures (5-min and 1-hr) in a supine position in individuals with SCI.