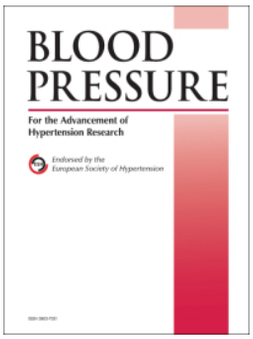

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The inverse and non-linear association between central augmentation index and heart rate variability in a cohort of male British combat personnel-findings from the ADVANCE study

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Abstract

Purpose: The central augmentation index (cAIx) is an indirect measure of arterial stiffness. The influence of heart rate variability (HRV) on cAIx remains unexplored in a military cohort and was the aim of this analysis.

Method: The first follow-up data from the ArmeD serVices trAuma rehabilitatioN outComE (ADVANCE) study were analysed. Participants were male British servicemen who served in Afghanistan (2003-2014) and were divided into two groups at recruitment: injured (who sustained severe combat injury) and uninjured. The uninjured were frequency-matched to the injured by age, rank, role-in-theatre and deployment. HRV was reported as root-mean-square-of-successive-differences (RMSSD) using a five-minute single-lead electrocardiogram. The cAIx was measured using pulse waveform analysis and was adjusted for heart rate at 60 beats/minute (cAIx@60). Effect modification by injury was assessed via interaction analysis. Linear models reported the association between RMSSD (HRV) and cAIx@60 adjusting for a priori confounders.

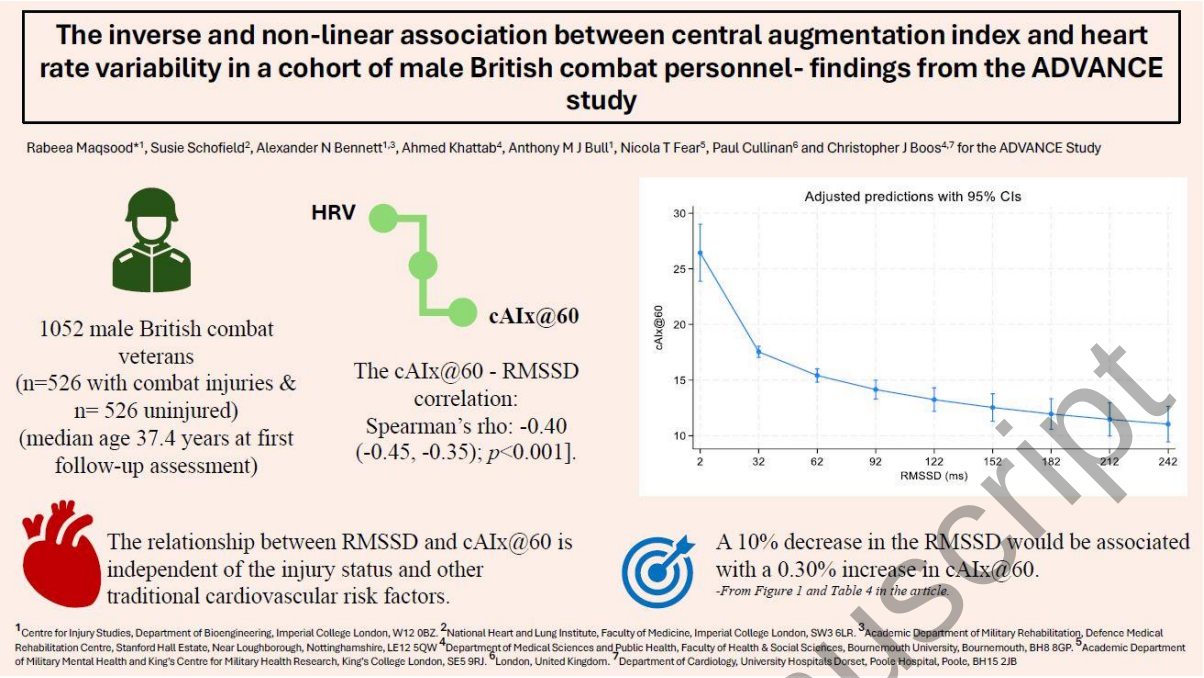
Results: 1052 participants (injured n=526; uninjured 526; median age at follow-up 37.4 years) were examined. Effect modification by injury was not statistically significant; therefore, was adjusted for along with other confounders. RMSSD and cAIx@60 exhibited a moderate inverse correlation (-0.40; $p<0.001$). The association between natural log-transformed RMSSD (LnRMSSD) and cAIx@60 was non-linear and statistically significant, suggesting that a 10% decrease in LnRMSSD would be associated with 0.30% increase in cAIx@60.

Conclusion: Lower RMSSD (HRV) is associated with an increase in cAIx@60, independent of injury status and other traditional cardiovascular risk factors. The efficacy of positive HRV modification on cardiovascular risk in military populations needs to be examined.

Plain Language Summary

The hardening of arteries can lead to serious cardiovascular sequelae and adversely affect health and wellbeing of an individual. One way to measure hardening of arteries (also known as arterial stiffness) is through the use of central augmentation index (cAIx). The cAIx measures the extra 'workload' the heart faces due to 'augmented pressure' or the 'extra pressure' exerted on the heart due to hardening of arteries. Previous research has not investigated whether there is a relationship between cAIx and heart rate variability (a measure of autonomic function), especially in a contemporary military cohort. This is crucial as servicemen may have an increased cardiovascular disease risk due to combat exposure and combat injuries, and studying this association may have important implications for their recovery. Therefore, the goal of this analysis was to explore the association between cAIx and HRV. With multivariable regression analysis and after controlling for confounders, we found an inverse and non-linear association between cAIx and HRV in a sample of British military cohort. It suggests that an increase in cAIx leads to a decrease in HRV but not in a linear way. Leveraging on this association, the results suggest the need to explore HRV elevating activities to mitigate the risk of arterial stiffness in military populations.

Graphical Abstract



Keywords: Arterial stiffness, HRV, vagal tone. Military, Trauma

Introduction

Heart rate variability (HRV) is a non-invasive and objective measure of cardiac autonomic regulation, indicating balance of underlying sympathetic and parasympathetic activity [1, 2]. HRV is a recognised cardiovascular risk marker; lower HRV is associated with a broad range of cardiovascular risk factors such as type-2 diabetes mellitus [3], obesity [4], and hypertension [5] in healthy and patient populations. Furthermore, among patients with established cardiovascular disease, lower HRV is associated with a worse prognosis [6].

It has also been shown that HRV is inversely associated with arterial stiffness [7]. However, most of the previous research relates to pulse wave velocity (PWV) as a measurement of arterial stiffness. The central arterial augmentation index (cAIx) is another indirect measure of arterial stiffness that reflects the additive effect of reflected arterial pressure waves, at sites of flow impedance, upon central arterial systolic pressure [8]. Stiffer arteries lead to earlier and greater augmentation pressure and cAIx [9]. Higher cAIx leads to increased left ventricular afterload and eventually left ventricular hypertrophy and diastolic dysfunction [10].

The cAIx is under the influence of autonomic nervous system control; increased sympathetic activation leads to arterial vasoconstriction and would be expected to increase central augmentation pressure and cAIx [11]. Unfortunately, the association between HRV and cAIx and the influence of autonomic changes on this relationship have been barely explored [12, 13, 14].

There is evidence to suggest that combat-related traumatic injury (CRTI) is associated with increased cardiovascular risk [15, 16]. Baseline data from the ArmeD serVices trAuma rehabilitatioN outComE (ADVANCE) study has shown significantly lower HRV [17] and higher cAIx in individuals with CRTI versus their uninjured counterparts of similar age, sex (all men), rank and deployment period (Afghanistan) [15]. However, the influence of CRTI on the relationship between HRV and arterial stiffness has not been examined. This is an essential step to determine the potential causal relationship between autonomic (dys)function and increased cAIx.

This study aimed to examine the association between HRV and cAIx (as a measure of arterial stiffness) independent of other cardiovascular risk factors in a cohort of injured and frequency-matched uninjured servicemen. We hypothesised that lower HRV would be

associated with higher cAIx, independent of the injury status and traditional cardiovascular risk factors.

Methods

Study design and setting

The ADVANCE study is a prospective longitudinal cohort study. It aims to investigate the long-term psychological and physiological health impacts of CRTI in a cohort of British military combat veterans and personnel over the course of 20 years [18]. The ADVANCE study completed baseline data collection in August 2020. We undertook the analysis of the first follow-up data of the ADVANCE study. A total of 1053 participants attended the first follow-up assessment (lost to follow-up/deceased/declined follow-up, n=92) as compared to the baseline assessment (n=1145). One participant from the uninjured group sustained a non-military related injury and was excluded in the present analysis to avoid bias, leaving the total sample size of 1052.

The ADVANCE study has full ethics approval from the UK Ministry of Defence Research Ethics Committee (MoDREC) (protocol no:357/PPE/12). All participants voluntarily took part in the study and provided their informed and written consent on the day of the data collection. The data used in the present study was collected between October 2019 and August 2023 by trained research nurses at the Defence Medical and Rehabilitation Centre, Stanford Hall, Loughborough, UK. The full protocol of the ADVANCE study can be found elsewhere [18].

Study population

Participants were male servicemen who had served in the UK Armed Forces during Operation HERRICK in Afghanistan (2003-2014). The participants were divided into two groups: injured (those who sustained a serious CRTI and required aeromedical evacuation to a UK hospital) and uninjured. The uninjured participants were frequency-matched to the injured participants based on age, rank, role-in-theatre, and deployment period [18]. None of the participants had an established cardiovascular, renal, or liver disease or diabetes mellitus prior to the injury/deployment. However, as the present analysis reports on the first follow-up data, the effect of chronotropic medication (beta-blockers) on the reported association between cAIx and HRV was assessed by excluding those participants who consumed beta-blockers in the sensitivity analysis to report unbiased results.

Data collection

Participants visited Stanford Hall, Loughborough for a single study visit which lasted for 6 hours and 30 minutes, on average, including the periods of rest and break between assessments. Participants were requested to refrain from caffeine, alcohol, and food intake for at least eight hours and from smoking for at least four hours prior to data collection. After seeking participants informed written consent, fasting venous blood samples were taken and processed by a local hospital laboratory. These blood samples were used to measure glycated haemoglobin [(HbA1c) as a measure of average glucose control over the preceding three months], high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides.

Following this, pulse wave data for pulse wave analysis (PWA) and pulse wave velocity (PWV) were collected using the Vicorder^{TM} device (Skidmore Medical Limited, Bristol, UK) with participants at rest in the supine position. Brachial arterial systolic and diastolic blood pressures, and central arterial augmentation index (cAIx) were measured using the Vicorder^{TM} device during the PWA in the morning. The primary outcome of the present analysis was cAIx which was calculated using the average of three cAIx measurements. However, a measurement differing by more than two-fold from the other two measurements in sequence, was treated as an outlier and excluded. Each reading was adjusted for a heart rate of 60 beats/minute (denoted as cAIx@60, %) as previously validated and described [15, 19].

This was immediately followed by HRV assessment. HRV was measured using the Bittium Faros^{TM} ECG device (Mega Motion Faros 180 recorder; Mega, Finland) with participants in the supine position at rest on a hospital bed in a noise free and temperature-controlled room. A continuous single-lead electrocardiogram (ECG) was recorded for 15-20 minutes. After the initial few minutes of setup adjustment and familiarisation, data were collected under spontaneous breathing for five minutes, which was then immediately followed by a slow-paced breathing protocol for five minutes. The primary independent variable of interest in the present analysis was the root mean square of successive differences (RMSSD), a time-domain measure of HRV that represents parasympathetic or vagal tone [1, 20] obtained via above-described five-minute ECG recording. The five-minute RMSSD is a gold-standard measure of short-term HRV and has been considered the best measure for autonomic nervous system activity [21]; it is less susceptible to respiratory changes and has better statistical properties which allow robust calculation [20]. In the present analysis, the five-minute HRV

data recorded under spontaneous breathing protocol was used and reported based on our previous work [22].

Data on socio-demographic, lifestyle (including smoking status), medication history, mental and physical health were collected from participants' clinical interview with a trained research nurse and validated questionnaires as described in the ADVANCE study protocol [18].

In the second half of the study day, data from Dual-Emission X-ray Absorptiometry (DEXA), spirometry, 6-mninute walk test and audiometry were collected. In this analysis, the six-minute walk distance test (6MWD-T) and total Metabolic Equivalents (METs) from all activity sources derived from the International Physical Activity Questionnaire (IPAQ) were used to reflect physical function and physical activity, respectively. The study day ended with a participant debrief with a member of ADVANCE research staff [18].

Injury severity was assessed using the New Injury Severity Scores (NISS) (range 1-75); which were calculated using the Abbreviated Injury Score (2008 updates provided by the UK joint Trauma Theatre Registry; JTTR). The NISS were categorised into higher injury severity (NISS>25 and lower injury severity (NISS<25) based on the previous evidence supporting its link to all-cause mortality [23].

The injury mechanism was categorised into blast versus gunshot wounds and others. 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, OR2-4), middle (senior non-commissioned officers, OR5-9), and senior (commissioned officers, OF1-6) [24] ranks, as previously used [15]. Ethnicity was categorised into two groups: White and other ethnic groups.

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.

HRV data analysis

The ECG recordings were analysed using Kubios Premium (Kubios Premium V.3.2, The Biomedical Signals Analysis and Medical Imaging Group, University of Kuopio, Finland) for HRV measurement [17, 25]. ECG data were visually inspected for ectopic beats and noise and were manually corrected wherever applicable. This was also supplemented with noise set

to medium and automatic correction. Throughout the analysis, the following operators were kept constant: the smoothness priors (500), interpolation (cubic spline: 4 Hz with 50 ms R-R threshold), and the noise level (medium).

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 histograms and QQ plots.

The association between RMSSD and cAIx@60 was assessed using linear models and was tested for linearity using the 'boxtid' command where $p < 0.05$ was considered to indicate a non-linear association. A statistically significant non-linear relationship between RMSSD and cAIx@60 was observed. Both splines and log-transformation were used to model the non-linear relationship between RMSSD and cAIx@60. As log-transformation produced lower Bayesian Information Criterion (BIC) than spline, RMSSD was naturally log-transformed (hereby referred to as LnRMSSD) and reported in the the multivariable regression analysis. All models were adjusted for a priori confounders (injury status, age, rank, ethnicity, HDL-C, LDL-C, systolic blood pressure, diastolic blood pressure, triglycerides, and smoking), based on previous literature [13, 14]. The regression coefficient for the association between LnRMSSD and cAIx@60 was interpreted as for each 10% decrease in RMSSD the outcome (cAIx@60) increased by x units calculated by coefficient multiplied by log(1.10), as described [26]. Additional models were run adjusting for injury mechanism, amputation status and injury severity. The relationship between cAIx@60 and RMSSD was visualized using the 'f_able' and 'margins' commands in Stata. The 'vif' command in Stata was used to rule out any multi-collinearity.

CRTI was assessed as an effect modifier of the association between LnRMSSD and cAIx@60. An interaction term between CRTI and LnRMSSD was considered [27] adjusting for a priori confounders. In further exploratory analyses, the role of injury mechanism, amputation and injury severity as effect modifiers was also examined via interaction. A sensitivity analysis was performed to exclude those on beta-blockers (n=11) in the model adjusted for injury status and other confounders

RMSSD had 78 (7.4%) missing data points (HRV testing not implemented at the time of assessment n=66; technical issues in ECG recordings n=11; an uninjured participant excluded due to a non-military injury n=1) and there were four missing values (0.4%) for cAIx@60.

The other missing data points were systolic blood pressure (n=1; 0.09%), diastolic blood pressure (n=1; 0.09%), HDL-C (n=23; 2.18%), LDL-C (n=47; 4.46%), triglycerides (n=21; 1.99%), HbA1c (n=24; 2.28%), and smoking (n=26; 2.47%). Multiple imputations were performed using the multiple imputations by chained equations (MICE) algorithm under the assumption that the data were missing at random (MAR) [28] with regress (cAIx@60, HDL-C, LDL-C, LnRMSSD), predictive mean matching (triglycerides, HbAIc, systolic and diastolic blood pressures) and logit methods (smoking) depending on the variables [28, 29]. Auxiliary variables (mean heart rate from the pulse wave velocity measurement and body mass index adjusted for amputation) were added to the imputed model due to their high correlation with the missing data (RMSSD) in the model. These auxiliary variables plus all the variables included in the final model were used in the imputed model. The fit of the imputed model was visually inspected using the 'midiagplot' command in Stata.

The number of imputed datasets (m=50) was decided following the fraction of missing information in the post-model estimates. Complete case and multiple imputed models are reported. The significance level was set at $p<0.05$. All statistical analyses were conducted in Stata V.17.0 (StataCorp, College Station, Texas, USA).

Results

Sample characteristics

In total, 1052 participants were included; 526 were injured [including 140 amputees] and 526 were uninjured. The median age of participants at the first follow-up assessment was 37.4 years (IQR: 34.15, 41.20). Most of the participants were White and held a junior rank at injury/deployment. Blast was the most common mechanism of injury (71% of the injured). The median NISS was 12 (IQR 5, 22). The injured group had a significantly lower 6MWD and had a significantly higher abdominal waist circumference than the uninjured group (Table 1).

Effect modification by injury status

The effect modification by CRTI on the association between LnRMSSD and cAIx@60 was non-signficant [coefficent –0.23 (95% CI -1.78, 1.31); p =0.76]. The same non-signficant effect modification was observed for higher injury severity [coefficent 0.91 (95% CI -1.73, 3.57), p =0.49], amputation [coefficent 0.61 (95% CI -1.76, 3.00); p =0.61], and injury mechanism (blast) [coefficent 0.13 (95% CI -1.54, 1.82); p =0.87].

Association between RMSSD and cAIx@60

There were no significant differences in systolic and diastolic blood pressures, lipid profile (LDL-C, triglyceride), cAIx (adjusted and unadjusted), and average blood glucose levels (HbA1c) between the injured and the uninjured groups. HDL-C was significantly lower in the injured than in the uninjured (p=0.01). The injured group had a significantly higher resting mean heart rate and lower RMSSD than the uninjured group (Table 2). For the total sample, the cAIx@60 had a significant and negative correlation with RMSSD [Spearman's rho: -0.40 (-0.45, -0.35); p<0.001] (Table 3).

After adjusting for a priori confounders, a10% decrease in RMSSD was associated with a 0.30 increase in cAIx@60 (%) (Table 4). Similar results were observed when excluding those taking beta-blockers (n=11) and with the imputed model (Table 4). There was an inverse and non-linear relationship between cAIx@60 and RMSSD (Figure 1). The additional models adjusted for injury severity, amputation, and injury mechanism also showed a similar coefficient (Table 4).

Discussion

The principle finding of this analysis is that lower short-term RMSSD (HRV) is inversely correlated and significantly associated with higher cAIx@60 (arterial stiffness, a cardiovascular risk factor). The relationship between RMSSD and cAIx@60 was independent of the injury status and other traditional cardiovascular risk factors. Our findings indicate that a 10% decrease in the RMSSD would be associated with a 0.30% increase in cAIx@60.

The inverse relationship between RMSSD (HRV) and cAIx@60, suggesting that lower HRV is associated with higher arterial stiffness, is not surprising. While the evidence on the topic is scarce, some general trends are comparable. For example, a significant and inverse association between the standard deviation of normal-normal intervals (SDNN) and AIx@75 has been reported in type-1 Diabetes patients [13]. However, our findings are contradictory to the findings of another study [12]. The authors reported a significant positive association between high frequency (LnHF) and AIx@75 in a much smaller study in men (n=39) but not women (n=41) during paced breathing at 12 breaths/minute. Whereas, in another study (n=107) this same group observed that higher heart rate recovery (suggestive of increased cardiac vagal activity) was associated with lower AIx@75 [30]. However, the direct comparison of our findings with other studies is limited given the heterogeneity in HRV measures reported (RMSSD, SDNN, and HF), population type (healthy, diabetes patients and servicemen with and without injury), and adjustment of AIx (at heart rate).

From a physiological standpoint, the precise mechanism of how lower RMSSD (HRV) is associated with higher cAIx@60 is unclear. From the lens of autonomic imbalance, lower RMSSD reflects vagal withdrawal [20], which may result in elevated sympathetic activity [31]. The positive correlation between sympathetic activity and AIx [32] may be a plausible explanation for this observation. Furthermore, increased vasoconstriction due to enhanced sympathetic activity affects vascular tone [11, 33] and leads to relative vascular impedance and increased augmentation index. The relationship between HRV and cAIx@60 may be bidirectional, given the suggestion of a bidirectional relationship between HRV and PWV-another marker of arterial stiffness [34]. The possibility of the influence of mediating factors (not included in this analysis) such as depression, anxiety, and physical function on the causal pathway between RMSSD and cAIx@60 cannot be excluded.

The observation that combat injury (and other injury variables) did not significantly moderate the relationship between cAIx@60 and RMSSD was unexpected. Our findings indicate that a

decrease in RMSSD would be associated with an increase cAIx@60 to almost the same degree in the injured as in the uninjured. While in the present analysis, the injury status was included as a confounder rather than a moderator, it will be the subject of our future work to investigate if this is a recurring trend in the upcoming follow-ups of the ADVANCE study.

Another finding of this analysis is the non-linear and inverse relationship between RMSSD and cAIx@60. The cAIx@60 appears to have a steeper decline at the lower end of the scale with narrow CIs and then becomes less steep as RMSSD increases with relatively wider CIs. The explanation of this trend is limited given the paucity of evidence on the link between RMSSD and cAIx@60.

To the authors' knowledge, it is the first study to have provided preliminary evidence on the association between HRV and cAIx@60 independent of traditional cardiovascular risk factors in a contemporary military cohort of injured and uninjured veterans and personnel. Another key strength of this analysis is its large sample size as compared to previous studies with a sample size of <550 [13, 30]. An exploratory analysis was conducted to examine the influence of other aspects of injury (mechanism, severity and amputation status) for a comprehensive assessment of the RMSSD-cAIx@60 association. A standardised protocol of data collection was used for all variables of interest indicating the internal validity of this analysis. Multiple imputation was also performed to account for the missing data and to report unbiased results.

The generalisability of results may be limited to populations similar in characteristics to participants included in this study – adult, mainly White male servicemen. The sensitivity analysis showed similar results when excluding those taking beta-blockers (n=11). Given this, participants taking beta-blockers were included in the reported analysis. Given the negative correlation between heart rate and AIx [19], AIx was adjusted at a standard heart rate of 60; however, the AIx and RMSSD models were not adjusted for heart rate to protect and maintain the intrinsic physiological influence of heart rate on RMSSD (HRV).

It may be early to ascertain the clinical significance of our findings given the exploratory nature of this analysis. Nonetheless, our findings present important implications for practice and research and should be contextualised for interpretation. Our findings suggest that increasing HRV levels in combat veterans might mitigate the risk of early atherosclerosis (cAIx@60) and potentially a later cardiovascular event. While HRV stands out as a robust non-invasive biomarker of cardiovascular health, its link with AIx and other lifestyle factors

remains under-represented in current research. Given this, we call for further research in the field to investigate the link between cAIx@60 and HRV in military cohorts as well as in other vulnerable and under-represented populations to generate comparable evidence. The causal pathway between cAIx and HRV also warrants exploration to fully understand the effect of HRV on cAIx in combat veterans and other populations.

In summary, in a large, combined group of injured and uninjured servicemen, lower RMSSD was associated with cAIx@60 in a non-linear pattern, independent of injury status and traditional cardiovascular risk factors.

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Author contribution: RM and CJB contributed to the study conception and design. AK acquired the funding for RM's PhD studentship at Bournemouth University. Material preparation and data management were performed by RM. HRV analysis was performed by RM and supervised by CJB. Statistical analyses were performed by RM and supervised by SS. The first draft of the manuscript was written by RM. The project was overall supervised by AK and CJB. The manuscript was critically reviewed and suggested improvements related to intellectual content by ANB, SS, NTF, AMJB, PC, and AK. All co-authors read and approved the final version of the manuscript.

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

Data availability statement: Given the unusually sensitive nature, data have not been made widely available. Requests for data will be considered on a case-by-case basis and subject to UK Ministry of Defence approval and clearance. The ADVANCE study protocol is available online.

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Table 1: Demographic and lifestyle characteristics of the participants by injury status.

	Total	**Injured**	**Uninjured**
Number	1052	526	526
Age at FU1 assessment, years	37.44 (34.15, 41.20)	37.17 (34.09, 41.18)	37.86 (34.22, 41.21)
Amputee, n (%)			
-Yes	-	140 (26.6%)	
-No	-	386 (73.3%)	-
Injury mechanism, n (%)			
-Blast	-	375 (71.29%)	-
-GSW and others	-	151 (28.71%)	
NISS-2008, median (IQR)		12 (5, 22)	-
Injury severity (NISS-2008), n (%)			
-Lower (NISS<25)	-	412 (78.33%)	-
-Higher (NISS>25)	-	114 (21.67%)	
Ethnicity, n (%)			
-White	957 (90.97%)	479 (91.06%)	478 (90.87%)
-Other ethnic groups	95 (9.03%)	47 (8.94%)	48 (9.13%)
Rank at sampling†, n (%)			
-Junior (OR2-4)	677 (64.35%)	368 (69.96%)	309 (58.75%)
-Middle (OR5-9)	242 (23.00%)	100 (19.01%)	142 (27.00%)
-Senior (OF1-6)	133 (12.64%)	58 (11.03%)	75 (14.26%)
Smoking status‡, n (%)			
-Never	481 (46.88%)	244 (48.13%)	237 (45.66%)
-Ex-smoker	380 (37.04%)	195 (38.46%)	185 (35.65%)
-Smoker	165 (16.08%)	68 (13.41%)	97 (18.69%)
Height, cm	179.21+6.59	179.46+6.91	178.95+6.25
Weight, kg	89.63+14.23	88.97+15.35	90.30+12.99
BMI adjusted for amputation, kg/m^2	28.33+3.87	28.48+4.07	28.17+3.66
Abdominal waist circumference‡, cm	97.09+10.61	97.82+11.26	96.36+9.88
Total weekly activity from all sources‡, METs	5850 (2784, 10776)	5868 (2340, 10836)	5811 (2977, 10737)
6MWD-T‡, meters	625.69+104.71	597.15+114.95	652.59+85.85

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

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

-FU1, first follow-up assessment; GSW, gunshot wound; NISS, New Injury Severity Score; BMI, Body Mass Index; 6MWD-T, 6-minute walk distance test; METs, Metabolic Equivalents.

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

‡Missing data (6MWDT n= 38; all activities METs n=54; abdominal circumference n=9, smoking status n=26)

Table 2: Haemodynamic, HRV, and cAIx data of the participants by injury status.

	Injured	**Uninjured**
Number	526	526
Systolic blood pressure†, mmHg	134.13+11.24	134.07+10.95
Diastolic blood pressure†, mmHg	70.34+9.17	69.69+8.87
cAIx† (unadjusted), %	17.63+7.16	17.69+7.50
cAIx† (adjusted at heart rate 60), %	17.35+8.34	16.51+8.70
Mean heart rate†, BPM	59.38+9.85	57.18+9.65
RMSSD†, ms	37.71 (25.37, 55.93)	42.07 (27.74, 62.44)
HDL-C†, mmol/L	1.29+0.31	1.34+0.29
LDL-C†, mmol/L	3.16+0.85	3.23+0.80
Triglycerides†, mmol/L	1.12 (0.8, 1.6)	1.13 (0.8, 1.61)
HbA1c†, mmol/mol	34.61+6.44	34.67+5.16

-Data presented as mean±SD or median (inter-quartile range; IQR) for highly skewed data.
*Based on the comparison between injured and uninjured participants using appropriate equality test based on normality.
-cAIx, central Augmentation Index; RMSSD, Root Mean Square of Successive Difference; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol, HbAIc; Glycated haemoglobin.
†Missing data (systolic/diastolic blood pressures n=1; cAIx (unadjusted) n=1; cAIx@60 (adjusted at heart rate of 60/minute) n=4; HDL-C n=23; Triglycerides n=21; LDL-C n=47; HbA1c n=24; Heart Rate, n=32; RMSSD n= 78).

Table 3: Correlation between cAIx@60 and RMSSD.

	Correlation between RMSSD and cAIx@60 (95% CI and *p*-value)
Total sample	-0.40 (-0.45, -0.35) *p*<0.001
Injured	-0.42 (-0.50, -0.35) *p*<0.001
Uninjured	-0.37(-0.45, -0.30) *p*<0.001

Table 4: Multivariable regression analysis of the cAIx@60 with RMSSD.

	Complete case analysis (n=926)										
	Univariable model	**Model 1 (Injury status)†**		**Model 2 (Injury status†)** excluding beta-blocker‡ users (n=915).		**Model 3 (Injury Severity†)**		**Model 4 (Amputation†)**		**Model 5 (Injury Mechanism†)**	
	Unadjusted unstandardised coefficient. (95% CI)	Adjusted unstandardised coefficient. (95% CI)	*p*-value	Adjusted unstandardised coefficient. (95% CI)	*p*-value	Adjusted unstandardised coefficient. (95% CI)	*p*-value	Adjusted unstandardised coefficient. (95% CI)	*p*-value	Adjusted unstandardised coefficient. (95% CI)	*p*-value
LnRMSSD*	-5.09 (-5.88, -4.30)	-3.21 (-4.06, -2.36)	<0.001	-3.23 (-4.09, -2.38)	<0.001	-3.22 (-4.06, -2.37)	<0.001	-3.17 (-4.02, -2.32)	<0.001	-3.20 (-4.05, -2.35)	<0.001
	Multiple imputed model (n=1053)										
LnRMSSD*	-5.27 (-6.07, -4.48)	-3.67 (-4.51, -2.82)	<0.001	-3.66 (-4.52, -2.81)	<0.001	-3.67 (-4.51, -2.82)	<0.001	-3.61 (-4.46, -2.77)	<0.001	-3.65 (-4.50, -2.80)	<0.001

*Ln, Natural log of RMSSD used in the regression. Interpreted as: coefficient*log(1.n) where n= point e.g 3.21*log(1.10) = 0.30 difference in cAIx@60 in response to 10% decrease in RMSSD in the injury status model. Uninjured were included as reference category in all injury-related models.
†Model further adjusted for age at the first follow-up assessment, ethnicity, rank at sampling, systolic and diastolic blood pressures, HDL-C, LDL-C, triglycerides, HbA1c, and smoking.
‡In the imputed model without beta blocker users, missing data assumed as non- beta blocker user.

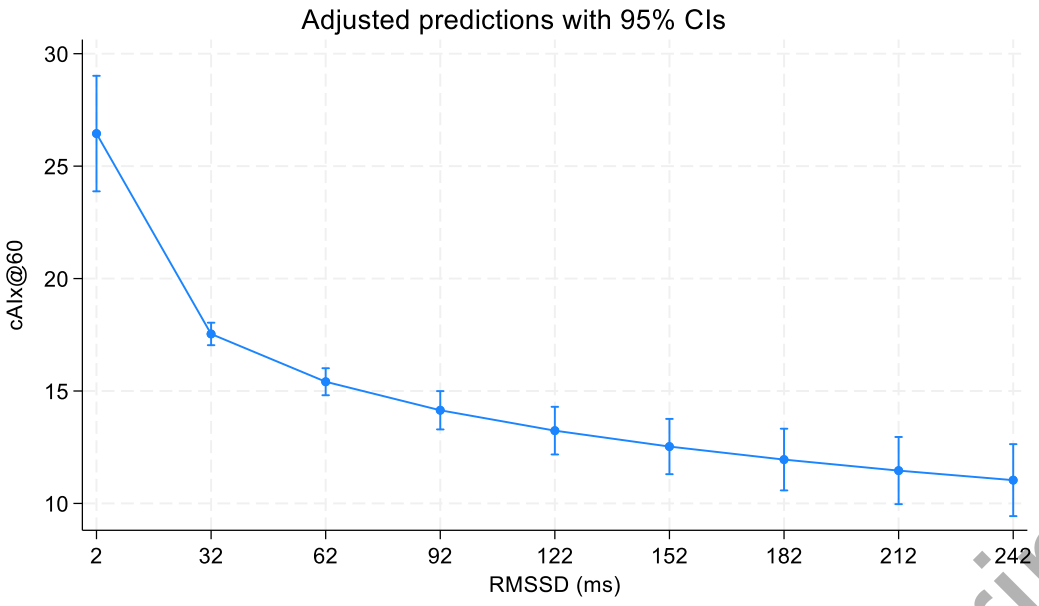

Figure 1: An inverse and non-linear association between cAIx@60 and RMSSD (adjusted for injury status and other confounders).