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Exploratory analysis of spontaneous versus paced breathing on heart rate variability in veterans with combat-related traumatic injury

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Abstract

Background: Respiration is a crucial determinant of autonomic balance and heart rate variability (HRV). The comparative effect of spontaneous versus paced breathing on HRV has been almost exclusively explored in healthy adults and never been investigated in an injured military cohort.

Objective: To examine the effect of spontaneous versus paced breathing on HRV in veterans with combat-related traumatic injury (CRTI).

Design: Observational cohort study.

Setting: ArmeD serVices trAuma rehabilitatioN outComE (ADVANCE) study, Stanford Hall, UK.

Participants: The sample consisted of 100 randomly selected participants who sustained CRTI (eg, amputation) during their deployment (Afghanistan 2003–2014) and were recruited into the ongoing ADVANCE prospective cohort study.

Intervention: Not applicable.

Main Outcome Measure: HRV was recorded using a single-lead ECG. HRV data were acquired during a sequential protocol of 5-minute spontaneous breathing followed immediately by 5 minutes of paced breathing (six cycles/minute) among fully rested and supine participants. HRV was reported using time domain (root mean square of successive differences), frequency domain (low frequency and high frequency) and nonlinear (sample entropy) measures. The agreement between HRV during spontaneous versus paced breathing was examined using the Bland–Altman analysis.

Results: The mean age of participants was 36.5 ± 4.6 years. Resting respiratory rate was significantly higher with spontaneous versus paced breathing (13.4 ± 3.4 vs. 7.6 ± 2.0 breaths/minute; $p < .001$), respectively. Resting mean heart rate and root mean square of successive differences were significantly higher with paced breathing than spontaneous breathing ($p < .001$). Paced breathing significantly increased median low frequency power than spontaneous breathing ($p < .001$). No significant difference was found in the absolute power of high frequency between the two breathing protocols. The Bland–Altman analysis revealed poor agreement between HRV values during spontaneous and paced breathing conditions with wide limits of agreement.

Conclusion: Slow-paced breathing leads to higher HRV than spontaneous breathing and could overestimate resting "natural-state" HRV.

INTRODUCTION

Heart rate variability (HRV) is a noninvasive marker of autonomic activity[1] and an increasingly recognized indicator of physical and mental health.[2,3] One of the major areas of uncertainty around HRV analysis relates to the optimal breathing modality used during HRV measurement. HRV is under the constant influence of respiration and its associated modulation of vagal nerve innervation of the sinoatrial node.[4] With inspiration, there is a shortening of RR interval (RRi) and an increase in heart rate whereas the opposite occurs during expiration and is termed respiratory sinus arrhythmia.[4,5] These breathing-related fluctuations in RRi are affected in response to central respiratory drive and the lung inflation reflex.[4]

Short-term (5–10 minutes) HRV is typically quantified during either spontaneous or paced breathing.[6] At present, there is a lack of consensus as to which method of HRV assessment is preferable. The normal respiratory rate of a rested healthy individual during spontaneous breathing is typically 10–20 per minute.[7,8] In contrast, with paced breathing, respiration is maintained at a constant and slower rate in response to auditory and/or visual prompts.[8] The rationale is that by standardizing the breathing pattern, including the inspiratory and expiratory time, HRV measurement reproducibility is improved. However, paced breathing particularly at 9–10 cycles per minute (cpm) is associated with increased HRV[9,10] and could potentially bias the estimation of HRV and genuine physiological interactions between respiration and HRV.

Research comparing the HRV measured during spontaneous versus paced breathing has been predominantly done in healthy adults[11–14] with a paucity of data relating to "nonhealthy" populations in which the relative influence of respiration (in response to cardiorespiratory disease, chronic pain, and anxiety states) on HRV may be greater. One area of recent interest has been in relation to the physical and psychological consequences of traumatic injury in military servicemen. Recently published baseline data from the ArmeD serVices trAuma rehabilitatioN outComE (ADVANCE) study has suggested that combat-related traumatic injury (CRTI) and worsening injury severity are associated with increased cardiovascular risk, plausibly explained by factors such as increased systematic inflammation, lower physical activity, and greater visceral fat area.[15] Further to this, CRTI and higher injury severity were also found to be significantly associated with lower ultra-short-term HRV measured during spontaneous breathing.[16] However, to date, the comparative influence of breathing protocol (spontaneous versus paced) on short-term HRV among injured military servicemen has not been investigated.

In this study, we sought to expand on previous research by exploring the comparative effect of spontaneous and paced breathing on HRV among combat veterans with CRTI. We hypothesized that HRV would be higher in paced breathing protocol as compared to spontaneous breathing.

METHODS

Study setting and design

This study is based on the first follow-up data from participants recruited into the ongoing ADVANCE study.[17] ADVANCE is a 20-year-long prospective cohort study investigating the effect of combat injury on psychophysiological outcomes in male military personnel and veterans deployed to Afghanistan during 2003–2014. The baseline data collection was completed in 2020. The data used for the present study were collected between November 2019 and 2021. The protocol for the ADVANCE study can be accessed.[17] The ADVANCE study has full ethics approval from the UK Ministry of Defence Research and Ethics Committee (protocol no: 357/PPE/12).[17]

Study population

Participants were randomly selected from the injured cohort of the ADVANCE study. Injured participants were defined as those who sustained a physical CRTI (e.g., gunshot wounds, burns, and amputation etc) during their deployment (UK-Afghanistan War 2003–2014) and required an aeromedical evacuation to a UK hospital for treatment and rehabilitation. The average time from injury or deployment to their first follow-up assessment was approximately 11 years.[16] The participants had no previous history of cardiovascular, renal, or liver disease prior to inclusion. Participants included in this study did not have any oral rate-controlling medicines such as beta-blockers or rate-limiting calcium channel blockers.

Sample size

We calculated our sample size using summary data from a previous study looking at spontaneous versus paced breathing in 30 healthy White military servicemen aged 33.3 ± 7.7 years at near sea level and at high-altitude.[14] Based on these data coupled with published data from our baseline ADVANCE cohort,[16] we estimated that a sample size of 100 (paired spontaneous versus paced) would have ≥80% power to detect a difference between average root mean square of successive differences scores of ≥5.5 ms

(SD of difference 30 ms), assuming a correlation of 0.79 and a significance level (alpha) of .05 (two-tailed) (GraphPad StatMate; GraphPad Software, San Diego, CA, USA). Based on this, we included a random sample of 100 out of the first 248 participants with CRTI who had been included in the first follow-up of the ADVANCE study at the time of the present study.

Study variables

The primary independent variable was the breathing protocol (spontaneous and paced breathing). Our primary outcome variable was HRV in which time-domain, frequency domain, and nonlinear measures were reported. Root mean square of successive differences was reported as a time-domain HRV measure and is conventionally considered an indicator of parasympathetic tone.[1,6] The frequency-domain measure of HRV included low frequency and high frequency powers.[1,6] The low frequency power is influenced by both sympathetic and parasympathetic branches of HRV along with baroreceptors.[1,6] The high frequency power has been traditionally used as an indicator of vagal activity.[1,6] Sample entropy is a nonlinear measure of HRV that indicates the complexity of heart rate signal.[1,6]

Ranks were classified into three main groups: senior rank (commissioned officers), midrank (senior noncommissioned officers), and junior rank (junior noncommissioned officers and other lower ranks) as described previously.[15,16,18] Ethnicity was reported as White and other ethnic groups. Injury severity (at the time of injury) was quantified using the New Injury Severity Score (NISS).[17] NISS was calculated using the Abbreviated Injury Score (2008 update), provided by the UK Joint Theatre Trauma Registry. Other reported variables included participants' smoking status, height, and abdominal circumference.

HRV data collection

Participants fasted for at least 8 hours prior to the data collection. The participants had been fully rested for at least 15 minutes prior to HRV measurement. HRV measurements were performed in the supine position in a temperature and noise-controlled room during the daytime.[17] The participants were encouraged to refrain from talking or sleeping during the measurement. Only a research nurse and the participant were present in the room to minimize distraction as recommended.[19]

A total of 10–15 minutes of HRV data were collected using a single-lead electrocardiogram (ECG) device (Mega Motion Faros 180 recorder: Mega Electronics Ltd., Pioneerinkatu, Finland). The participants followed a fixed breathing protocol in which at least 8 minutes of spontaneous breathing (to allow adequate familiarization) was immediately followed by a 5-minute paced breathing protocol. The last 5-minute epoch of spontaneous breathing protocol was compared to the 5-minute paced breathing protocol. For spontaneous breathing, the participants were encouraged to relax and breathe normally. For paced breathing protocol, the participants were encouraged to follow the auditory cadence to control breathing at a rate of six breaths per minute for 5 minutes.

HRV data analysis

Data collection and analysis methods were conducted in full compliance with the recommended HRV checklist.[19] HRV analysis was conducted using Kubios HRV Premium Software version (3.5) (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). The RR series were corrected by the Kubios HRV Premium "automatic correction method." All ECG recordings were also visually inspected to screen for ectopic beats[20] and analyzed by a single data analyst (R.M.). The smoothness prior method (set at 500; interpolation; cubic spline: 4 Hz with 50 ms R-R threshold) was used to remove very low frequency (<0.04 Hz) trend components from the RRi series. The noise level was set at medium.[21] Time and frequency domain measures of HRV were calculated in accordance with the HRV Task Force Guidelines.[1] HRV spectral measures were calculated using the Fast-Fourier-Transform model. The reported frequency ranges were 0.04–0.15 Hz for low frequency and 0.15–0.40 Hz for high frequency.[1]

Statistical analysis

Continuous data were presented as mean and standard deviation or median and interquartile range for normally distributed and skewed data, respectively. Histograms and QQ plots were used to assess the normality along with skewness and kurtosis tests. Categorical data were presented as number (%). HRV indices were naturally log-transformed (hereafter referred to as Ln) for correlation analyses. Appropriate parametric or nonparametric paired *t*-test was used for paired comparison of HRV indices between spontaneous and paced breathing.

Pearson's r and Spearman's rho were reported as correlation coefficients for log-transformed HRV and nonnormative HRV, respectively (data not shown here but can be requested from the authors). These

correlation coefficients were interpreted as weak (0.10–0.39), moderate (0.40–0.69), strong (0.70–0.89), and very strong (0.90–1.00) as previously described.[22] As correlation does not show an agreement,[23] the Bland–Altman analysis was used to assess the agreement between the two breathing protocols.[24] Linear regression was run to detect proportional bias for HRV indices (with absolute values), revealing significant proportional bias for all HRV variables except the root mean square of successive differences. However, a visual inspection of the Bland–Altman plot of the root mean square of successive differences indicated heteroscedasticity. Considering the increasing standard deviation with concentration, we presented the Bland–Altman plots using the absolute values and percent difference as recommended.[25] Average difference (mean bias %), 95% confidence interval (CI), and limits of agreement (LoA) were reported.[25] Statistical significance was set at $p < .05$ for all tests.

RESULTS

Sample characteristics

The mean age of the participants was 36.5 ± 4.6 years. The participants sustained a CRTI at the mean age of 25.7 years. The majority of participants were White (96%), were nonsmokers (51%), and had lower rank (67%) (Table 1). The mean height and abdominal circumference of participants were 179.7 ± 6.5 and 96.3 ± 11.4 cm, respectively. Of the 100 injured participants, 34 (34%) were amputees. The median NISS for the injured participants was 15 (interquartile range: 9, 27). The mechanism of injury for most of the participants was blast (81%).

TABLE 1 Demographics of study participants.

	Participants
Number	100
Rank at sampling	
Junior	67 (67%)
Middle	24 (24%)
Senior	9 (9%)
Age at injury/deployment, years	25.7 ± 4.6 (Range 18–39)
Age at follow-up 1 assessment, years	36.5 ± 4.6 (Range 28–51)
Ethnicity	
White	96 (96%)
Other	4 (4%)
Amputation status	
Amputee	34 (34%)
Nonamputee	66 (66%)
Injury mechanism	
Blast	81 (81%)
Gunshot wounds and others	19 (19%)

Note: Data presented as mean ± SD or number (%).

Comparison of HRV indices between spontaneous and paced breathing protocols

The mean respiratory rate and frequency were higher during spontaneous (13.5 ± 3.4 cpm; 0.22 ± 0.05 Hz, $p < .001$) versus paced breathing (7.6 ± 2.0 cpm; 0.12 ± 0.03 Hz, $p < .001$). Resting heart rate was significantly lower with spontaneous breathing (58.11 ± 8.85 bpm; $p < .001$) than with paced breathing (61.06 ± 9.52 bpm). Root mean square of successive differences and low frequency power were found to be significantly lower and sample entropy was significantly higher with the spontaneous compared with the paced breathing protocol (Table 2). The absolute power of high frequency was significantly lower with paced breathing protocol whereas a significant difference was observed for normally log-transformed high frequency. Root mean square of successive differences and high frequency values from spontaneous breathing showed a strong correlation with their paced counterparts whereas low frequency and sample entropy had moderate and weak correlations, respectively (data not shown but can be requested from the authors).

Agreement between spontaneous and paced breathing protocols

The Bland–Altman analysis showed poor agreement between spontaneous and paced breathing protocols (Figure 1). For the root mean square of successive differences, the mean bias was −23.27% (± LoA: −84.91%, 38.35%). A mean bias of −117.95% (± LoA: −228.30%, −7.60%) and 23.23% (± LoA: −132.86%, 179.33%) was observed for low frequency and high frequency powers, respectively. For sample entropy, the mean bias was estimated as 48.48% (± LoA: −1.88%, 98.85%). Overall, the upper and lower LoAs were wide indicating poor agreement between the two breathing protocols (Table 3).

DISCUSSION

Clinically, HRV is an objective marker of autonomic function.[1] Lower HRV levels have been associated with a higher risk of all-cause mortality and increased cardiovascular risk.[26] This highlights the importance of investigating HRV profile in individuals who have been

TABLE 2 Difference in HRV between spontaneous and paced breathing protocols.

Measure	Spontaneous breathing ($n = 100$)	Paced breathing ($n = 100$)	Mean difference ± SD or Median difference (interquartile range)	*p* value[a]
Respiratory rate, cpm	13.49 ± 3.45	7.65 ± 2.07	5.84 ± 3.93	$p < .001$
Mean HR, bpm	58.11 ± 8.85	61.06 ± 9.52	−2.94 ± 4.35	$p < .001$
Root mean square of successive differences, ms	43.72 (27.25, 61.04)	56.79 (36.90, 81.41)	−9.37 (−23.48, −3.20)	$p < .001$
Low frequency power, ms^2	1137.88 (382.11, 2033.10)	4897.17 (2467.32, 9391.62)	−3111.34 (−7523.82, −1450.85)	$p < .001$
High frequency power, ms^2	507.02 (251.32, 1301.46)	460.11 (148.48, 1050.25)	45.34 (−186.76, 390.36)	$p = .14$
Sample entropy	1.66 ± 0.29	1.01 ± 0.23	0.65 ± 0.34	$p < .001$

Note: Data presented as mean ± standard deviation or median (interquartile range).
Abbreviations: bpm, beats per minute, cpm, cycles per minute; HR, heart rate; HRV, heart rate variability; ms, millisecond; ms^2, milliseconds square.
[a]Based on the comparison between spontaneous and paced breathing using an appropriate parametric or nonparametric test.

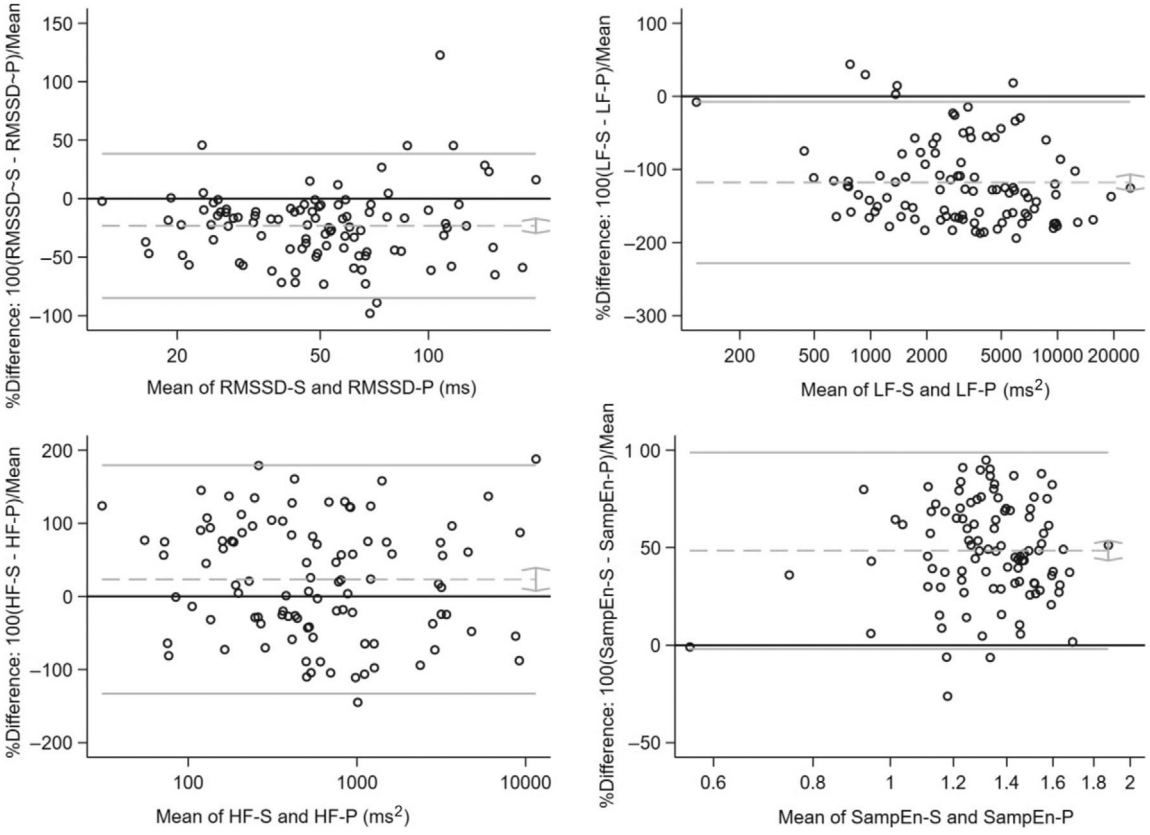

FIGURE 1 The Bland–Altman analysis of the agreement in HRV indices between spontaneous and paced breathing protocols. HF, high frequency; HRV, heart rate variability; LF, low frequency; ms, millisecond; ms^2, milliseconds squared; P, paced; RMSSD, root mean square of successive differences; S, spontaneous; SampEn, sample entropy. Absolute values have been used in the Bland–Altman percent plots. The *x*-axis represents the mean of the HRV index from spontaneous and paced breathing (S + P/2), and the *y*-axis represents the percentage of the difference in HRV index between spontaneous and paced breathing (100 × S−P)/mean. Gray dotted lines denote mean bias (%), and gray solid lines are 95% confidence intervals of bias (lower and upper limits of agreements).

reported to have a higher prevalence of cardiovascular disease risk (eg, arterial stiffness) as a result of CRTI.[15]

In this study, we examined, for the first time, the effect of spontaneous and paced breathing on HRV in veterans with CRTI. Our hypothesis was confirmed that HRV was significantly lower with spontaneous breathing conditions as compared to paced breathing; this was further confirmed by the Bland–Altman analysis indicating that both protocols may not be used interchangeably in this sample.

Overall, we observed that low frequency power was significantly skewed in the paced breathing protocol as compared to spontaneous breathing. Although this agrees with other studies reporting an increase in low

TABLE 3 The Bland–Altman analysis of the agreement in HRV indices between spontaneous and paced breathing protocols.

HRV measure	Mean bias ± SD (%)	95% CI (mean% difference)	95% LoA (%)
Root mean square of successive differences, ms	−23.27 ± 31.44	−29.51 to −17.03	−84.91 to 38.35
Low frequency, ms^2	−117.95 ± 56.30	−129.12 to −106.78	−228.30 to −7.60
High frequency, ms^2	23.23 ± 79.64	7.43 to 39.03	−132.86 to 179.33
Sample entropy	48.48 ± 25.69	43.38 to 53.58	−1.88 to 98.85

Note: Data presented for absolute value and as mean bias ± SD, interpreted in percentage.
Abbreviations: CI, confidence interval; HRV, heart rate variability; LoA, limits of agreement; ms, millisecond; ms^2, milliseconds square.

frequency[27,28] and a decrease in high frequency power in healthy participants[27] observing paced versus spontaneous breathing, our findings are contradictory to those of Solinsky and colleagues.[29] They reported a marked decrease in low frequency power when following a paced breathing protocol. We appreciate that the reason for this contradiction may stem from differences in mechanism and type of trauma (spinal cord vs. combat injury) and the use of different respiratory frequency set for paced breathing in Solinsky and colleagues' study[29] (0.25 Hz/15 cpm) as compared to our study (0.1 Hz/6 cpm).

The greater low frequency power during paced breathing is expected as the respiratory sinus arrhythmia amplitude maximizes[9] and HRV amplitude increases as breathing and heart rate synchronize typically at 0.1Hz[27] or 6 cpm otherwise also known as coherent or resonance frequency.[27] However, in our study, the participants were simply asked to pace their breathing at 6 cpm – not with the aim of finding their resonance frequency per se. Interestingly, although the average breathing rate was significantly lower with paced breathing, it was noted that a minority of participants (5%) unconsciously breathed at ≤0.11 Hz during the spontaneous breathing protocol. This is in line with the existing evidence.[30,31] However, this was beyond the scope of control and did not seem to affect the overall results.

The interpretation of elevated low frequency power (representative of sympathetic domination) and high frequency power (a conventional indicator of parasympathetic activity) during paced breathing is not straightforward and rather complex. Different views have been reported in the literature. First, the elevated low frequency may be attributed to sympathetic activation caused by the cognitive task of correctly controlling the breathing rate as per cadence.[12,32] Second, low frequency power may have a parasympathetic origin given the changes in the low frequency range as a result of a parasympathetic blockade vs. a sympathetic blockade[33]; thus low frequency power may be an indicator of cardiac vagal activity when the breathing frequency is <9 cpm.[10] It has also been suggested to redifferentiate the low frequency power band in two zones: lower (0.06–0.1 Hz) and upper (0.1–0.15 Hz) – reflecting activity from sympathetic and parasympathetic branches to provide better information on underpinning mechanisms.[30] On the other hand, high frequency power may not be an accurate indicator of vagal activity when the breathing frequency is <9 cpm.[34] We observed greater high frequency power during spontaneous as compared to paced breathing – though the difference was insignificant. Although the difference was significant for normally log-transformed high frequency power, the wide LoA on the Bland–Altman plot showed that high frequency from spontaneous and paced breathing protocols may not be used interchangeably.

A significant decrease in sample entropy was observed during paced breathing as compared to spontaneous breathing. This concurs with other studies conducted in healthy participants.[28,35] Entropy measures of HRV such as sample entropy are regarded as the indicators of irregularity of RRi. This is significant because estimating sample entropy using paced breathing alone would represent lower complexity in RRi in contrast to spontaneous breathing; this might mask the "true" physiological effect on HRV.

Breathing at 0.1 Hz has been known to induce beneficial effects including cardiovascular[27,28] and elevated autonomic activity including higher HRV values.[9,10] Given that CRTI has been associated with lower HRV (ultra-short term),[16] we stress the use of spontaneous breathing protocol if the objective is to understand the baseline effect on HRV. We cannot exclude the possibility that by being an intervention, paced breathing could affect the genuine physiological relationship between CRTI and HRV. Second, using the paced breathing protocol, it may not be possible to truly reflect vagal activity using high frequency power as it can only reflect vagal activity when the respiratory rate is ≥9 cpm.[10,34] It is noteworthy that in our study, the compliance with the "6 cpm" protocol during paced breathing was not precise as the average respiratory rate during the paced breathing was 7.65 ± 2.07 cpm. Only 34% of participants managed to follow the exact "0.10 Hz/6 cpm" protocol (data not shown in the table). This highlights the issue of compliance with the prescribed breathing rate that might have originated from difficulty in hearing the auditory cadence. Lastly, there has been some debate around the interpretation of high frequency and root mean square of successive differences in paced breathing conditions. We observed a significantly higher root mean square of successive differences in paced

breathing conditions as compared to spontaneous breathing. Although the root mean square of successive differences has been known to be less affected by respiratory rate as compared to high frequency,[34] it has been recently reported that root mean square of successive differences may not be a valid measure of parasympathetic activity during slow deep breathing given its correlation with low frequency.[36] This further restricts the use of paced breathing if the aim is to analyze the true physiological effect on HRV.

This study has several additional strengths and weaknesses that should be acknowledged. We included standard measures of HRV from both linear and nonlinear domains in order to obtain a comprehensive analysis. HRV was measured under standardized conditions. For example, we followed a set breathing protocol in the order of 5 minutes of spontaneous breathing followed by 5 minutes of paced breathing to minimize biases. This is important because using the reverse order – paced and then spontaneous breathing – is likely to affect the HRV results as lower respiratory frequency has been reported under spontaneous breathing condition after having performed longer paced breathing.[37] Moreover, our sample size ($n = 100$) is larger than several previous comparative studies of healthy[11–14] and post-trauma populations ($n = <60$).[29] Our cohort was also broadly consistent with the full injured ADVANCE cohort in terms of age at injury/deployment, ethnicity, and proportion of amputees.[15]

The findings of our study are not directly comparable with other studies due to the heterogeneity in respiratory frequency chosen for paced breathing, population, and methodological differences (eg, maneuvers and postures). We did not report if the participants followed nasal or oral breathing during the paced breathing protocol. Given the optimizing effect of nasal breathing on brain function,[38] this might ultimately affect HRV. Our population consisted of mostly White males limiting the generalizability of the results. It would be interesting to investigate the comparative effect of breathing on HRV in female combatants as well.

This study offers significant implications for future research and practice. The reported differences in HRV between two breathing protocols hold clinical significance. For example, in our study, median root mean square of successive differences was 9.37 ms higher with paced breathing as compared to spontaneous breathing. This difference is crucial when the aim is to understand the effect of CRTI on the "natural state" HRV. Based on the findings of this study, in the future, we aim to study HRV profile of injured and uninjured veterans from the full ADVANCE cohort ($n = 1144$) along with investigating the role of HRV in predicting cardiovascular risk given its correlation with higher cardiovascular risk.[39]

We suggest that paced breathing might be more suitable for studies investigating HRV Biofeedback (HRVB) therapy, especially for the rehabilitation of individuals with trauma. HRVB is a technique to promote emotional well-being by promoting HRV and eventually mitigating depression and anxiety.[9,27] Given the burden of depression, anxiety, and post-traumatic stress disorder (PTSD) in the ADVANCE cohort at baseline,[18] HRVB may be considered as an intervention for the rehabilitation of military personnel following combat trauma, given the increasing interest in the use of breathing techniques and wearable devices to manage selected trauma such as traumatic brain injury and PTSD in veterans.[40] However, this remains unexplored in this population and warrants further research. Lastly, following this study, we also intend to assess the effects of age, time since injury, injury severity, and comorbidities on HRV in relation to CRTI using the larger sample from the first follow-up data from the ADVANCE study.[17]

CONCLUSION

Slow-paced breathing at a programmed rate of 6 cpm was associated with a significant increase in HRV in veterans with combat injury compared to spontaneous breathing. Further, our findings suggest paced breathing might overestimate the "natural state" HRV. As paced breathing increases HRV, it may hold promise in biofeedback therapy although further investigation is needed.

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ETHICS STATEMENT

This study had full ethical approval from the Ministry of Defence Research and Ethics Committee (protocol no:357/PPE/12). All participants in this study undertook full informed written consent. This study was conducted in compliance with the Declaration of Helsinki (1964).

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