The long-term impact of combat-related traumatic injury on heart rate variability- findings from the ADVANCE study

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Data availability statement: The data that support the findings of this study are available from the ADVANCE study, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the corresponding author upon reasonable request (subject to the UK Ministry of Defence clearance) and with permission of the ADVANCE study.

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Abstract

Introduction: Combat-related traumatic injury (CRTI) has been associated with adverse cardiovascular outcomes in veterans. However, the long-term impact of contemporary CRTI on heart rate variability (HRV, a marker of autonomic function) has never been investigated in combat veterans and personnel. This analysis aimed to examine the association between CRTI and short-term HRV in a contemporary cohort of British servicemen.

Methods: This analysis utilised the first follow-up data from the ArmeD serVices trAuma rehabilitatioN outComE (ADVANCE) prospective cohort study. Participants comprised 469 injured (who sustained serious physical CRTI whilst on deployment in Afghanistan) and 506 uninjured servicemen [who were uninjured and frequency-matched to the injured based on age, sex (male), rank, and deployment: Afghanistan 2003-2014 at recruitment]. Resting HRV was measured (5-minute electrocardiogram) in the supine position. Root-mean-square-of-successive-differences (RMSSD) and the sympathetic nervous system (SNS) index were reported as measures of parasympathetic and sympathetic activity, respectively. Multiple linear regression models reported the association between CRTI and HRV, adjusting for age, rank, and ethnicity.

Results: Participants' median age was 37.5 years. The time from CRTI/deployment was approximately 11 years. Median RMSSD was significantly lower in the injured versus uninjured [37.7ms, (IQR: 25.3, 55.9) versus 41.9ms (IQR: 27.7, 62.2); p=0.01]. After confounder adjustment, CRTI was significantly associated with lower RMSSD [Geometric Mean Ratio: 0.92 (95%CI: 0.85, 0.99) and relatively higher SNS index [coefficient: 0.19 (95% CI: 0.05, 0.34)] in the injured versus uninjured. Blast injury and traumatic amputation were associated with significantly lower RMSSD and greater SNS activity.

Conclusion: CRTI is associated with greater relative autonomic imbalance. These findings may help understand the recovery pathway following CRTI in wounded combat veterans and personnel.

Keywords: HRV, RMSSD, autonomic function, vagal tone, combat injury, trauma.

Key messages

What is already known on this topic:

- Acute trauma has been associated with lower heart rate variability (HRV; indicative of autonomic imbalance). Within this scope, the long-term impact of combat-related traumatic injury (CRTI) on HRV (autonomic function), several years post-injury, has not been investigated in a contemporary military cohort so far.

What this study adds:

- Using the data from the ArmeD SerVices TrAuma and RehabilitatioN OutComE (ADVANCE) study, we found that combat injury, blast injury, and traumatic amputation were independently and significantly associated with lower root mean square of successive differences (RMSSD; a marker of parasympathetic tone) and higher sympathetic nervous system index (SNS index; a marker of sympathetic activity).
- This is the first study that reports that even approximately 11 years after combat injury/deployment, the injured servicemen appear to have relatively lower vagal tone (indexed via RMSSD) and higher sympathetic activity (indexed via SNS index) versus the frequency-matched (age, sex [male], rank, deployment to Afghanistan) uninjured servicemen.

How this study might affect military practice or policy:

- While causality was not assessed, it is anticipated that these findings might be relevant for military healthcare professionals and practitioners to inform future recovery pathways for injured combat veterans and design interventions targeted at long-term health benefits in this population.

Introduction

The variation in the time interval between consecutive heartbeats, known as heart rate variability (HRV), is a non-invasive measure of autonomic function [1]. HRV is influenced by the antagonistic action of sympathetic and parasympathetic branches of the autonomic nervous system. The parasympathetic or vagal tone appears dominant under resting conditions slowing heart rate [1]. With this inherent characteristic, HRV offers diagnostic applications as a marker of cardiovascular disease (CVD) risk [2], stress and adaptability [3], and physical health [4] among others.

The assessment of HRV in military combat settings remains limited to acute trauma which is associated with marked alterations in autonomic balance and reduced HRV [5]. The longer-term effects of non-acute combat-related traumatic injury (CRTI) on HRV are unknown. The recent conflict in Afghanistan has brought this issue into sharp focus given the large number of veterans who survived CRTI [6]. A better understanding of the effects of CRTI and its severity on HRV could create opportunities to refine the physical and mental rehabilitation of our combat veterans to improve their long-term health outcomes following CRTI. Within this scope, the utility of HRV as a marker of autonomic function to reflect recovery and resilience via enhanced parasympathetic tone following CRTI warrants further exploration.

Using the ADVANCE study baseline data, we have previously reported that CRTI and higher injury severity are independent predictors of reduced ultra-short-term HRV [7]. However, currently, there is a need to examine the longer-term impact of CRTI on autonomic function measured using the 'gold-standard' ECG-derived short-term HRV measurement [1]. Following up on the injured participants approximately three years after their baseline visit would enrich our understanding of the longer-term impact of CRTI on HRV.

Hence, this analysis aimed to examine the relationship between CRTI and short-term ECG-measured HRV in a cohort of injured versus uninjured combat veterans at the first follow-up time point of the ADVANCE Cohort study. The secondary aims were to investigate the effect of injury severity, mechanism and amputation on HRV. We hypothesised that short-term HRV would be lower in the injured as compared to the uninjured. We further hypothesised that lower short-term HRV would be explained by higher injury severity, blast injury, and amputation.

Methods

Study design and setting

This analysis used the first follow-up data from the longitudinal ArmeD serVices trAuma rehabilitatioN outComE (ADVANCE) prospective Cohort study, UK. The ADVANCE study aims to investigate the effect of CRTI on psycho-social and physiological health outcomes in a cohort of male British military veterans and personnel at >20 years post-exposure to injury/deployment. The detailed protocol of the ADVANCE study can be accessed elsewhere [8].

Ethics

The ADVANCE study has full ethics approval from the Ministry of Defence Research Ethics Committee (protocol no:357/PPE/12). All participants were given the participant information sheet to fully understand the ADVANCE study aims and data collection protocol. Participants read the consent form in the presence of a research nurse. All participants voluntarily took part in the study and had given their informed and written consent on the study data. Participants held the right to withdraw from the study data at any point they wish without giving any formal explanation or having any impact afterword. All data were collected and stored safely and secure in accordance with the GDPR 2018 and the UK Data Protection Act 2018. Only pseudonymised data were made available to authorised researchers (RM, CJB) following clearance from the ADVANCE study board.

The present analysis used the data collected between October 2019-August 2023 by trained research nurses at Defence Medical and Rehabilitation Centre, Stanford Hall, Loughborough, UK.

Data management

This study was conducted in compliance with the Declaration of Helsinki (1964). Study data were collected and managed using Research Electronic Data Capture (REDCap) tool hosted at Imperial College London. REDCap is a secure, web-based software platform designed to support data capture for research studies. Only authorised authors (RM and CJB) had access to the data reported in this analysis in a password-protected format.

Study population

The participants were male servicemen who had served in the UK Armed Forces during Operation HERRICK in Afghanistan (2003-2014). At recruitment, set inclusion and exclusion criteria were followed [8]. The participants were divided into injured and uninjured groups. The injured group consisted of those who had sustained a serious physical CRTI (e.g. blast injury, burns, lacerations, fractures, amputations) during their deployment to Afghanistan and required aeromedical evacuation for the management of their injuries. The uninjured participants were frequency-matched to the injured group based on age, sex (male), rank, role-in-theatre, and deployment period. The participants had no past history of established cardiovascular, renal, or liver disease or diabetes mellitus before recruitment to the ADVANCE study [8].

Sample size

The total number of participants who attended their first follow-up assessment after three years from baseline was 1053 (deceased/lost to or declined follow-up 8.03%; n=92); 975 participants of these had complete ECG data available for HRV analysis and were included in the present analysis (Figure 1).

Study variables

The main exposure variables were combat injury status (yes/no), injury severity, injury mechanism, and amputation. Injury severity was scaled using the New Injury Severity Scores (NISS) which is calculated as the sum of the squares of the three most severe Abbreviated Injury Scores (AIS) injuries, independent of anatomical position of the injury. The NISS ranges from a score of 1 to 75 with a score of 1 reflecting the least severe and 75 representing the most severe injury [9]. The NISS were calculated based on the 2008 updated AIS provided by the UK Joint Trauma Theatre Registry (JTTR)- a database of every service casualty admitted to a UK medical facility. Injury severity was dichotomised into higher (NISS≥25) versus lower (NISS<25) based on a previously reported link between a higher NISS≥25 (injury severity) and all-cause mortality [10]. Injury mechanism data were extracted from the JTTR dataset and categorised into blast versus gunshot wounds and others (burns, fractures, assaults, etc). The missing data on injury mechanism (n=40) were extracted from the baseline patient medical history. Amputation status was defined as injured non-amputees and injured amputees.

Rank at sampling was used as a proxy measure of socioeconomic status and was classified into junior (junior non-commissioned officers and other lower ranks), middle (senior non--commissioned officers), and senior (commissioned officers) [11] as previously described and used [12]. Ethnicity was categorised into two groups: White and other ethnic groups.

The primary outcome was short-term HRV- reported using the time-domain, frequency-domain, and non-linear measures under the European HRV Task Force recommendations [1]. Resting mean heart rate (HR), root mean square of successive differences (RMSSD), and standard deviation of normal-to-normal intervals (SDNN) were reported as time-domain measures. Low frequency (LF; 0.04-0.15Hz), high frequency (HF; 0.15-0.4Hz) LF/HF ratio, and total power represented frequency-domain measures using the Fast Fourier Transform analysis. The non-linear measures were SD1, SD2, sample entropy (SampEn), acceleration capacity, and deceleration capacity. RMSSD and HF are conventional markers of parasympathetic tone whereas SDNN and LF represent both sympathetic and parasympathetic tones in short-term recordings [13]. SD1 and SD2 represent RMSSD/HF and LF, respectively. Lastly, SampEn reflects signal complexity, usually larger values indicating lower RRi fluctuations [13]. The sympathetic nervous system (SNS) index (computed with mean HR, stress index, and SD2) and parasympathetic nervous system (PNS) index (computed with mean RR, RMSSD, and SD1) were also reported and interpreted using the HRV software guidelines [14].

Systolic and diastolic blood pressure were measured using the Vicorder™ device (Skidmore Medical Limited, Bristol, UK) during pulse wave analysis [8]. Anthropometric data (height, weight, body mass index adjusted for amputation, abdominal circumference) were collected using a standard protocol as described elsewhere [8]. The six-minute-walk-distance test (6MWD-T) was used to assess participants' physical function and the self-reported analogue visual scale from the EQ-5D-5L was used to assess the health-related quality of life (QoL) [8]. Metabolic equivalent (MET) minutes were used to quantify the weekly physical activity from all sources [8].

Participants' medication data and self-reported history of COVID-19 were also recorded and included in the sensitivity analyses.

HRV Data Collection and Analysis

Participants were requested to fast for at least eight hours and refrain from smoking before HRV assessment. All HRV data were collected during daytime in a quiet and temperature-maintained room (average 20.4±1.5 °Celsius). Participants were fully rested and in the supine position on a hospital bed.

The RRi from the P-QRS-T complex in a cardiac cycle were recorded using the Bittium Faros^{TM} ECG device (Mega Motion Faros 180 recorder; Mega, Finland). Two surface electrodes were separately placed under the right and left clavicles and the third under the rib frame. The HRV recordings were on average 15-minutes long. The first few minutes of the ECG recording were for adjusting to the setup and ensuring smooth recording. The last 10 minutes were under spontaneous and paced breathing (six cycles/minute) protocols with a 5-minute epoch for each. The ECG recording of the first five minutes under spontaneous breathing condition was used for HRV measurement in this analysis, based on our recent work [15].

The ECG recordings were analysed in Kubios Premium (Kubios Premium V.3.2, The Biomedical Signals Analysis and Medical Imaging Group, University of Kuopio, Finland) for HRV measurement [7, 16]. While beat correction was set to automatic in Kubios, the signals were further visually inspected for noise, ectopic beats, or 'spikes' (not positioned in a normal cardiac cycle) and were manually corrected wherever needed. The following operators were constant throughout the analysis: the smoothness priors (500), interpolation (cubic spline: 4 Hz with 50ms R-R threshold), and the noise level (medium).

All HRV measures included in this analysis had excellent intra- and inter-rater reliability in our cohort [17]. All ECG recordings were analysed by a single data analyst (RM), blinded to the injury status during analyses.

Statistical analysis

Results are presented as mean and standard deviation (SD) for normally distributed data or median and interquartile range (IQR) for skewed data. All data were visually inspected for normality using the histogram and QQ plots. Following assumption checks, the two-sample *t*-test and Mann-Whitney-U tests were used to study the differences by injury status for parametric and non-parametric equality analysis, respectively.

Following univariable analysis, multiple linear regression analyses were run separately to examine the association between the exposure (injury status, injury severity, injury mechanism, amputation status) and two selected HRV primary outcomes (RMSSD and SNS index). All models were adjusted for apriori confounders (age at the first follow-up assessment, rank at baseline, and ethnicity). The regression models were assessed for heteroscedasticity; which if detected, robust standard errors were reported using the vce(robust) command. Multicolinearity was inspected via the 'vif' command.

RMSSD data was naturally log-transformed before being entered into the linear regression model. The coefficient was exponentiated to give the geometric mean ratios (GMR)- a ratio of two geometric means- as the outcome.

The missing data on the outcome (HRV) was 7.40% whereas negligible on the other variables (systolic and diastolic blood pressure n=1). Multiple imputation analysis was performed for missing HRV data using the predictive mean matching (pmm) and regress algorithm with 50 imputations. The mean HR from the pulse wave analysis was included as an auxiliary variable given its good correlation with logRMSSD (r=-0.60) and the SNS index (r=0.79) [18]. As the data were missing only for the outcome variables and the imputed and non-imputed models were similar, results from the non-imputed models with complete case analysis were presented

[18]. The significance level was set at $p<0.05$. All statistical analyses were conducted in Stata V.17.0 (StataCorp, College Station, Texas, USA).

Sensitivity analyses were conducted excluding the users of anti-hypertensives, β-blockers, statins, anti-depressants, and COVID-19 cases.

Results

Sample characteristics

A total of 975 participants were included with a median age of 37.5 (IQR: 34.2, 41.2) years at the time of the first follow-up assessment. Of these, 469 were injured (n=113 amputees) and 506 were uninjured. Fewer than half of the participants were still serving in the Forces (45.05%, n= 437). The injured and uninjured were of similar age, height, weight, and ethnicity (Table 1). Participants with CRTI were more often of junior rank and had lower QoL and 6MWD but had greater abdominal waist circumference despite similar total physical activity scores to the uninjured (Table 1).

The injured participants sustained CRTI at a median age of 25 (IQR 22, 29) years and the average time from injury/deployment to the first follow-up assessment was approximately 11 years. For the injured group, the median NISS was 12 (IQR: 5, 22). Blast was the commonest injury mechanism and was observed in 69.7% of the injured group. Overall, 19.6% of the injured participants had a NISS≥25. The blast injury group had a higher NISS score than the other injury group (12 [IQR 6, 22] versus 9 [IQR 4, 14]; $p<0.001$).

HRV measures by injury status

HRV data was of high quality with artefact correction performed in <0.4% of ECGs. The respiratory rate was on average 0.22Hz (13.2 breaths/minute). No significant differences were found in brachial systolic and diastolic blood pressures between the injured and uninjured. Similar results were seen when excluding those on anti-hypertensives including β-blockers

(Supplementary Table S1-S2). RMSSD and HF power exhibited a strong correlation (Spearman's rho= 0.94; p<0.001) (Supplementary Figure S1). Mean HR and RMSSD/HF were inversely correlated (both p<0.001). The injured had a significantly higher mean HR than uninjured servicemen (59.8±9.3bpm versus 57.4±8.8bpm; p<0.001). Median RMSSD was significantly lower in the injured versus uninjured servicemen (37.7ms [IQR 25.3, 55.9] versus 41.9ms [IQR 27.7, 62.2]; p=0.01). Among the frequency domain HRV measures, only HF power demonstrated a significant difference between the two groups. The SNS index was significantly higher (p=0.001) whereas the PNS index was lower in the injured than in the uninjured (p<0.001). All non-linear HRV measures showed statistically non-significant differences between the two groups except for SD1 and SampEn (Table 2).

Association between CRTI, injury severity, injury mechanism, amputation, and HRV

After confounder adjustment, injured servicemen had an 8% lower geometric mean RMSSD than those who were uninjured (GMR 0.92, 95%CI 0.85, 0.99). Blast injury was associated with a 10% and significantly lower geometric mean RMSSD than the uninjured. Injured amputees had a 17% lower RMSSD than the uninjured (GMR 0.83, 95%CI 0.74, 0.94). Compared to the uninjured, higher injury severity scores (NISS≥25) were associated with a non-significant 10% lower RMSSD (GMR 0.90, 95%CI 0.78, 1.02) (Table 3, Figure 2).

CRTI was significantly associated with a greater SNS index in the adjusted model [coefficient: 0.19, 95% CI (0.05, 0.34)]. Higher injury severity (NISS≥25), blast injury, and limb amputation were significantly and independently associated with a greater SNS index (Table 4; Figure 2). Sensitivity analyses excluding the users of β-blockers, anti-hypertensives, anti-depressants, statins, and COVID-19 cases showed similar results (Supplementary Table S3; Supplementary Figures S2-S3 for β-blockers).

Discussion

Using the three-year follow-up data from the ADVANCE study, we found that CRTI was associated with significantly lower parasympathetic tone (RMSSD) and higher sympathetic activity (SNS index) than those of a frequency-matched group of uninjured servicemen. Higher injury severity (NISS≥25), amputation, and blast injury were independent predictors of greater SNS activity. Conversely, CRTI, blast injury, and amputation were independently associated with lower short-term RMSSD (HRV) whereas NISS≥25 was not.

Lower short-term RMSSD, suggestive of relatively lower vagal tone, among the injured versus uninjured is consistent lower ultra-short-term HRV reported at ADVANCE baseline [7]. Our data suggest several plausible factors that might explain this observation. The injured servicemen had significantly lower physical function (the 6MWD-T [mean difference: -48.91meters; $p<0.001$]) and relatively higher abdominal obesity (greater abdominal circumference [mean difference: 1.37cm; $p= 0.04$]) than the uninjured servicemen. This could subsequently affect HRV [19]. Lower health-related QoL in the injured versus uninjured group might also contribute to the observed relationship given that HRV attenuates with low health-related QoL [20]. The possibility of other factors e.g. sleep and anxiety disorders, influencing the reported associations cannot be excluded. These factors could also explain the relatively elevated SNS index in the injured versus uninjured, though under normal ranges as per the guideline [14].

We found that blast injury was associated with relative lower vagal tone (RMSSD). This may be explained by endothelial dysfunction as a result of damage to the vascular endothelial barrier due to blast injury; which may reduce vagal activity [21]. Blast waves may also cause traumatic brain injury (TBI), ultimately affecting brain stem activity and autonomic function [22]. Thus, the possibility of TBI's deteriorating effect on HRV [23] cannot be excluded. As TBI assessment was beyond the scope of this analysis, it will be separately assessed within the ADVANCE cohort [8].

The higher injury severity (NISS$\geq$25) showed a non-significant association with lower RMSSD but a significant association with a higher SNS index in the injured than the uninjured. The underlying mechanisms explaining this beyond that already discussed remain uncertain. The longitudinal impact of CRTI and its severity on both vagal (RMSSD) and sympathetic activity is unknown and is the subject of our future work.

There is a dearth of research exploring the relationship between combat-related traumatic limb amputation and HRV. In the only previous comparable study, Peles et al. found no significant group differences in HF power between 52 servicemen with traumatic amputation compared with 53 uninjured controls. However, LF power was significantly greater among the amputees [24]. These observations may not be directly comparable to ours given that their population was older (aged 50-65 years) and their injuries were sustained >40 years ago. Additionally, their control group was combat naïve and selected from the general population. Moreover, as other studies have investigated the influence of phantom limb pain on the link between HRV and amputation [25], their comparison with our data is limited.

Lower HRV measured using 5-minute RMSSD has been independently linked to increased all-cause mortality in both healthy adults and other patient populations [26]. Unfortunately, given the influence of age, sex [27] breathing protocols [28], and patient characteristics on HRV, it is not possible to define a specific HRV cut-off that can be used to specifically infer increased clinical risk or conclude its physiological meaningfulness at this point. The comparison of RMSSD reported in our injured (37.7 ms) and uninjured groups (41.9 ms) with the population-derived normative median RMSSD reported in healthy adults (42 ms; range 19-75 ms) [29] should be interpreted in this context.

To the authors' knowledge, this is the first study to have comprehensively assessed the association between short-term HRV and all aspects of CRTI in a contemporary military cohort and represents data more than a decade post-injury/deployment. Our results are based on a significantly larger sample size (n=975) than the only previous military comparative study (n=<110) [24] and consist of high-quality data. This analysis offers good internal validity as a standardised protocol of HRV data collection was used [8] followed by the determination of breathing protocol [15] and reliability of reported HRV measures [17]. We conducted sensitivity analyses excluding those who consumed medications that might have affected HRV (such as anti-hypertensives, β-blockers, statins, and anti-depressants) to report unbiased results. We also undertook additional COVID-19 sensitivity analysis to rule out the potential effect of COVID-19 on the reported association.

The findings reported in this analysis are subject to a few limitations. HRV data were not available for some participants; however, this was addressed via the multiple imputation analysis which showed similar results to the complete case analysis. Participants taking β-blockers (n=11) were included in the present analyses to include those who might be at elevated CVD risk. However, a sensitivity analysis revealed non-significant differences in the sociodemographic, anthropometric, and HRV data between β-blocker users and non-users (Supplementary Table S1-S2). Only RMSSD and the SNS index were modelled as primary outcomes as indicators of parasympathetic and sympathetic tone, respectively. This was decided because some participants could involuntarily breathe in the LF range (0.04-0.15 Hz) while following spontaneous breathing protocol which may affect HF results. Overcoming this, RMSSD is considered less prone to respiratory changes and correlates with HF [13] and SD1 [30]. We used the SNS index to quantify the sympathetic activity instead of the LF/HF ratio; which as a marker of sympatho-vagal balance remains controversial [13]. No significant group differences were observed in other reported HRV measures; this may be due to their sensitivity to certain methodological factors i.e. duration of recording and breathing protocol [13]. The use of rank as a measure of socio-economic status may have yielded some residual confounding. The external validity of this study may be limited to those similar in characteristics to our population i.e. predominantly White male servicemen with and without CRTI. Given that RMSSD and HF (parasympathetic markers) appeared less affected by the correction of concurrent HR [31], the models were not corrected for concurrent HR. Moreover, the cross-sectional associations reported in this analysis should not be interpreted as causal. We have previosuly explored the mediating effect of physical and mental health factors on the CRTI and HRV relationship using the baseline data from the ADVANCE study [32]. However, as this was beyond the scope of present analysis, it will be subject of our future research.

Our findings offer important implications. Regarding practice, it is anticipated that injured participants with suppressed HRV may benefit from lifestyle measures known to improve HRV, i.e. enhanced physical function and weight loss, as a part of their recovery to regain parasympathetic dominance and suppress sympathetic activity following CRTI. Daily HRV tracking may also be helpful to identify the baseline HRV 'norm' of an individual and adjust the physiological response in real-time by practising slow-paced breathing (six cycles/minute) in response to periods of 'fight or flight'. In future research, we aim to explore the temporal changes in HRV relating to CRTI with the ADVANCE study's 2nd follow-up data and investigate the impact of potential mediators such as the 6MWT on the CRTI-HRV relationship- which was beyond the scope of this analysis. Future reseearch may also consider pre-injury/deployment assessment of HRV to better capture the trajectory of change in autonomatic function following injury/deployment. Lastly, given the limited evidence on the CRTI-HRV link and age-corrected HRV normative data, we call for further research to generate comparable evidence across international military cohorts to understand the HRV norms for military populations.

Conclusion

In this first follow-up of the ADVANCE Cohort study, CRTI was associated with a greater relative autonomic imbalance (indexed via lower parasympathetic tone and higher sympathetic activity) in the injured compared to that of a frequency-matched uninjured population. These findings suggest that even 11 years post-CRTI/deployment, the injured servicemen may still have a dominant 'fight or flight' response and a suppressed 'rest and digest' response- the latter should be enhanced for recovery and cultivating resilience following CRTI.

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Supplementary Material

Table 1: Socio-demographic, anthropometric, and lifestyle characteristics of the sample, by injury status.

	Injured	**Uninjured**
Number	469	506
Age at FU1 assessment, years	37.29 (34.33, 41.26)	37.87 (34.22, 41.27)
Amputee -Yes -No	 113 (24.09) 356 (75.91)	 -
Injury mechanism -Blast -GSW and others	 327 (69.72) 142 (30.28)	 -
NISS-2008	12 (5, 22)	-
Injury severity (NISS-2008) -Lower (NISS<25) -Higher (NISS≥25)	 377 (80.38) 92 (19.62)	 -
Ethnicity, -White -Other ethnic groups	 426 (90.83) 43 (9.17)	 460 (90.91) 46 (9.09)
Rank at sampling† -Junior (OR2-4) -Middle (OR5-9) -Senior (OF1-6)	 333 (71.00) 89 (18.98) 47 (10.02)	 298 (58.89) 137 (27.08) 71 (14.03)
Smoking status -Ex-smoker -Never smoked -Smoker	 180 (38.38) 228 (48.61) 61 (13.01)	 182 (35.97) 229 (45.26) 95 (18.77)
Height, cm	179.42±6.76	178.90±6.32
Weight, kg	89.06±15.42	90.38±13.01
BMI adjusted for amputation, kg/m^2	28.44±4.05	28.20±3.63
Abdominal circumference‡, cm	97.79±11.34	96.42±9.85
Total weekly activity from all sources‡, METs	5796 (2352, 10836)	6073.5 (2977.5, 10821)
6MWD-T‡, meters	603.65±112.67	652.56±85.55
Self-rated health score‡ (EQ-5D-5L)	80 (70, 90)	82 (74.5, 90)

Notes:

-Data presented as mean±SD or number (%) or median (inter-quartile range; IQR) for highly skewed data.

-FU1, first follow-up assessment; GSW, gunshot wound; NISS, New Injury Severity Score; 6MWD-T, 6-minute walk distance test; METs, Metabolic Equivalents; EQ-5D-5L; EuroQol's health-related quality of life (visual analogue scale *"how good or bad your health is today?"*)

†Junior non-commissioned officers and other lower ranks (OR2-4), senior non--commissioned officers (OR5-9), commissioned officers (OF1-6)

‡Missing data (abdominal circumference n=5; all activities METs n=48; EQ-5D-5L n=4; 6MWD-T n=32).

Table 2: Comparison of haemodynamic and HRV data, by injury status.

	Injured	Uninjured	p-value*
Number	469	506	-
Respiratory rate, Hz	0.22±0.05	0.22±0.05	0.26
Systolic blood pressure†, mmHg	134.21±11.33	134.15±10.92	0.92
Diastolic blood pressure†, mmHg	70.60±9.17	69.75±8.81	0.14
Heart Rate, BPM	59.82±9.35	57.46±8.81	p<0.001
RR intervals, ms	1027.11±158.40	1068.80±165.39	p<0.001
RMSSD, ms	37.71 (25.37, 55.93)	41.95 (27.74, 62.25)	0.01
SDNN, ms	41.53 (28.98, 55.50)	42.76 (31.16, 59.24)	0.14
HF power, ms^2	454.82 (215.70, 1033.79)	599.85 (243.95, 1218.17)	0.02
LF power, ms^2	733.44 (367.84, 1553.97)	803.01 (402.63, 1614.36)	0.49
Total power, ms^2	1481.85 (763.16, 2804.51)	1585.05 (882.11, 3134.58)	0.12
LF/HF	1.60 (0.82, 3.29)	1.37 (0.67, 2.96)	0.05
AC, ms	-36.35 (-55.35, -23.94)	-36.33 (-52.51, -25.05)	0.81
DC, ms	35.36 (22.52, 56.86)	37.05 (24.59, 53.98)	0.67
SD1, ms	26.70 (17.97, 39.62)	29.71 (19.64, 44.11)	0.01
SD2, ms	51.80 (36.51, 66.34)	51.61 (38.53, 69.03)	0.42
SampEn	1.65±0.25	1.69±0.26	0.008
SNS Index	-0.44 (-1.19, 0.53)	-0.64 (-1.34, 0.13)	0.001
PNS Index	0.41 (-0.45, 1.32)	0.67 (-0.14, 1.73)	p<0.001

Notes:

-Data presented as mean±SD or median (IQR) for highly skewed data.

-HRV, heart rate variability; Hertz; BPM, beats per minute; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal to normal intervals; LF, Low Frequency; HF, high frequency; LF/HF, low frequency/high frequency ratio; AC, acceleration capacity; DC, deceleration capacity, SD1; in Poincaré plot, the standard deviation perpendicular to the line-of-identity; SD2, in Poincaré plot, the standard deviation along the line-of-identity; SampEn; Sample entropy; SNS index, sympathetic nervous system index; PNS, parasympathetic nervous system index.

*Based on the comparison between injured and uninjured participants using appropriate equality test based on normality.

†Missing data (n=1).

Table 3: Multivariable regression analysis of logRMSSD with injury status, injury severity, injury mechanism, and amputation status at the first follow-up assessment.

	Univariable	Model 1 (CRTI) n=975	Model 2 (Injury severity) n=975	Model 3 (Injury mechanism) n=975	Model 4 (Amputation) n=975
	Unadjusted GMR	**Adjusted GMR**	**Adjusted GMR**	**Adjusted GMR**	**Adjusted GMR**
Injury status					
Uninjured	Ref	Ref	-	-	-
Injured	0.90 (0.84, 0.98)	**0.92*** (0.85, 0.99)	-	-	-
Injury Severity (NISS-2008)					
Uninjured	Ref	-	Ref		-
NISS<25	0.91 (0.83, 0.98)	-	0.92 (0.85, 1.00)		-
NISS≥25	0.90 (0.78, 1.03)	-	0.90 (0.78, 1.02)		-
Injury mechanism					
Uninjured	Ref	-	-	Ref	-
Blast	0.89 (0.82, 0.97)	-	-	**0.90*** (0.83, 0.98)	-
GSW and others	0.93 (0.83, 1.04)	-	-	0.95 (0.85, 1.06)	-
Amputation					
Uninjured	Ref	-	-	-	Ref
Injured non- amputee	0.93 (0.85, 1.01)	-	-	-	0.94 (0.87, 1.02)
Injured amputee	0.83 (0.73, 0.94)	-	-	-	**0.83**** (0.74, 0.94)

Notes: -Each model has been adjusted for age at the first follow-up assessment, rank at sampling, and ethnicity.

-RMSSD, the root mean square of successive differences; CRTI, combat--related traumatic injury; GMR, geometric mean ratio; GSW, Gunshot Wounds, NISS, New Injury Severity Score; Ref, reference category.

-* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, $p \leq 0.0001$.

Table 4: Multivariable regression analysis of SNS index† with injury status, injury severity, injury mechanism, and amputation status at the first follow-up assessment.

	Univariable	**Model 1 (CRTI) n=975**	**Model 2 (Injury severity) n=975**	**Model 3 (Injury mechanism) n=975**	**Model 4 (Amputation status) n=975**
	Unstandardised coefficient	**Adjusted unstandardised coefficient**	**Adjusted unstandardised coefficient**	**Adjusted unstandardised coefficient**	**Adjusted unstandardised coefficient**
Injury status					
Uninjured	Ref	Ref	-	-	-
Injured	0.24 (0.09, 0.40)	**0.19**** (0.05, 0.34)	-	-	-
Injury Severity (NISS-2008)					
Uninjured	Ref	-	Ref		-
NISS<25	0.21 (0.05, 0.37)	-	**0.16*** (0.00, 0.31)		-
NISS≥25	0.39 (0.12, 0.66)	-	**0.35**** (0.06, 0.63)		-
Injury mechanism					
Uninjured	Ref	-	-	Ref	-
Blast	0.29 (0.12, 0.46)	-	-	**0.24**** (0.07, 0.41)	-
GSW and others	0.15 (-0.07, 0.38)	-	-	0.08 (-0.12, 0.29)	-
Amputation					
Uninjured	Ref	-	-	-	Ref
Injured non- amputee	0.16 (0.00, 0.33)	-	-	-	0.11 (-0.04, 0.27)
Injured amputee	0.50 (0.25, 0.75)	-	-	-	**0.46***** (0.21, 0.71)

Notes: - Each model has been adjusted for age at the first follow-up assessment, rank at sampling, and ethnicity.

-†Raw SNS index was used in the regression analysis and skewness was handled by *estat (hettest)* and *vce(robust)* commands in Stata.

-CRTI, combat-related traumatic injury; GMR, geometric mean ratio; GSW, Gunshot Wounds; NISS, New Injury Severity Score; Ref, reference category.

-* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, p≤ 0.0001