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Does Military Combat-related Traumatic Injury Increase Cardiovascular Disease Risk?

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Copyright Statement

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ABSTRACT

Background

Cutting edge battlefield trauma care has allowed military personnel to survive complex injuries that historically would not have been possible. There is largely historical evidence suggesting that combat related traumatic injury (CRTI) is associated with an increased cardiovascular (CV) disease (CVD) risk. However, data from a contemporary military population with CRTI is lacking.

Hypotheses

The main hypotheses addressed in this thesis are:

1. Military servicemen who have had suffered severe CRTI with limb amputations have increased arterial stiffness compared to those less severely injured and non-injured servicemen.
2. Combat-related traumatic amputation is associated with greater vascular inflammation and a more adverse lipid profile than that observed with less severe combat related injuries and with non-injured servicemen exposed to the same operational environment.

Methods

This was a cross-sectional observational study that included the first 699 male British military personnel who took part in the ongoing ADVANCE (**A**rme**D** Ser**V**ices Tr**A**uma Rehabilitatio**N** Out**C**om**E**) study project. CRTI veterans with limb amputations (CRTI-A, n=105) and CRTI veterans without limb amputations (CRTI-NA, n=200) were compared with a frequency-matched healthy control population (n=394) of servicemen of similar age and operational exposure (Afghanistan 2003-14). Fasting serum lipids, glucose and Hs-CRP levels were measured using venous blood. Arterial stiffness (expressed as central [AIx] and peripheral [pAI] Augmentation Index), pulse wave velocity (PWV), brachial and central aortic blood pressure, subendocardial viability ratio (SEVR), an indirect marker of myocardial blood perfusion, were measured using the Vicorder device. Injury severity was determined using the New Injury Severity Score (NISS) and the association of injury severity score and CVD risk profile was assessed.

Results

The mean age was 33.9 (± 5.40) [range 23-60] years with the time from injury or deployment to examination averaging 90.4 (± 20.99) months. The NISS were higher in the CRTI-A vs CRT-NA vs control 36.75 (± 18.01) vs 20.11 (± 17.9) vs 0; $p<0.001$ respectively. Resting heart rate ($minute^{-1}$) was significantly higher amongst those with CRTI-A, 63.67 (± 12.53) vs CRTI-NA group 58.35 (± 9.41) and, healthy controls 56.39 (± 9.24); $p<0.001$, with no significant difference in respective HDL Cholesterol levels 1.120 (± 0.27) vs 1.249 (± 0.33) vs 1.252 (± 0.27) mmol/l; $p<0.001$. PWV, AIx or central blood pressure. The SEVR values, 194.0 (± 43.32) vs 201.3 (± 45.07) vs 208.5 (± 63.04); $p=0.0101$) respectively, were significantly lower whereas Hs-CRP, 2.60 (± 3.81)mg/l vs 1.96 (± 2.68)mg/l vs 1.44 (± 1.69)mg/l; $p=0.0166$ respectively and total cholesterol/HDL ratio 4.49 (±1.25) vs 4.31 (±1.82) vs 4.19 (±1.48); $p=0.012$, respectively, were significantly greater among the CRTI-A compared with the CRTI-NA and healthy controls. These differences were most marked with proximal bilateral amputations.

Conclusion

Servicemen who have sustained severe CRTI-A have a worse CVD risk profile with evidence of greater vascular inflammation and a more adverse lipid profile than those without amputations or injury. However, there was no significant difference in arterial stiffness. These findings confirm the need for a long-term cohort study to assess whether these differences in risk profile will translate into meaningful variance in CVD outcomes.

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List of Abbreviations

BMI	Body mass index
6MWT	6-minute walk test
aDBP	Aortic diastolic blood pressure
ADVANCE	ArmeD SerVices TrAuma RehabilitatioN OutComE
AIx	Central augmentation index
AKA	Above knee amputation
AKI	Acute Kidney Injury
ANOVA	One-way Analysis of Variance
aSBP	Aortic systolic blood pressure
AWC	Abdominal waist circumference
BF%	Body fat percentage
BMI	Body mass index
CAC	Central arterial compliance
CAD	Coronary artery disease
cAP	Augmentation Pressure
CASP	Critical Skills Appraisal Programme
CFR	Coronary flow reserve
CHD	Coronary Heart Disease
CoI	Conicity index
CI	Confidence interval
CINAHL	Citation Index for Nursing and Allied Health Literature
CKD	Chronic Kidney Disease
cPP	Pulse Pressure
CRP	C-reactive protein
CRTI	Combat related traumatic injury
CRTI-A	Combat related traumatic injury with limb amputation
CRTI-NA	Combat related injured - no amputation
CV	Cardiovascular
CVD	Cardiovascular disease
DALYS	Disability Adjusted Life Years
DM	Diabetes Mellitus
DMRC	Defence Medical Rehabilitation Centre

DMS	Defence Medical Services
eGFR	Estimated glomerular filtration rate
HC	Healthy control
HDL-C	High-Density Lipoprotein (HDL) Cholesterol
HBAKA	Hypertensive BAKA
HR	Heart Rate
HRQOL	Health-related quality of life
Hs-CRP	High-sensitivity C reactive Protein
HTN	Hypertension
ICD	International Classification of diseases
IHD	Ischaemic Heart Disease,
IHD	Ischaemic heart disease
ISS	Injury Severity Score
JTTR	Joint Theatre Trauma Registry
LDL	Low-density lipoprotein
LDL-C	Low Density Lipoprotein (LDL) Cholesterol
MAP	Mean Arterial Blood Pressure
MEDLINE	Medical Literature Analysis and Retrieval System Online
MeSH	Medical Subject Headings
MetS	Metabolic syndrome,
NCEP	National Cholesterol Education Program
NIDDM	Non-insulin-dependent diabetes mellitus
NISS	New Injury Severity Score
NO	Nitric oxide
Non-A	Non-amputee
OR	Odds Ratio
pAI	Peripheral augmentation index
PICO	Patient/population, Intervention, Comparison/control, Outcome
PP	Pulse pressure
PTSD	Post-traumatic stress disorder
PWV	Pulse wave velocity
ROS	Reactive oxygen species
RHR	Resting heart rate
SEVR	Subendocardial viability ratio
SM	Service Members
TC	Total Cholesterol
TG	Triglycerides

TIA	Transient ischaemic attack
TLLAP	Traumatic Lower Limb Amputee
TTA	Trans tibial amputation
UBEA	Unilateral Below Elbow CRTI-A,
WC	Waist circumference
WHR	Waist-to-hip ratio
WHtR	Waist to height ratio

Chapter 1
General Introduction

1.1 Background and rationale

Between 2003 and 2014, the battlefields of Afghanistan (Op Herrick, 2016) and Iraq (Op Telic, 2016), left the Defence Medical Services (DMS) with significant numbers of military personnel who sustained severe complex injuries which, without the use of body armour and cutting-edge battlefield trauma care, they would previously not have survived (Ministry of Defence, The National Archives). The rate of war-related amputations is now twice that experienced by military personnel in previous wars (Robbins et al., 2009). Consequently, a relatively large population of young service members was created with unique healthcare and rehabilitation needs. There is emerging evidence that combat related traumatic injury (CRTI) is associated with an increased risk of CVD. With heart and circulatory disorders being responsible for 1 in 4 deaths in the United Kingdom (UK), there is a need for further exploration into the risk of cardiovascular disease (CVD) development in these individuals.

The effects of combat related traumatic injuries (CRTI) on contemporary and subclinical CVD risk factors should be examined and determined to facilitate early identification and mitigation strategies before the onset of clinical disease to minimise the impact of CVD long term. If severe traumatic injury and limb amputations are linked to an increased risk of CVD then it could be argued that an increased burden of identifiable subclinical cardiovascular risk factors, such as raised fasting blood glucose, lipid profile and high sensitivity C reactive protein (Hs-CRP) would be present prior to the onset of clinical CVD (Kotsis et al., 2017, Antunes et al., 2016).

An indirect assessment of the impact of atherosclerotic risk factors acting on the arterial tree is now possible using a variety of methods. Non-invasive methods include the quantification of arterial stiffness (using pulse wave velocity [PWV] and pulse wave analysis [PWA] to measure the arterial augmentation index [AIx] (Magalhaes et al., 2011). These evolving techniques have enriched the understanding and quantification of CVD risk and improved the ability to detect subclinical cardiovascular risk.

Data from both military and civilian cohort studies suggest that CRTI resulting in limb amputations (CRTI-A) are linked to an increased risk of developing CVD (Modan et al., 1998,

Kunnas et al., 2011, Robbins et al., 2009). These findings were however refuted by Mundell and colleagues who concluded that those with trauma-related limb amputations were at no higher risk of a cardiac event than their able bodied controls (Mundell et al., 2018). Mundell et al also found that the provision of a prosthesis was not associated with a reduced risk of developing CVD (Mundell et al., 2018).

One of the principle limitations of the current studies that have investigated the plausible link between CRTI, and cardiovascular risk is that their data mainly relate to retrospective analysis of injuries sustained in historical wars fought more than 40 years ago. Furthermore, the majority of studies were cross sectional studies with poorly defined comparative controls that were often civilians of dissimilar ages and background (Hrubec and Ryder, 1980, Kunnas et al., 2011, Modan et al., 1998), leading to considerable bias in reported outcomes. Limited studies are also available about other types of traumatic injuries with the focus primarily on established CVD risk markers such as hypertension and diabetes (Rose et al., 1987).

Previous cohorts have not been as severely injured as the UK DMS cohort. Other limited and insufficient studies were in long term health related outcomes such as bone health (osteoarthritis, osteopenia/osteoporosis and fracture), all-cause mortality and long term vocational and quality of life (QOL) outcomes and not transferable to our current uniquely severely injured population of casualties (Dutta et al., 2008, Kang and Bullman, 2001, Kulkarni et al., 1998, Shahriar et al., 2009, Vollmar et al., 1989). Other shortcomings of previous studies include their retrospective design and the fact that cardiovascular risk factors were not collected at baseline.

This MPhil study is a cross sectional evaluation of the baseline data of the prospective longitudinal ADVANCE (ArmeD SerVices TrAuma RehabilitatioN OutComE) Study conducted by the DMS. The ADVANCE Study was designed to examine the medium- and longer-term outcomes of British combat casualties who sustained traumatic injuries during recent military operations in Afghanistan (www.advancestudydmrc.org.uk). Rehabilitation of these casualties takes place at Defence Medical Rehabilitation Centre (DMRC), previously residing at Headley Court and now at Stanford Hall, where the long-term outcomes of this cohort of severely injured UK armed forces casualties are investigated. Rehabilitation is regarded as a crucial step in achieving optimal long-term outcomes and quality of life after amputations. Therefore, there is a need to gain as much information as possible to improve the potential for optimal rehabilitation of military personnel with combat-related traumatic amputations. An increased understanding of the health issues facing the CRTI-NA military

personnel, and other CRTI-A, would be invaluable to enhance the long-term function and quality of life after amputation.

The DMS has undertaken to ascertain various aspects of the health and psychosocial characteristics of service personnel by means of prospective, longitudinal studies. This will provide information for future conflicts and offer appropriate and timely support for serving and discharged members alike.

1.2 Epidemiology of combat related traumatic injuries and amputations

A study by Chandler and colleagues confirmed that for the UK military population, extremities are involved in the vast majority of combat injuries, which resulted in a large surgical workload required for their treatment (Chandler et al., 2017). Therefore, the UK Military Joint Theatre Trauma Registry was searched for survivors of extremity injuries during the period between 19th March 2003 (Invasion of Iraq) and 27th October 2014 (cessation of combat operation in Afghanistan), excluding casualties killed in actions or who died from their wounds.

It was found that of 2348 UK combatants who survived injury in Iraq and Afghanistan, 1813 (77%) had extremity injuries with 205 (11%) having at least one amputation at the wrist or ankle or more proximal. Trans-tibial was the most common level of limb loss. Eighty-five casualties lost two limbs, with 83 of these (98%) lost both lower limbs and 17 lost three limbs. A prospective study of the UK Naval Services to determine casualties, injury patterns, recovery and residual burden from conflicts between March 2003 and April 2013 revealed there were 277 casualties in the study period of which 63 (23%) were fatalities. 23% were medically discharged and 9% were placed in reduced fitness categories in continued military service. 46% returned to full service. Injuries to the lower limb were the most common cause for medical downgrading or discharge with upper limb traumatic injuries the next most frequent. The conclusion was that extremity injuries were the most challenging in rehabilitation and reconstruction services with the aim to achieve maximal outcomes for the injured personnel (Penn-Barwell et al., 2014).

Examining US injury trends between 2005-2009, information was extracted from the Joint Theatre Trauma Registry (JTTR) to obtain all relevant injury information about all US military. A total of 1,992,232 military service members were deployed with 7,877 combat casualties resulting in 29,624 distinct combat wounds. The mean age of the combat casualty cohort was 26.0 years old (98.8% male). Combat wounds were distributed as follows:

head/neck, 28.1%; thorax, 9.9%; abdomen, 10.1%; and extremities, 51.9%, which confirms that the majority of combat related injuries could involve amputation or at least compromised ambulation. Explosive related injuries were more prevalent than gunshot injuries (74.4% vs 19.9%, $p<0.001$). Explosive related injuries became more common in Iraq than Afghanistan between 2005-07 equaling towards 2009.The conclusion was that wounding patterns in Afghanistan and Iraq differed from previous US conflicts with explosive mechanisms accounting for 74.4% of combat casualties (Belmont et al., 2012).

Further traumatic injury statistics are: 1530 fractures with 501 (33%) involving upper limbs and 1029 (67%) lower limbs and pelvis. The tibia was reported to be the most frequently fractured bone. 597 (58%) of lower limb fractures were open compared to 344 (69%) in upper limbs. An average of seven surgical procedures was reported per limb.

While mortality rates were lower in current than in past conflicts, the survivors were left with complex soft and bone tissue damage following traumatic injuries resulting in multiple surgeries. These interventions often lead to infection complicating the recovery and care process (Weintrob et al., 2018). Such infections are associated with the development of CVD (Khademi et al., 2019). It was reported that one third of combat injuries from Iraq and Afghanistan developed infection during their initial hospitalisation (more than 52,000 US military were wounded in action). Injury severity, amputations and blood transfusions were associated with risk of infection (Weintrob et al., 2018). Infection therefore played a significant role in the development of cardiovascular disease risk. During 2009-12, 1807 US casualties were evacuated with 34% developing infection of which 50% was skin, soft tissue or bone infections (Weintrob et al., 2018).

To stratify injury severity for The ADVANCE Study, the New Injury Severity Score (NISS) was applied. The New Injury Severity Score is defined as the sum of the squares of the Abbreviated Injury Scale scores of each of the patient's three most severe injuries, regardless of the body region in which they occur (Samin, 1999). Equally, the New Injury Severity Score can be applied for predicting the length of hospital stay in multiple trauma patients (Salehi et al., 2016). The NISS, associated with 50% chance of survival, increased each year from 32 in 2003 to 60 in 2012 concluding an improvement in survival during a ten-year period, confirming the increased potential of survival relative to the severity of the injuries sustained on the battlefield (Penn-Barwell et al., 2015). This finding confirms the need for measuring performance of combat casualty care systems to include measures of morbidity, functional recovery and survival (Penn-Barwell et al., 2015).

1.3 Cardiovascular disease risk associated with combat related traumatic injuries

CVD refers to cardiac and vascular system disorders. It is a set of heterogenous conditions of which atherosclerosis is one of the principal pathogenic causes. They are chronic diseases which develop gradually through life and is usually asymptomatic for a long time. By the time symptoms develop the condition is typically in its more advanced stages (US Department of Health, 2011).

A previous retrospective study investigating CVD following 667 wounded or injured Finnish World War II veterans (injured 1939-45) recorded in 1980, delivered outcome data for only 102 of the 667 participants (Kunnas et al., 2011). The risk of coronary artery disease (CAD) related death with injured war veterans increased 1.7-fold compared to the uninjured (95% CI 1.1-2.5; p = 0.01) and there was a significant risk of depression (Kunnas et al., 2011). However, the study lacked detail in order to be compatible with today's requirements. For example, data on baseline risk factors, population, age and CVD events such as stroke, peripheral vascular disease etc., were not defined. In addition, the types of injuries were not recorded to allow meaningful comparisons to current UK military battlefield casualties.

Modan et al evaluated the 24-year mortality rates of male traumatic lower limb amputees (n=201) of the Israeli army in a longitudinal study (Modan et al., 1998). These soldiers were wounded between 1948 and 1974 and were compared with a sample representing the general population (n = 1,832). The conclusion was that the incidence of CVD was doubled in amputees compared with controls. Mortality rates were also significantly higher (21.9% vs 12.1%; $p<0.001$) in amputees compared with controls due to increased CVD-related mortality (8.9% vs 3.8%; $p<0.001$). However, only selected risk factors for CVD were available on the 101 surviving amputees (aged 50 to 65 years) therefore the risk increase is poorly defined. The control sample (n = 96) were matched by age and ethnic origin.

An increase in CVD risk factors with traumatic lower limb amputees may explain an increase in recorded mortality. The fact that the study was an evaluation of only a 24-year mortality rate of male lower limb amputees and limited defined risk factors were recorded for survivors and not for those who died, makes the results extremely difficult to apply to current CRTI-A.

On top of the physical trauma, the patient group assessed was faced with social and psychological challenges, including high rates of depression and anxiety disorders, post-traumatic stress disorder (PTSD), body-image anxiety and reduced social functioning, social

discomfort and negative sense of self and identity (Melcer et al., 2012, Melcer et al., 2010, Sandweiss et al., 2011). Research in this area is limited, and the majority of the literature is from general population samples (Horgan and MacLachlan, 2004). Although only cardiovascular risk factors were considered in our study, psychological trauma should not be discounted between comparative groups as it could impact on cardiovascular health.

To fully understand the type of injuries with resulting implications on cardiovascular health, we researched publications by means of a systematic review that analysed the injury records of these recent conflicts. We also wanted to learn how injuries suffered during recent conflicts compare with historic wars, for which there are publications available relating to CVD risk development due to amputations. We researched which type of injuries were more prevalent and at what scales. Although we are analysing injury statistics for UK military personnel, we also looked into the US statistics to help us appreciate the scale of the injury problem caused by modern conflicts.

In general, CVD is associated with reduced quality of life and is not only the most important cause of premature mortality but also of Disability Adjusted Life Years ("DALYS") in Europe (Reiner et al., 2011a).

CVD is caused by invariable and/or variable risk factors. Invariable or fixed risk factors (age, gender, genetic heritage) cannot be changed, while variable risk factors such as smoking tobacco, physical inactivity, poor eating habits, elevated blood pressure, type 2 diabetes, dyslipidemia and obesity can be modified by changing bad habits (Reiner et al., 2011b). The goal of managing variable risk factors is to reduce CVD morbidity and mortality. CVD mortality can be reduced 45-75% by controlling risk factors and 25-55% by proper CVD treatment (Reiner et al., 2011a).

1.4 Cardiovascular disease risk markers

Cardiovascular disease risk markers investigated in this study were physiological and biochemical as well as anthropometrical and lifestyle markers of CVD risk.

1.4.1 Physiological markers of CVD risk

(a) Hypertension (High blood pressure)

Hypertension, at an estimated cost of £2bn to the NHS (Ettehad et al., 2016) , is defined as office systolic pressure (SBP) values ≥140 mmHg and/or diastolic BP (DBP) values ≥90 mmHg (Lurbe et al., 2016). Elevated blood pressure (BP) was not only the leading contributor to premature death in 2015 (10 million deaths) but also accounted for 200 million disability-adjusted life years (Forouzanfar et al., 2017). CVD risk is doubled with every 10mmHg increase in diastolic and 20mmHg in systolic blood pressure (NICE, 2019). Optimal BP is defined as systolic BP<129 mmHg and diastolic BP <80 mmHg (Jelakovic et al., 2007). BP has an independent and continuous relationship with the incidence of cardiovascular events such as haemorrhagic stroke, ischaemic stroke, myocardial infarction, sudden death, heart failure, peripheral artery disease (PAD) and end-stage renal disease (Lewington et al., 2002). Epidemiological associations between BP and cardiovascular risk extend from very low levels of BP [i.e. systolic BP (SBP) >115 mmHg]. However, 'hypertension' is defined as the level of BP at which the benefits of treatment (either with lifestyle interventions or drugs) unequivocally outweigh the risks of treatment, as documented by clinical trials (Mancia et al., 2013).

Hypertension is rare to occur as a risk factor in isolation and clusters with other risk factors such as dyslipidaemia and glucose intolerance (Bhatt et al., 2006, Berger et al., 2010). This clustering of metabolic risk factors exerts a multiplicative effect on the cardiovascular risk-burden (Berry et al., 2012).

(b) Heart rate variability

Heart rate variability (HRV) is discussed here as a novel CVD risk factor; however, it was not included in this study. It forms part of the longitudinal ADVANCE study and therefore a short synopsis explaining HRV is included.

HRV is the physiological phenomenon of variation in the time interval between heartbeats and is controlled by a primitive part of the nervous system called the autonomic nervous system (ANS)(Kubota et al., 2017). It works regardless of our desire and regulates, among

other things, our heart rate, blood pressure, breathing, and digestion. The ANS is subdivided into two large components, the sympathetic and the parasympathetic nervous system, also known as the fight-or-flight mechanism and the relaxation response (Thayer et al., 2010). The hypothalamus, through the ANS, sends signals to the rest of the body either to stimulate or to relax different functions in order to maintain a balance between the two nervous systems. HRV is a non-invasive way to identify ANS imbalances(Thayer et al., 2010). If a person's system is in more of a fight-or-flight mode, the variation between subsequent heartbeats is low. If one is in a more relaxed state, the variation between beats is high. A low HRV is associated with an increased risk of death and cardiovascular disease while a high HRV may be related to greater cardiovascular fitness and an increased resilient to stress (Kubota et al., 2017).

A low HRV is associated with a 32-45% increased risk of a first cardiovascular event in populations without prior CVD history. An increase in the standard deviation of the inter beat intervals (IBI) of normal sinus beats (SDNN) of 1% results in an ~1% reduction in risk of fatal or non-fatal CVD (Hillebrand et al., 2013).

(c) Arterial stiffness

Arterial stiffness, characterised by increased carotid-femoral pulse wave velocity (PWV), is a known independent predictor of cardiovascular events and mortality alongside the traditional classical risk factors, even in healthy individuals (Zoungas and Asmar, 2007, Vlachopoulos et al., 2010, Magalhaes et al., 2011, Vasan et al., 2019). Carotid-femoral PWV could therefore be considered as a surrogate endpoint for CVD and applied in risk stratification for CVD (Laurent et al., 2006). Furthermore, Kotsis and colleagues concluded that vascular measurements may be a better indicator of CVD progression from a young healthy vessel to an aged diseased vessel responsible for adverse clinical events (Kotsis et al., 2017).

Patients with increasing prevalence of metabolic syndrome (increased BP, high blood sugar, excess abdominal fat, and abnormal cholesterol or triglyceride levels) irrespective of age, gender and BP, were found to have stiffer arteries with impaired endothelial function, which is associated with an increased CVD risk (Gong et al., 2019).

Impaired vascular function, expressed as elevated arterial stiffness, a haemodynamics biomarker, is associated with target organ damage and related CVD mortality (Vasan et al., 2019, Morgillo et al., 2019). Arterial (vascular) stiffness is determined by using numerous techniques including assessments of central haemodynamics, aortic arterial stiffness, indices

of aortic wave reflection, endothelial dilatation, circulating serum calcium and by means of measuring PWV, which is associated with 10-year CVD risk estimates (Park and Lee, 2019).

Large artery stiffness leads to increased PWV and accelerated wave reflections causing an increase in myocardial work and central systolic BP, along with a decrease in coronary artery perfusion pressure (Salvi, 2017). This increase in PWV as a result of increased arterial stiffness is associated with many of the common cardiovascular risk factors, such as age, high blood pressure, smoking, cholesterol levels and obesity, and these lead to a dramatic increase in risk of heart attack, stroke and heart failure (London et al., 2004).

Arterial stiffness is expressed as either peripheral augmentation index (pAI) where the reflected wave is a percentage of the forward wave or as central augmentation index AIx where the augmented central pressure is a percentage of the central pulse pressure (Salvi et al., 2008). An increase in AIx, reflected by an increase in PWV, relates to the speed at which the pulse waves travel down the arteries away from the heart. Increased stiffness in the Aortic wall also decreases the sensitivity of the baroreceptors, which impacts negatively on blood pressure adjustment. Increased PWV predicts cardiovascular morbidity and mortality independently in several population groups, including healthy controls (Cruickshank et al., 2002, Laurent et al., 2001, London et al., 2004, Shechter et al., 2009, Sutton-Tyrrell et al., 2005, Weber et al., 2004).

(d) Subendocardial viability ratio (SEVR)

Subendocardial viability ratio (SEVR) represents a non-invasive measure of myocardial perfusion related to the work of the heart (Ekart et al., 2016). SEVR is calculated through pulse wave analysis and is an index to verify myocardial oxygen supply and demand (Tsiachris et al., 2012). A study by Tsiachris and colleagues (n=24) showed that there was a significant association between SEVR ($r=0.651$; $P<0.01$) and coronary flow reserve (CFR) with hypertensives (Tsiachris et al., 2012). Estimation of SEVR could therefore be applied to assess coronary microcirculation where coronary arteries are normal but myocardial ischaemia is present to verify CVD risk. Equally with a significant correlation between SEVR and central arterial compliance, SEVR could be a useful tool to reflect on the balance between coronary perfusion and ventricular afterload (Tagawa et al., 2018). With resistance training-induced decrease in central arterial compliance associated with changes in SEVR, this phenomenon could be present bearing the nature of our study cohorts in mind where participants are generally training to be combat-ready. The comparative non-injured cohort who are still serving are assumed to be undertaking regular training, where some of the injured cohort are

taking part in the Invictus Games and are therefore exposed to regular strenuous workouts. Training-induced changes in central arterial compliance were significantly associated with changes in SEVR in young men (Tagawa et al., 2018). With proteinuria and estimated glomerular filtration rate (eGFR) being regarded as markers of cardiovascular disease, eGFR below 30 mL/min was linked to significantly lower SEVR (Ekart et al., 2016).

(e) Endothelial nitric oxide

Cardiovascular risk factors such as hypercholesterolemia, hypertension, and DM enhance reactive oxygen species (ROS) generation, resulting in oxidative stress (Li et al., 2014). This leads to oxidative modification of lipoproteins and phospholipids, mechanisms that contribute to atherogenesis and vascular damage and arterial stiffening. Nitric oxide (NO) is a multifunctional signalling molecule involved in the maintenance of metabolic and cardiovascular homeostasis (Li et al., 2014). Physical exercise and especially aerobic training were found to be effective interventions in the prevention and treatment of hypertension and cardiovascular disease via NO production and reduction in oxidative stress (Korsager Larsen and Matchkov, 2016).

1.4.2 **Biochemical markers of CVD risk**

(a) Inflammatory biomarkers

C-reactive protein (CRP) is a substance produced by the liver that increase in the presence of inflammation in the body. An elevated C-reactive protein level, identified by blood tests, can signal flare-ups of inflammatory diseases such as rheumatoid arthritis and vasculitis. CRP is one of the proteins called “acute phase reactants” produced to heal inflammation. Although CRP is not a test for heart disease, it is an indicator of cardiovascular disease (Brito et al., 2015, Li et al., 2017). One of its most useful clinical assets is its potential utility to assist in risk prediction for heart disease and stroke in people who don't already have heart disease (Brito et al., 2015, Li et al., 2017, Berger et al., 2010).

A variation of the CRP test, the high-sensitivity CRP (Hs-CRP) test represents the accurate quantification of CRP levels within the previously defined normal range with greater precision. Elevated Hs-CRP can independently be used to find the risk for heart disease and stroke in people previously undiagnosed with heart disease (Berger et al., 2010, Li et al., 2017). PWV was notably higher in a cohort suffering from inflammation with increased CRP than a healthy control (0.7 (SD 0.3-1.4) versus 0.4 (SD 0.2-0.7) mg/dl; P=0.03) (Shen et al., 2015, Dregan, 2018). There is however insufficient evidence of sufficiently powered clinical

trials to evaluate the incremental impact of Hs-CRP on CVD risk (Lin et al., 2018). Similar statements were made for fibrinogen (Kaptoge et al., 2007).

(b) Lipids

Lipids (cholesterol and triglycerides) circulate as lipoproteins in blood plasma. Most of the circulating cholesterol is in the form of low-density lipoproteins (LDL), which are atherogenic. Lipid values are independently related to blood pressure (Mancia et al., 2005). Total LDL cholesterol is positively associated with CVD risk and characterized by hypercholesterolemia (Perk et al., 2013, Piepoli et al., 2017).

Dyslipidemia can be hereditary or caused by external factors such as a diet rich in saturated fats and carbohydrates, reduced physical activity, chronic kidney disease, certain medication, smoking, stress, or a genetic predisposition (Goldberg, 2018). A lipid analysis estimates CVD risk whereby Total Cholesterol (TC), Low Density Lipoprotein (LDL), Triglycerides (TG) and High-Density Lipoprotein (HDL) is measured. LDL and TC are closely related to CVD risk (Reiner et al., 2011b).

(c) Triglycerides

Epidemiological evidence indicates that high levels of plasma Triglycerides (TG) levels predict cardiovascular disease (Harchaoui et al., 2009, Xiaofeng, 2019). It was however found that for CVD risk estimation it is recommended to take both high TG and low HDL-C levels into account (Harchaoui et al., 2009). Possible causes of hypertriglyceridemia are obesity, non-insulin-dependent diabetes mellitus (NIDDM), excessive alcohol consumption, simple carbohydrate-rich diets, chronic kidney disease, hypothyroidism, pregnancy, autoimmune diseases, drugs (corticosteroids, estrogens, tamoxifen, beta blockers, thiazides, isotretinoin, cyclosporine, antiretroviral drugs, psychotropic drugs), but also genetic predisposition (Reiner et al., 2011b).

(d) Estimated glomerular filtration rate (eGFR)

Estimated glomerular filtration rate (eGFR) is a test to measure the level of kidney function and determine the stage of kidney disease. Estimated GFR can be calculated from the results of a blood creatinine test, age, body size and gender. Modest reductions in eGFR from the normal range variably predict cardiovascular morbidity (Mathisen et al., 2011). According to NICE guidelines, people with an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 m2 and/or albuminuria are at increased risk of CVD (NICE, 2014).

(e) Creatinine

Elevated creatinine levels are indicative of compromised kidney function. Elevated creatinine levels predict adverse cardiac outcomes and even minor changes in renal functions are associated with adverse cardiac outcomes (Smith et al., 2003). However, Patients with traumatic amputations have significantly lower creatinine levels compared to the general population and could overestimate renal function if applied as an indicator (Im et al., 2012).

1.4.3 Glycaemic indices

CVD is the most significant cause of mortality linked to diabetes (DM) (Ryden et al., 2013). The World Health Organisation and the American Diabetes Society classify DM as either type l, type ll and "other specific types" DM and gestational DM (Ryden et al., 2013).

A healthy diet plays a significant role with respect to CVD. Among men, high glycaemic load, glycaemic index, and high carbohydrate and starch intake, were associated with increased risk of CVD (Burger et al., 2011). Furthermore, HbA1c is a reliable risk factor of all-cause and cardiovascular mortality in both diabetics and non-diabetics (Cavero-Redondo et al., 2017).

1.4.4 Anthropometric markers of CVD risk

(a) Abdominal waist circumference

Sedentary lifestyle and bad nutrition habits cause obesity (increased BMI and abdominal waist circumference [AWC]) which has a linear association with CVD mortality. People with a sedentary lifestyle or physical inactivity have their CVD risk increased 1.5 times (Perk et al., 2013). Healthy eating habits are the basis of CVD prevention as it affects the levels of fat and blood sugar, blood pressure and body weight (Perk et al., 2012, Piepoli et al., 2017). The lowest mortality rate in BMI ranges from 20 to 25 kg/m2 (Piepoli et al., 2017). Other obesity indices are conicity index (CoI), AWC, waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) (Caitano Fontela et al., 2017, Motamed et al., 2015, Segura-Fragoso et al., 2019).

(b) Conicity index

Conicity index (CoI) is an index of abdominal adiposity, which is significantly associated with cardiovascular risk indicators. The conicity index has several advantages over the WHR: (i) it has a theoretical (expected) range; (ii) it includes a built-in adjustment for waist circumference, height and weight, allowing direct comparisons of abdominal adiposity between individuals or even between populations; and (iii) it does not require the hip circumference to assess fat distribution (Valdez et al., 1993).

In comparison with the Framingham 10-year CVD risk, it was found that CoI, WHR and WHtR were superior obesity indices compared to BMI in predicting the 10-year CVD risk (Motamed et al., 2015, Segura-Fragoso et al., 2019).

(c) Waist to height ratio (WHtR)

Madruga et al described a positive association between WHtR and the presence of hypertension in adolescents (Madruga et al., 2016). A study of the anthropometric data of Chinese adults (n=1022, ages 18-69) comparing body mass index (BMI), waist circumference (WC), waist to height ratio (WHtR), waist-hip-ratio (WHR) and body fat percentage (BF%) found that WHtR is the best index in predicting the risk of hypertension. It was also noted that men are more likely to hoard fat in the abdomen compared to women who hoard fat in the thighs, making abdominal fat an easy indicator for CVD risk (Fan et al., 2018).

1.5 Aims

The main aim of this study is to investigate whether military servicemen who have experienced serious CRTI demonstrate a subclinical adverse cardiovascular risk profile than that of less and non-injured servicemen exposed to the same operational environment at the same time.

1.6 The research hypotheses are as follows:

1. Military servicemen who have had suffered severe CRTI with limb amputations have increased arterial stiffness compared to those less severely injured and non-injured servicemen.
2. Combat-related traumatic amputation is associated with greater vascular inflammation and a more adverse lipid profile than that observed with less severe combat related injuries and with non-injured servicemen exposed to the same operational environment.

1.7 Summary

In summary, previous studies of CVD risk with Vietnam and World War II veterans were inconclusive, focused on PTSD and were retrospective cross-sectional studies. Methodologically robust, longitudinal cohort studies are required to better understand CVD risk in those who have taken part in modern armed conflicts.

Chapter 2
Systematic Review (Published 2019)

A systematic review of the literature on cardiovascular risk and combat related traumatic injuries with and without amputations

2.1 Introduction

In this chapter a systematic literature review is described to examine the published medical literature relating to combat related traumatic injuries leading to amputation and future cardiovascular disease risk.

Cutting edge battlefield trauma care has allowed military personnel to survive complex injuries in recent years that previously would have been fatal. There is some evidence to suggest that CRTI is associated with an increased CVD risk (Boehmer et al., 2004, Kunnas et al., 2011, Modan et al., 1998), which could be mitigated if there was a better understanding of these risk factors within an injured population. Studies have been performed after previous wars (Boscarino, 2006, Bramsen et al., 2007, Kang and Bullman, 2001, Kunnas et al., 2011, Modan et al., 1998, Watanabe and Kang, 1996, Fett et al., 1987). However, this data was retrospectively collected, the injuries less severe, and the baseline cardiovascular risk factors were not defined. In some cases, only survivors were studied and not those who had died due to their injuries, or afterwards, and the cohort was for less than 24 years from injury (Modan et al., 1998).

The comparative and in some cases, the compounding effects of the roles of the risk factors involved in the developing mechanisms of these enhanced morbidities has not been studied comprehensively and the only two literature reviews that were published about this topic were in 2008 and 2009 (Naschitz and Lenger, 2008, Robbins et al., 2009). At the time of these reviews, only five studies were available, and none had appropriate comparative controls (Naschitz and Lenger, 2008). Of the five, only one study revealed an increase in cardiovascular morbidity after amputation.

The impact of CRTI, without the impact of PTSD, on cardiovascular outcomes and its associated risk factors has not been widely examined. The objective of this review is to

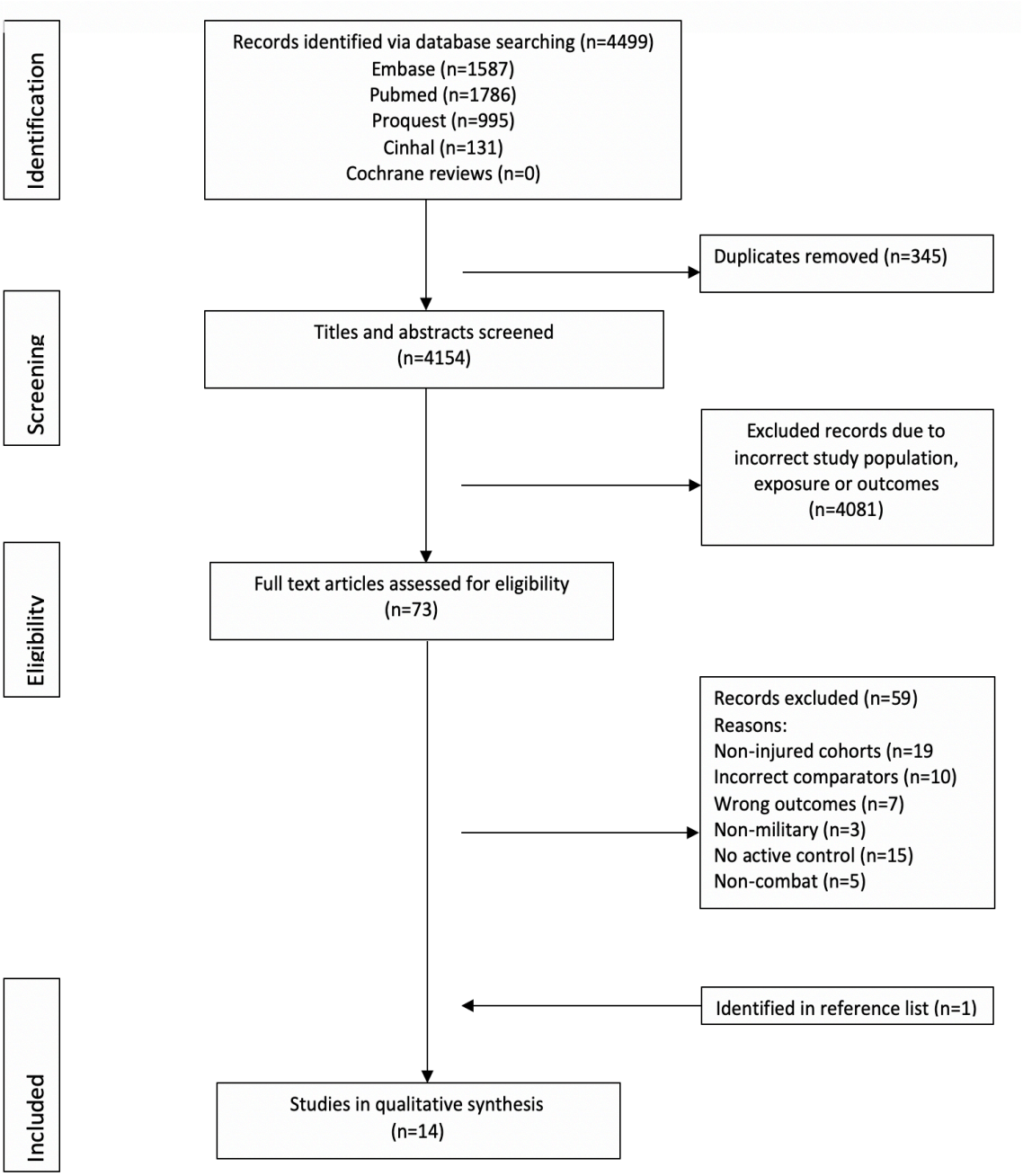
systematically search, summarise and compare current understanding of cardiovascular risk factors and the mechanisms (not psychological) contributing to increased cardiovascular morbidity ensuing serious battlefield injuries and to determine if there is sufficient evidence to support this link.

2.2 Literature search strategy

The strategies applied to the structure of this systematic review evolved from the quest to explore what links have been identified between combat related traumatic injuries and cardiovascular disease risks. Advanced searches for peer reviewed, full-text empirical literature published preferably in English since 1980 were undertaken using the comprehensive electronic discovery service provided by EBSCO. Key health and social care databases such as MEDLINE (Medical Literature Analysis and Retrieval System Online) and CINAHL (Citation Index for Nursing and Allied Health Literature) were searched applying Medical Subject Headings (MeSH) or subject terms as well as appropriate keywords. Studies that evaluated the impact of combat related traumatic injuries on future cardiovascular risk factors were of specific interest and therefore selected and examined.

The systematic review was conducted and reported according to the PRISMA guidelines (Stovold et al., 2014). (See Fig 2-1).

Figure 2-1: PRISMA Flow Chart: the phases of the review



The search strategy adhered to the PICO (patient/population, intervention, comparison/control, outcome) tool (Eriksen and Frandsen, 2018), to ensure quality of the literature search. Eligibility, according to inclusion criteria, was initially assessed by the title, followed by examination of the abstract. Full text versions of probable articles were obtained for further examination.

Search terms and keywords, according to the PICO tool, are listed in Table 2-1. The searches were structured and applied in the MEDLINE and CINAHL databases. The Cochrane database was searched for similar current systematic reviews.

2.2.1 Study selection

Only observational studies (any language) reporting on CVD and cardiovascular risk factor-related outcomes following military and combat-related injuries and exposure, were included. Individual case reports and conference abstracts were excluded. The populations for eligible studies had to include currently serving military servicemen or ex-military/veterans, who were exposed to combat operations. A non-exposed comparator group had to be identified in the study to act as control cohort and more than one year was required from exposure to outcome observation. Eligible studies had to comprise more than 50% combat-related traumatic injured participants in the active cohort, in order to be categorised as a combat-related injury study.

Keywords in titles and abstracts were searched to identify further records for exclusion. Excluded Keywords were: Non-combat, non-explosion injury, traumatic brain injury, PTSD, post-traumatic stress disorder, PTSD induced hypertension, Pre-deployment diabetes, Spinal cord injuries, paraplegia, quadriplegia, heat, children, animal, in vivo/vitro (Table 2-1).

Table 2-1: Search terms according to PICO

Population: MeSH	Intervention: MeSH	Outcomes
Military Military personnel Afghanistan Iraq Armed services Army Marines Infantry Veterans Soldiers servicemen	Traumatic Traumatic injury trauma trauma-related war related injuries amputation amputee Lower limb amputees wounds and injuries wounding wounded injured, injury war related injuries Combat warfare battlefield	Cardiovascular disease Cardiovascular Risk Cardiovascular Death Cardiovascular event Coronary heart disease Ischemic heart disease Coronary artery disease Coronary disease Coronary artery bypass Myocardial infarction or heart attack Acute coronary syndrome Peripheral arterial disease peripheral vascular disease Hypertension Blood pressure Atrial fibrillation Arterial hypertension Heart failure Carotid intimal thickness Stroke aortic aneurysm coronary artery intervention Percutaneous Coronary Intervention coronary artery stenting Metabolic syndrome Augmentation index Arterial stiffness Pulse wave velocity Obesity Diabetes Mellitus

2.2.2 **Quality Assessment**

The Critical Skills Appraisal Programme (CASP) (www.casp-uk.net, 2007), Oxford, UK was applied to critically appraise the shortlisted empirical studies. The CASP checklist is applied for appraising quantitative and qualitative research and literature reviews. The checklist can be tailored to the specific research paradigm. Reliability and validity of quantitative research or the authenticity and generalisability of qualitative methods could be appraised. The tool, as adapted for this systematic review, comprises 16 questions relating to study conduct (Table 2-2).

Table 2-2: Criteria for quality assessment of identified publications

Item	Criteria	Study description
1	Objective	Does the paper clearly state the objectives/hypotheses?
2	Study design	Is the study design described?
3	Target population/sample	Is the target population described? Male, corresponding with ADVANCE
4	Target population/sample	Is the target population representing a random sample of the population?
5	Target population/sample	Is a description of sample selection included?
6	Target population/sample	Is recruitment of the sample described?
7	Target population/sample	Are inclusion and/or exclusion criteria stated?
8	Target population/sample	Is the study sample described? (Minimum criteria = sample size, gender, age, military, veteran)
9	Target population/sample	Are numbers of samples for each stage defined? (Numbers recruited, groups, eligible, lost)
10	Variables	Are cardiovascular risk factor measurements described?
11	Data sources	Are data sources described?
12	Data collection	Are the data collection methods described?
13	Measurement of data	Was reliability of the measurement of data referred to?
14	Data validity	Was validity of measurement or collection referred to?
15	Statistical methods	Were appropriate statistical methods applied and described? Were confounders addressed?
16	Statistical methods	Was missing data addressed and the methods for addressing missing data described?
17	Total score	

Included studies were assessed according to the criteria listed in Table 2-2 and a quality score out of 16 was allocated (Table 2-3, column 17). Most studies complied with at least 13 of the items, which is an acceptable score qualifying included studies (Zeng et al., 2015) with one study scoring 11.

Table 2-3: CASP quality assessment (Critical Skills Appraisal Programme, Oxford, UK) of publications/studies included per list per category (See Table 2-2)

	Year	Objective	Design	Target description	Target population	Sample selection	Sample recruitment	Inclusion/exclusion criteria	Sample description	Sample numbers for each stage	Variables -risk factor descriptions	Data sources	Data collection	Data measurement	Data validity	Statistical methods applied	Missing data addressed	Total
Item		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Etjahed	2017	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Hrubec	1980	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	15
Kunnas	2011	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	13
Labouret	1983	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	15
Linberg	2013	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	15
Magalhães	2011	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Modan	1998	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Peles	1995	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	15
Rose	1987	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Rose	1986	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Shariar	2009	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Stewart	2015	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
Yekutiel	1989	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	15
Volmar	1989	1	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0	11

2.2.3 Data Collection

To summarise and compare the evidence, a table was created to highlight the main characteristics of each study (Table 2-4). The study name and year, study design and duration, sample/population, age of population (years), hypothesis (relationship combat trauma and CVD risk, outcomes), potential confounders/source of bias, covariate adjustments and results were tabulated in Table 2-4. The summary of the characteristics of the studies aided the easy identification of trends, similarities in study methods and outcomes, populations, comparator cohorts and study durations. The summary was also used to assess adherence to selection protocol.

2.2.4 Results

The titles were assessed initially, followed by the abstracts and of the 4198 citations that remained, 73 studies were selected for full-text analysis plus one study identified from a reference list. A total of 14 studies were selected based on the inclusion criteria (see PRISMA flow chart, Figure 2-1) (Stovold et al., 2014). The reference sections of the full-text articles were scrutinised for identification of further suitable studies not identified under the PICO search strategy. One more study was identified.

Table 2-4: Characteristics of studies examining the association of Battlefield Related Traumatic Injury and cardiovascular risk

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
Yekutiel (1989)	Cross-sectional >20 years from injury 3 control subjects from birth year of sample	Israeli War Veterans (1948-49, 1956, 1967, 1973) 53 Traumatic lower limb amputees 159 control (age and sex matched) 77 SCIP	SCIP mean 40.4 years old TLLAP mean 57.2 years old Male	Spinal cord injured and traumatic lower limb amputees are more prone to develop hypertension, ischaemic heart disease and diabetes than healthy age matched controls	Shortening of lifespan after SCIP – 70% of cases had one disease entity – reason for CVD not necessarily related to cord injury. Increase in mortality 30 years after TLLAP due to cardiac and vascular diseases	Control group non exposed to combat	Unadjusted	SCIP: 34% HTN, IHD and DM 18.6% in controls Due to lack of physical activity – decreased HDLC TLLAP: CHD in amputee vs control (32.1% vs 18.2% p<0.01) DM (22.6% vs 9.4%) HTN: similar (35.8% vs 35.2%)	15
Kunnas (2011)	Prospective longitudinal	Finnish WWII Veterans (1939-45) Caucasian 102 Combat injured	55 years War-time activities from 17-20 years old Male	Wartime stress related to late-life all course mortality – wounded veterans have increased long-	Being physically wounded or injured in war may lead to increased CHD mortality	Injury – self report	Adjusted for BMI and depression	Wounded or injured: death 1.7 times more likely from CHD than control. Adjusted for BMI and depression. (CI 95%: 1.1 – 2.5; p = 0.02) HTN and cholesterol similar, DM 8% vs 4.6% with injured	13

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
		565 non-injured deployed veterans		term coronary heart disease and coronary death at age 55					
Hrubec (1980)	Retrospective cohort >30 years Cause of death analysed	US Military WWII (1944-5) 3890 proximal amputees 2917 distal amputees 3890 injured US population (age matched)	<30 years old at injury 3 groups age matched Male	Cause of death - no difference between proximal amputees either of the two groups Amputation leads to increase in cardiovascular disorder incidence	Proximal group – mortality increase with time Distal – similar to population		Adjusted all-cause and age	Proximal vs injured CI 90%: CVD (RR 1.58: 1.40-1.79) IHD (RR 1.56: 1.36-1.79) All cause (RR 1.36: 1.25-1.48) Proximal vs distal amputees and vs general population CVD (1.44: 1.26-1.64) IHD (1.45: 1.24-1.68) All cause (1.29: 1.18-1.41)	15
Etjahed (2017)	Cross-sectional 32.1 years from injury HRQOL was assessed Metabolic syndrome assessed	Iran-Iraq War Iranian veterans 235 Bilateral traumatic lower limb amputation with MetS vs general population	31.5 years at injury 52 years mean age at follow up Comparator age not reported Male	Lower limb amputations are related to HRQOL and MetS with veterans	Response rate 40.7% Amputees have significantly higher plasma insulin levels and insulin response to oral glucose	Very low response rate; poorly defined unexposed (combat control group)	Adjusted for age, BMI, smoking, physical activity, blood pressure and cholesterol	2x increase in MetS with Lower limb amputation (obesity, hypertension, hyperlipidaemia and hyperinsulinemia MetS among amputees 61.2% (CI95%: 55.9% - 68.4%) MetS 27% in Iranian population	16

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
Modan (1998)	Retrospective cohort study 24 years Wounded 1948-74	Israeli army 201 male traumatic lower limb amputees – veterans Vs 1832 general population	<40 years in age (50%) Male	Increased mortality rates with traumatic lower limb amputees	Amputees - CVD main cause for mortality with amputees Hyperinsulinemia, increased coagulation, increased sympathetic and parasympathetic responses	No adjustment for time, ethnicity, smoking status	Adjusted for age	Mortality increased 2-fold with amputees vs general population (21.9% vs 12.1%, $p < 0.001$) due to CVD Amputees - higher plasma insulin during fasting Amputees - increased coagulation Ischaemic heart disease - the same Mean age similar, but age distribution differed	16
Labouret (1983)	Cross-sectional >15 years Equal diastolic BP in both groups	WWI French veterans n = 23 WWI 1914 n = 67 WWII 1939 n = 16 Other 106 Combat related amputation (49 AKA) 184 Controls age matched, no HTN	Age compared 10-year cohorts from 40-89 years Male	Systolic hypertension in patients with combat-related amputation	Increase in systolic pressure (not diastolic) with amputees, significant effect for each age decade	No adjustments Poor definition of how hypertension define; no blinding	Unadjusted	Higher systolic with amputees vs controls (56% vs 29%; $p \leq 0.02$) Change in viscoelastic properties of arteries related to amputation could explain the increase in systolic pressure	15

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
Stewart (2015)	Retrospective cohort Critically wounded military personnel 1.1-4.3 years follow-up	US Military Iraq/Afghanistan Wars 2002-11 3846 severe traumatic injuries Control = Millennium Cohort	25-29.2 years 98% male	Relationship between severity of injury and subsequent development of HTN, CHD, DN and CKD	Severity of combat-related injuries is related to subsequent development of HTN, CHD, DM and CKD. AKI increased the rate of HTN and CKD by 66% and 479% respectively	Retrospective study Adjusted for covariates Observational evidence does not prove causality	Adjusted for age, race, MAP, heart rate, presence of burn injury and AKI	Each 5-point increase in ISS relates to a 6%, 13%, 13% and 15% relative increase in the rate of HTN, CHD, DM and CKD respectively. Adjusted risks CI 95%: HTN (OR 1.06; 1.02-1.09; p=0.003), CHD (OR1.13; 1.03-1.25; p=0.01), DM (OR1.13; 1.04-1.23; p=0.003) Increased risk vs Millennium control	16
Peles (1995)	Cross-sectional Plasma insulin response, autonomic nervous system response 33 ± 11 years after amputation	Israel Defence Force veterans 1948-74 52 amputees lower limb 53 non-military controls, matched for age and ethnic group	50-65 years Male	Insulin resistance and autonomic nervous system activity in male lower limb amputation	Two groups had similar atherosclerotic cardiovascular risk, BP, plasma lipoprotein profile, smoking, physical activity and ethnic origin.	Adjusted for co-variates	Adjusted for age, obesity and lipid levels	Increased insulin resistance in amputees independent of CVD risk factors (HTN, obesity and lipid profile)	15

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
Rose (1987)	Cross-sectional ≥ 15 years Age similar at study and amputation	US Vietnam War veterans 19 BAKA (10 normotensive and 9 hypertensive) 12 UBEA, age matched controls	20-22 at injury 35-36 years at analysis Male Caucasian 1 Black	Traumatic leg amputation (not arm) is associated with IHD mortality in veterans with a mean age of 35-36 years and 14-15 years after injury	BAKA had increased IHD UBEA not significant increased IHD rates	Unadjusted	Unadjusted	Hypertensive BAKA has a significant increase in glucose after glucose intolerance test, increase in hypertension and obesity. Normotensive BAKA had similar cardiovascular risk factors than control	16
Shariar (2009)	Cross-sectional 22.3 years mean follow-up	Iranian Wars 327 Bilateral lower limb amputees Control Iranian general population (undefined)	42 years at analysis 20.6 years at injury Control group age not specified Male	Cardiovascular risk incidence with bilateral lower limb amputation	95.4% had one modifiable risk factor Most common – abdominal obesity Susceptibility to CVD in near future is highly likely	Adjusted for co-variates	Adjusted for abdominal obesity and CVD risk factors	CHD similar to general population Higher prevalence CVD risk factors with bilateral lower limb amputees Hypertension 28.5% vs 20.4%: $p<0.05$ Obesity 82.5% vs 14.4%: $p<0.05$ TCL 36.5% vs19.3%: $p<0.05$ Smoking 31.8% vs 22.3%	16
Rose (1986)	Cross-sectional Caucasian males (one black) 21 years at injury	US Vietnam War veterans 19 BAKA (10 normotensive and 9 hypertensive)	Mean age 36 Male Caucasian 1 Black	Combat-related bilateral above-knee amputees are at increased risk of	9 leg amputees were hypertensive 1 arm amputee was hypertensive	Unadjusted	Unadjusted	Insulin response and body fat content correlate with diastolic BP ($r=0.55$, $p<0.01$) Mean diastolic BP: HPT-BAKA 139 ± 4 mm Hg (systolic), 101 ± 2 mmHg (Diastolic)	16

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
		12 UBEA, age matched controls		cardiovascular mortality	Insulin response increased: Hypertension leg amp 260 ± 60 μU/ml Normal BP leg amp 125 ± 24 μU/ml Arm amp 101 ± 20 μU/ml Increased body fat content 37.2% compared to 23.2% and 22.6% respectively			NOR-BAKA 123 ± 2 mm Hg (sys), 80 ± 2mm Hg (diastolic) UBEA 126 ± 3 mm Hg (sys), 83 ± 3 mm Hg (diastolic)	
Magalhães (2011	Cross-sectional 8 years after injuries	Angola Males 60 Unilateral TLLAP 86 - Control age matched non-amputee, non-military	48 ± 6 mean age Male	Increased PWV related to increased cardiovascular mortality	Higher arterial stiffness in amputees after adjustment for confounders Association between unilateral LL amputation and aortic	Control civilians Small sample size Male only cohorts Causality not established with arterial	Adjusted for age, mean BP, BMI, HTN, smoking, alcohol consumption, TCL, TG, glucose, uric acid, LDL, heart rate, waist	Amputees increased: Uric acid (7.1 vs 6.2, p < 0.01) LDL/HDL 3.8 vs 2.8, p<0.01) Controls increased: HDL (45 vs 40, p<0.01) Creatinine (1.14 vs 1.03, p <0.01) HTN - Amputees higher proportion of hypertension (P <0.05)	16

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
					stiffness in males	stiffness relative to multiple covariates	measure (p = 0.047)	Smokers and alcohol consumption – similar both groups (P > 0.05) PWV – Amputees vs non-A (10.8 vs 9.9 p < 0.05) Diastolic BP, PP, HR – statistically similar (p > 0.05)	
Linberg (2013)	Cross-sectional 6-minute walk test performance	US Military active and retired SMs. 118 Traumatic lower limb 97 Without LL	18-40 years Male	Comparative assessment guides of physical capabilities after lower limb to non-injured is required to help clinicians set achievable goals for optimum health	TTA had better 6MWT than all other amputation level Skilled rehabilitation improves walking ability Appropriate goals to be set for individuals to achieve 6MWT Preserve knee joint where possible	Self-motivation to cover distance in 6MWT	Adjusted for age, height, weight and waist circumference	Significant difference between non-LL and TTA (p < 0.001) Set appropriate goals for amputees to optimise movement to minimise CVD risk development	15
Volmar (1989)	Prospective cross-sectional Male war veterans	WW II veterans – both groups 329 AKA group I702	Male Age means 67.2SD 7.9 yrs and 68.1 SD 5.3 yrs	Change in unilateral flow after leg amputation causes	Increased level of amputation = increased level of	Diagnosis confirmed by arteriograp	Adjusted for Hypertension, hyperlipidaemia ,smoking, DM, obesity	5.8% aneurysms in amputees 1.1% aneurysms in non-amputees 84% of amputees had infrarenal aorta axial shifting – non-amputee = none	11

Study	Study design and duration	Sample/population	Age of population (years)	Hypothesis: Relationship combat Trauma and CVD risk	Outcomes	Potential confounders / source of bias	Covariate Adjustments	Results	Quality Score
	43.8 years average after injury	without amputation group II		asymmetrical flow patterns at the aorta bifurcation	haemodynamic changes Flow level in terminal aorta reduced by 25% Leg amputation causes more aneurysms than non-amputations	hy in all cases		12 patients with left leg amputation had leftward convexity of terminal aorta Same with right leg amputation = rightward convexity	

ICD International Classification of diseases **CVD** Cardiovascular Disease **CI** Confidence interval, **DM** Diabetes Mellitus, **HR** Heart Rate, **CKD** Chronic Kidney Disease, **AKI** Acute Kidney Injury, **CHD** Coronary Heart Disease, **HTN** hypertension, **IS** Ischaemic Stroke, **IHD** Ischaemic Heart Disease, **MAP** Mean Arterial Blood Pressure, **SES** socioeconomic status, **OR** Odds Ratio, **TLLAP** Traumatic Lower Limb Amputee, **HDLC** High Density Lipoprotein Cholesterol, **HRQOL** Health-related quality of life, **MetS** Metabolic syndrome, **AKA** Above knee amputation, **BAKA** Bilateral above knee amputations, **HPT-BAKA** Hypertensive BAKA, **NOR-BAKA** Normotensive BAKA, **UBEA** Unilateral Below Elbow Amputees, **ISS** Injury Severity Score, **TCL** Total Cholesterol, **Non-A** non-amputee, **PP** pulse pressure, **TTA** trans tibial amputation, **6MWT** 6 minute walk test, **SM** Service Members, **LL** limb loss

The initial search retrieved 4499 potentially relevant studies. The results were transferred to ENDNOTE 9 for Mac and searched for duplicates. A total of 345 were identified and removed. After further elimination, 4198 studies qualified for further title and abstract scrutiny, leaving 74 studies for full text eligibility assessment. 61 full-text articles failed to fully meet the systemic review inclusion criteria and were excluded (see Figure 2-1, PRISMA Flow chart). One additional study, which met the selection criteria, was identified from the reference lists (Figure 2-1) and included leaving a total of 14 studies for inclusion in this review.

2.2.5 Study characteristics

The studies were generally observational with nine being cross-sectional, one being prospective longitudinal and three being retrospective cohort studies (Table 2-4). Follow-up periods ranged from one to 44 years.

Sample sizes of CRTI-NA military personnel ranged from n=19 to 6807 participants. Five studies represented US military war veterans, three studies represented Israeli war veterans. Finnish, Iran/Iraq, Angolan and French war veterans were also represented (table 2-4).

Combat operations covered by the included studies were: World War I (1918) and II (1939-45), Iraq/Afghanistan conflicts (2002-11), Vietnam War (1961–1975), the Iran-Iraq Wars (1980-1988), Israeli Conflicts (1948-1974), Gulf War I (1991) and conflicts in Angola. Eight studies reported on traumatic lower limb amputations, three on traumatic above knee amputations and two on traumatic combat-related injuries.

Age range of the study population cohorts varied from 18 to 89 years. The populations were predominantly male (98-100%), Caucasian (62-100%) with the majority, where stated, of non-officer rank at time of combat injury.

A total of 12 of the included studies described amputations following traumatic injuries while two described traumatic injuries only (Table 2-4). Table 2-5 was designed to compare the outcomes of the included studies to help form a general assessment.

Table 2-5: Comparative assessment of included publication outcomes – cardiovascular risk factors following combat-related traumatic injuries

Study	Year	Cardiovascular mortality	CHD mortality	All-cause mortality	MetS	IHD / CHD	Hypertension	Obesity	DM	Plasma insulin	CHD	Aneurysm abdominal	Lipid profile	Arterial stiffness
Etjahed	2017				+		+	+		+			+	
Hrubec	1980	+	+	+		+								
Kunnas	2011	+	+				-		+				-	
Labouret	1983						+							+
Linberg	2013													
Magalhaes	2011						+						+	+
Modan	1998	+	-	+		-	-		+	+			-	
Peles	1995						-	-		+			-	
Rose	1987						+	+	+				-	
Rose	1986						+	+		+				
Shariar	2009	+	+				+	+	+		-		+	
Stewart	2015		+				+		+		+			
Yekutiel	1989		+				-		+					
Volmar	1989						-		-		-	+	-	
Score		4+ 0 -	4+ 1 -	2+ 0 -	1+ 0 -	1+ 1-	7+ 5-	4+ 1 -	6+ 1-	4+ 0 -	1+ 2-	1 +	3+ 5-	2+ 0 -

- = similar to comparator or no effect, **+** = Increased risk, **blank space** = risk not reported as affected
CHD coronary heart disease, **MetS** metabolic syndrome, **IHD** ischemic heart disease, **HTN** hypertension

2.2.6 Study outcomes

Four Studies reported an increase in cardiovascular and CHD mortality with one study reporting no increase in CHD mortality, while two studies found all-cause mortality increased (Table 2-5). Metabolic syndrome (MetS) increase was observed in one study. Hypertension was reported in 12 studies with an increase in seven studies and five studies showed no difference from control. Obesity increased in four studies with one study reporting no increase. Diabetes was reported in seven studies and plasma insulin in four. All the studies reported an increase in the two risk factors bar one study that found diabetes was unaffected. CHD was unaffected according to two studies, but an increase was reported in one other. Lipid profiles were reported in eight studies with three increased profiles and five had no difference compared to control. Abdominal aneurysms were reported in one study and increased arterial stiffness in two studies. The main outcomes such as cardiovascular mortality, CHD, mortality, hypertension, obesity and diabetes with lipid profile were inconclusive overall (Table 2-5).

2.2.7 Study quality

Following selection, the data for each study was extracted using the pre-designed data extraction form (Table 2-4), which included the year of publication and author, the study design and duration, the sample size with military conflict, age of sample, sex, study outcomes, covariate adjustments and results. The Critical Skills Appraisal Programme (CASP) was applied to critically appraise the quality of each of the 14 selected studies (Table 2-3). The CASP tool comprised 16 criteria (Table 2-2) relating to study conduct. Studies with a total score of >13 were considered to be high quality, those of 10-13 of moderate quality and those scoring <10 were deemed poor quality.

The quality scores for the 14 studies range from 11 to 16 (out of a maximum of 16; Table 2-3). The mean quality score for the 14 studies was 15.07 (SD±1.44). Seven of the studies met all the criteria, five had a score of 15 and two studies scored 13 and 11 respectively.

2.3 Clinical Outcomes

2.3.1 Cardiovascular Mortality

The outcome of cardiovascular mortality (Tables 2-4 and 2-5) was reported in four cohort studies. Hrubec et al observed an increased risk of adjusted all-cause and CHD-related death (RR=1.58: CI 1.40-1.79) among proximal CRTI-A vs injured controls in their retrospective analysis of a ≥30 year follow-up of injured World War II Veterans (Hrubec and Ryder, 1980). Modan reported a two-fold higher CVD mortality risk following a 24-year follow-up of wounded Israeli veterans with lower limb amputations (Modan et al., 1998).

Shahriar (2009) found that 95.4% (n=312) of bilateral lower limb CRTI-A had at least one modifiable cardiovascular risk factor 22.3 years (22.3 ± 3.9) after injury. A total of 10.7% of the injured cohort suffered from coronary artery disease (Shahriar et al., 2009).

2.3.1.1 Coronary Heart Disease (CHD) Mortality

CHD-related death was reported in four studies. Hrubec et al observed higher adjusted CHD related death amongst combat veterans with traumatic proximal amputations versus controls with disfigurement injuries (Hrubec and Ryder, 1980, Kunnas et al., 2011). Kunnas et al reported that wounded or injured veterans were 1.7 times more likely to die from CHD mortality than non-injured veterans from World War II conflicts (RR 1.7: CI 95%: 1.1 – 2.5; p=0.02). However, the nature and severity of the injuries were not defined and were dependent on self-report (Kunnas et al., 2011). Shariar et al reported a 10.7% incidence of coronary artery disease with bilateral lower limb CRTI-A in later life (Shahriar et al., 2009), while Yekutiel et al (1989) reported increased CHD with traumatic lower limb CRTI-A vs an age and sex matched control cohort, 32.1% vs 18.2%; p<0.01 (Yekutiel et al., 1989).

2.3.1.2 Atherosclerosis

One study compared proximal amputation (n=3887) to distal amputation (n=2917) and 3890 injured veterans, CI 95%, 1.64 vs 1.4 vs 1.55; p<0.001. However, Hrubec et al reflected that Enos et al reported evidence of atherosclerosis in young casualties of the Korean conflict. Enos et al found that 77.3% of 300 battle casualties with an average age of 21.1 years had gross evidence of coronary atherosclerosis (Hrubec and Ryder, 1980, Enos et al., 1986).

2.3.1.3 CHD and severity of injury

A further two studies reflected on CHD and injury severity. One reported an increased risk of CHD and the other was neutral. Shahriar and colleagues found CHD incidence with bilateral lower limb CRTI-A similar to the general population (Shahriar et al., 2009), while Stewart et al found the increase in risk of CHD related to the severity of traumatic injury, CI 95%, Odds Ratio 1.13; 1.03-1.25; p=0.01 (Stewart et al., 2015).

2.3.2 **Metabolic Syndrome**

Only one cross sectional study reported MetS as a specific outcome. Etjahed and colleagues observed a two-fold higher risk of MetS with Lower limb amputation (obesity, hypertension, hyperlipidaemia and hyperinsulinemia. Incidence of MetS among CRTI-A, CI (95%) 61.2%; (55.9% - 68.4%), compared to 27% in the Iranian population.

2.3.3 **Hypertension (HTN)**

Ten studies reported on HTN with five concluded an increase in HTN and five studies found HTN similar to controls. Peles et al found that an increase in insulin resistance appears independent of HTN and other cardiovascular risk factors (Peles et al., 1995). Yekutiel, Kunnas and colleagues both reported similar HTN between the traumatic injured cohort and the control irrespective of increase in other cardiovascular risk factors (Yekutiel et al., 1989, Kunnas et al., 2011).

Labouret et al observed that higher systolic blood pressure was observed with CRTI-A vs controls (56% vs 29%; $p \leq 0.02$) (Labouret et al., 1983). Magalhães and colleagues observed a higher proportional HTN ($P < 0.05$) with CRTI-A (Magalhaes et al., 2011). Bilateral above knee amputations presented hypertensive and normotensive with the same statistical regularity (Rose et al., 1987, Rose et al., 1986).

Etjahed and colleagues reported an increased HTN as a risk factor in the two-fold increase in MetS observed with CRTI-A (Ejtahed et al., 2017).

A higher prevalence of HTN with bilateral lower limb CRTI-A vs CRTI-NA (CI 95%, 28.5% vs 20.4%: $p<0.05$) was reported by Shahriar et al (Odds Ratio (OR) 1.06; 1.02-1.09; p=0.003) and by Stewart and colleagues (Shahriar et al., 2009, Stewart et al., 2015).

2.3.4 Diabetes Mellitus (DM)

Five studies reported on DM as a positive outcome. Kunnas and colleagues observed an increase in DM with combat injured vs non-injured (8% vs 4.6%) (Kunnas et al., 2011), while Yekutiel et al recorded (22.6% vs 9.4%) traumatic lower limb amputation vs control (Yekutiel et al., 1989).

Rose et al compared hypertensive bilateral above knee amputees (BAKA) with normotensive BAKA. The hypertensive BAKA had an increased prevalence of body fat and insulin response whereas the normotensive BAKA illustrated similar levels of cardiovascular risk factors as the control cohort of unilateral below elbow CRTI-A (Rose et al., 1986, Rose et al., 1987). The level of severity of injury related to the increase in prevalence of DM (Stewart et al., 2015).

2.3.5 Plasma Insulin

Four studies reflected on an increase in plasma insulin. Etjahed, Modan and Rose et al reported an increase in plasma insulin with traumatic amputation, while Peles and colleagues recorded an increase in insulin resistance (Ejtahed et al., 2017, Modan et al., 1998, Peles et al., 1995).

2.3.6 Obesity

Four studies reported an increase in obesity related to combat-related traumatic injury. Etjahed and colleagues related the increase in obesity with the two-fold increase in MetS with bilateral lower limb amputations, CI 95%: 61.2%, 55.9% - 68.4%; $p<0.05$, (Ejtahed et al., 2017). a total of 95.4% of the Iranian war veterans with bilateral lower limb amputations had at least one modifiable risk factor with the most common one abdominal obesity, 82.5% vs 14.4%; $p<0.05$ (Shahriar et al., 2009). Rose et al found that hypertension with BAKA correlates with increased body fat content and obesity (Rose et al., 1986, Rose et al., 1987).

2.3.7 Lipid Profile

Three studies reported an increase in lipoid profile while two studies found no increase compared to control. LDL/HDL ratio was increased with CRTI-A, 3.8 vs 2.8; $p<0.01$ while HDL was increased with the control group vs CRTI-A, 45 vs 40; $p<0.01$ (Magalhaes et al., 2011). Shahriar et al also observed an increase in total cholesterol (TCL) with bilateral lower limb amputees compared to the general population, TCL 36.5% vs 19.3%; $p<0.05$ (Shahriar et al., 2009). Etjahed and colleagues listed hyperlipidaemia under the two-fold increase in MetS with CRTI-A (Ejtahed et al., 2017).

Kunnas et al found the lipid profiles statistically equal between injured and non-injured cohorts while Peles and colleagues found no statistical differences in the lipid profiles between CRTI-A and non-military controls (Kunnas et al., 2011, Peles et al., 1995).

2.3.8 Arterial Stiffness

Two studies described an increase in arterial stiffness, derived from measuring pulse wave velocity non-invasively, with lower limb amputations due to a change in viscoelastic properties of the arteries, thereby raising BP (Labouret et al., 1983, Magalhaes et al., 2011).

2.3.9 Aneurysms

One study reported an increased incidence of abdominal aneurysms due to changes in aortic flow patterns associated with the level of leg amputations (Vollmar et al., 1989).

2.4 Discussion

The influence of combat exposure resulting in traumatic injury on cardiovascular mortality was assessed in this systematic review. An increase in cardiovascular and CHD mortality as well as an increase in cardiovascular risk factors associated with obesity and glucose intolerance is described in the studies. However, an increase in risk factors such as hypertension and lipid profile related to traumatic injury did not leave conclusive results. The effect of combat related traumatic injury on cardiovascular disease risk factors warrants further investigation.

The limitations evident in the selected literature are the lack of baseline risk descriptions, risk of bias created by self-reporting by participants, lack of adjustments for the influence of confounders, causality not proven by observation and comparator cohorts comprising civilians, who possibly had a different health report at baseline due to military enlisting requirements. Baseline health data captured for servicemen in earlier wars were less stringent than with current military enrolment as reported by Hrubec and Ryder reflecting on the evidence published by Enos et al that 77.7% of 300 battlefield casualties aged average 21 years old, were found to have gross evidence of coronary atherosclerosis (Enos et al., 1986, Hrubec and Ryder, 1980).

The majority of the studies included in this systematic review were retrospective and investigated relative CVD risk 15-43 years after injury. The veterans were often compared with a civilian control cohort with a different baseline demographic compared to the injured cohort.

A search of MEDLINE and CINAHL databases identified a systematic review relating to the long-term effects of traumatic amputation on cardiovascular risk factors (Naschitz and Lenger, 2008). They concluded that abnormalities in arterial flow proximal to the site of amputation might explain the subsequent scale of cardiovascular risk. For example; proximal leg amputation vs distal amputation had greater cardiovascular risk and similarly with bilateral vs unilateral amputation. Therefore, coronary risk in lower limb CRTI-A may be greater than predicted by algorithms, which are not bearing hemodynamic or psychological factors in mind. The level of amputation has been found to correlate with the level of haemodynamic changes, which leads to arteriosclerotic changes and eventual aneurisms in later life (Vollmar et al., 1989). Magalhães (2011) describes a change in arterial wall structure with CRTI-A in a small study (n=60), leading to increased pulse wave velocity (PWV) due to increased arterial

stiffness. PWV could be applied in routine assessment to stratify cardiovascular risk (Magalhaes et al., 2011).

Labouret (1983) described an increase in systolic BP but not diastolic with CRTI-A vs control and the increase specifically relates to each decade after the injuries (56% vs 29%; $p \leq 0.02$). Therefor longitudinal assessment of CVD risk development is essential. Labouret further hypothesises that the change in viscoelastic properties of the arteries after the amputation and with time could explain the increase in systolic pressure (Labouret et al., 1983). Another consideration is that systemic and/or regional haemodynamics (arterial stiffness) may be altered by trauma resulting in increased mortality rates (Modan et al., 1998, Yekutiel et al., 1989, Vollmar et al., 1989, Paes et al., 1990, Peles et al., 1995, Rose et al., 1987).

Pulse wave velocity (PWV) increases with increase in arterial stiffness. To be able to compare cardiovascular function and structure between individuals with varying body compositions, cardiovascular parameters have to be adjusted to compensate for differences in body size (Chantler and Lakatta, 2009). For example, the body weight for CRTI-A has to be estimated before the body mass index is calculated in order for the differences to be corrected. Magalhães found that by applying multivariate analyses, adjusting for the effects of major confounders, such as body mass index, the PWV remained high in the case of lower limb CRTI-A, however he notes that the results were inconclusive as not all the contributing factors were assessed in the study (Magalhaes et al., 2011).

A literature review published in 2009 reflects that CRTI-A are at a higher risk of developing CVD and that insulin may be associated with blood pressure regulation in maturity onset obesity (Robbins et al., 2009). However, injured civilian and military subjects were included in their study cohort while our systematic review focuses on injured military. Hyperinsulinemia, hypertension and early onset obesity are CVD risk factors, which could develop when servicemen/women are discharged and sensible diets and physical fitness maintenance are not adhered to as would have been the case while in the military (Robbins et al., 2009). This highlights the need for measuring the maintenance of physical ability as a contributor to developing CVD risk factors. Linberg and colleagues assessed the effect of rehabilitation and reaching pre-injury standards of movement on health outcomes, which will impact on development of cardiovascular risk factors (Linberg et al., 2013). Therefore, functional differences between comparator groups have to be considered for assessment.

The modern application of the term ‘MetS’ (Metabolic Syndrome) could have a different impact on outcomes compared to historic studies. Patients with MetS had increased weight, waist and hip circumference, fasting blood sugar, triglycerides, low density lipoprotein, and liver enzyme levels; $P<0.05$, (Ejtahed et al., 2017). The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines suggested that a diagnosis of metabolic syndrome (previously known as syndrome X) is reached where three or more of the following risk factors are present:

- central obesity
- elevated triglyceride
- low HDL
- raised blood pressure
- raised fasting plasma glucose

More recently the International Diabetes Federation have defined criteria for metabolic syndrome where it is diagnosed if the patient has a 'large waist' plus any other two risk factors (Gater et al., 2018).

If the hypothesis that CRTI is associated with increased CVD risk, prevalence of hypertension (a traditional CVD risk marker) would be expected to be evident with the majority CRTI participants. However, five studies found no increase in hypertension compared to seven which reported an increase.

Hrubec and colleagues found an increase in CHD while Modan et al found no increase, which adds another inconsistency to the hypothesis that CRTI adds to an increase in CVD risk (Hrubec and Ryder, 1980, Modan et al., 1998).

One study observed the increase in CVD risk factors in the short term (1.1-4.3 years follow-up) after combat injury relative to the severity of injury and reported subsequent development of risk factors. However, findings were observational, which does not necessarily prove causality (Stewart et al., 2015).

Veterans with PTSD were excluded from this study due to the links with alcohol and substance abuse affecting cardiovascular health as well as adverse effects on the central noradrenergic system contributing to CVD (Bedi and Arora, 2007, Gunawardena et al., 2007).

This review highlights the limitations in existing available literature. Study cohorts were from historical conflicts and were compared with civilian populations without known cardiovascular disease at baseline. Most of the studies were cross-sectional and retrospective as such with an element of risk of bias and no adjustment for important confounders. Confidence interval was not always reported, and population demographics were unclear.

A further limitation to the studies is that no more than 50% of cardiovascular events are in fact linked to cardiovascular risk factors and some people who experience cardiovascular events had no risk factors at all (Naschitz and Lenger, 2008). Some novel haemostatic risk factors such as fibrinogen, possibly factor VIII and von Willebrand factor, are associated with CHD but are not useful in predicting outcomes (Folsom et al., 1993). Further limitations with earlier studies are that haemodinamic factors were not included in CVD risk prediction models (Naschitz and Lenger, 2008).

The conclusion is that available literature does not overwhelmingly support the hypothesis that CRTI is associated with an increased risk of cardiovascular disease and outcomes following the increase in prevalence of a specific CVD risk factor (or risk factors). A prospective longitudinal study is required where exposed injured military subjects from contemporary conflicts are compared with uninjured military subjects recruited with similar exposure and health assessment, age, sex, physical and psychological condition at baseline. Traditional and novel cardiovascular risk factors, activity levels and psychological state of mind should be assessed at regular time intervals to establish any trends developing thereby creating an insight into the development of coronary heart disease risk factors, allowing for the development of early intervention protocols.

Chapter 3
Methods

3.1 Study design and population cohorts

This study is a cross sectional analysis of the first 699 participants recruited into the ongoing ADVANCE (**A**rme**D** Ser**V**ices Tr**A**uma Rehabilitatio**N** Out**C**om**E**) Study. The ADVANCE Study is a prospective longitudinal cohort study, designed to analyse and examine the medium to long term outcomes of British military personnel who sustained traumatic injuries (with and without amputations) during recent military conflicts. (https://www.advancestudydmrc.org.uk)

The ADVANCE Study is a collaboration between the Defence Medical Rehabilitation Centre (DMRC), Imperial College London and King's College London. The study, a 20-year cohort observation, investigates both the physical and psychosocial outcomes of battlefield casualties in the long-term and is the first prospective cohort study in this area. Evidence collected will provide unique information that will be essential to the care of survivors of serious injuries, military or otherwise, across the globe.

A total of 1,200 serving and discharged combat veterans will be recruited, with 6 appointments offered over a 20-year period. Participation involves a baseline visit and 5 subsequent follow-up visits at the Defence Medical Rehabilitation Centre (DMRC) Stanford Hall, where the study is based.

Several academic institutions are supporting the ADVANCE Study (Figure 3-1).

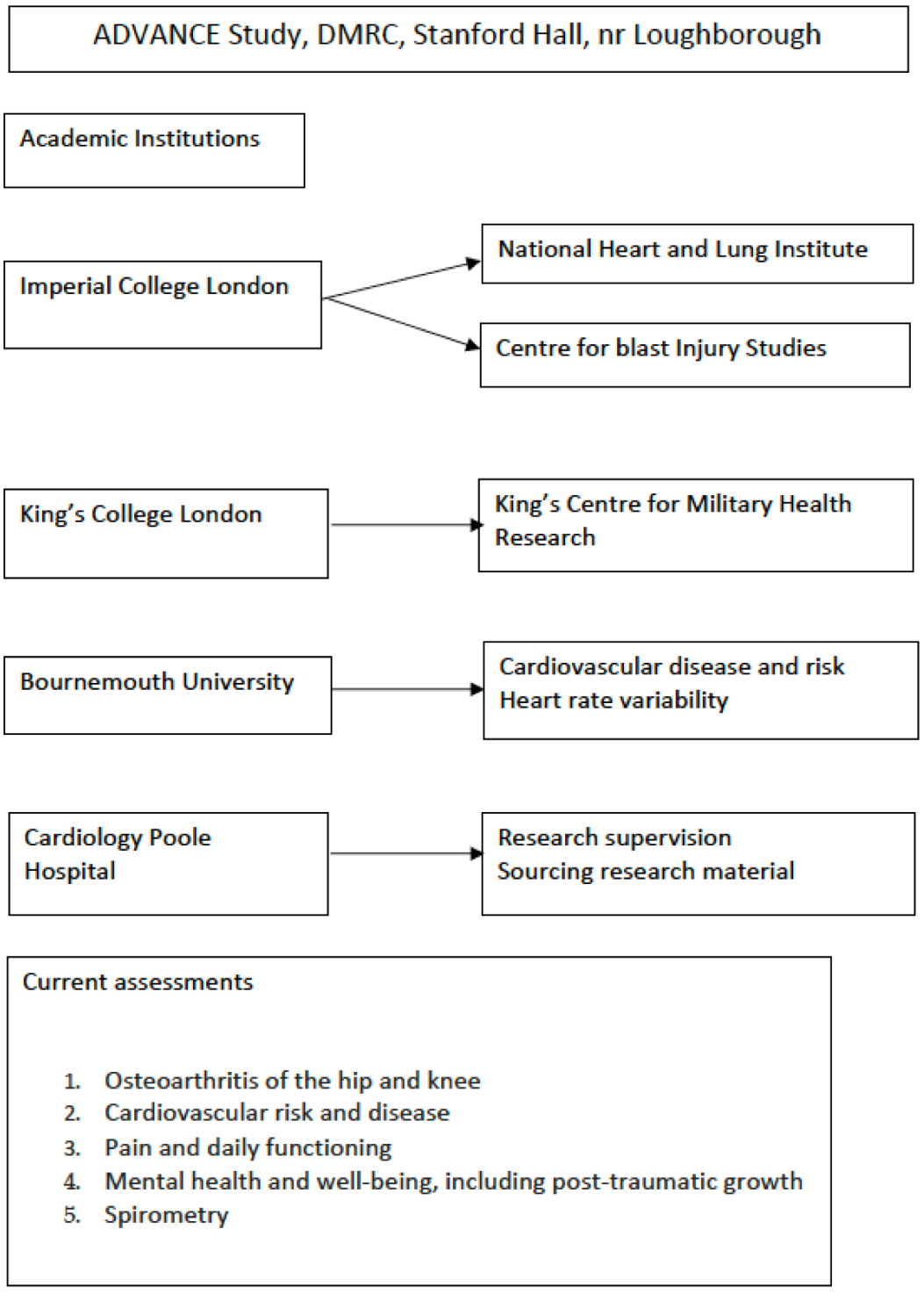


Figure 3-1: Academic Institutions participating in the ADVANCE Study

3.2 Data collection and access protocol

3.2.1 ADVANCE protocol

The data collection protocol was approved by the Ministry of Defence Research Ethics Committee (MoDREC Protocol No: 357/PPE/12, Appendix C). The data collection protocol followed by the research personnel employed by ADVANCE is described in paragraphs 3.2.1 and 3.2.2.

3.3 Study Design, Methodology and Data Analysis

3.3.1 Study Design

Prospective longitudinal cohort study with the exposure being CRTI.

Group 1: "Exposed Group": n >200 battlefield trauma casualties

Group 2: "Non-Exposed Group": n >200

Frequency matched for age, sex, service, rank, deployment and combat role.

3.3.2 Outcomes

Primary Outcomes

a. **Cardiovascular risk-** as determined by cross sectional differences augmentation index and PWV

Secondary Outcomes

a. Central blood pressure
b. Cardiovascular risk as determined by more traditional cardiovascular risk factors (e.g. blood pressure and diagnosis of hypertension, lipid profile, blood glucose/DM, smoking history, HsCRP and abdominal waist circumference)
c. Cardiovascular disease as determined by individual components of the primary composite CVD score

3.3.3 **Trial Subject Selection**

(a) Eligibility Criteria: Group 1: Exposed Group

Inclusion Criteria

a. UK Armed services personnel
b. Male
c. Sustaining physical battlefield trauma, while on deployment, requiring aeromedical evacuation and direct UK hospital admission
d. Injured during 2003 or after

Exclusion Criteria

a. Females
b. Patients who were unwilling or unable to give informed consent
c. Patients with established CVD (previous stroke or transient ischaemic attack [TIA], ischaemic heart disease [IHD], peripheral vascular disease)
d. Past medical history of DM
e. Past medical history of renal or liver disease
f. Aged <18 and >50 years
g. Active acute infection with systemic features of sepsis, at the time of recruitment, as defined below. Potential participant with active acute infection will be considered for recruitment once the acute illness is treated and resolved. (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. 1992):

2 of 3 of:

I. Temperature >38 ^{0}C or <36 ^{0}C
II. Heart rate >90beats/min
III. Respiratory rate >20 breaths/min

(b) Eligibility Criteria: Group 2: Non-Exposed Group

Frequency matched for age, sex, service, rank, deployment and combat role.

Inclusion Criteria

a) UK Armed services personnel
b) Male
c) Previously deployed.
d) No CRTI, as defined in the inclusion criteria.

Exclusion Criteria

a) Females
b) Patients who are unwilling or unable to give informed consent
c) Patients with established CVD (previous stroke or ischaemic heart disease [IHD], peripheral vascular disease)
d) Past medical history of DM
e) Past medical history of renal or liver disease
f) Aged <18 years and >50 years
g) Active acute infection with systemic features of sepsis, at the time of recruitment, as defined below. Potential participant with active acute infection will be considered for recruitment once the acute illness is treated and resolved. (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. 1992) :

2 of 3 of:

I. Temperature >38 ^{0}C or <36 ^{0}C
II. Heart rate >90beats/min
III. Respiratory rate >20 breaths/min

Recruitment

Potential battlefield trauma exposed volunteers were identified through DMRC Headley Court records and were recruited prospectively for newly injured patients and retrospectively back to and including 2003 as per the inclusion and exclusion criteria as detailed above. Volunteers suitable for the study were identified by their treating clinical team or through their medical records or with the assistance of Defence Medical Statistics data. These patients were then approached by a member of the research team. A verbal explanation of the trial and patient

information sheet was provided by the authorised trial clinician for the participant to consider. This included a detailed information about the rationale, design and personal implications of the study and include a participant invitation form, participant information sheet and participant consent form (Appendix A-C). Following information provision, participants were given at least 24 hours to consider participation and were given the opportunity to discuss the trial with their family and healthcare professionals before they were asked whether they would be willing to take part in the trial. This process was clearly documented into the participant's medical notes.

Non-exposed volunteers were recruited from the same military units as the battlefield exposed volunteers. Non-exposed volunteers were approached via poster, e-mail, and presentation once approval has been given through the chain of command of the units. Once matched non-exposed volunteers were identified, the same recruitment proceeds were followed as for the patients mentioned above.

Consent

Assenting participants were formally assessed for eligibility and invited to provide informed, written consent. The right of the participant to refuse consent without giving reasons was respected. Further, the participant was free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment. A copy of the consent will be given to the participant, one filed in the Trial Master File, one filed in the hospital. The written consent was taken by an authorized clinician or research nurse and the study was conducted in keeping with the Declaration of Helsinki.

Randomisation

Not applicable

Unblinding

Not applicable

3.4 Data Collection, Source Data and Confidentiality

General

All information collected during the course of the trial was kept strictly confidential.

Information was held securely on paper and on a secure electronic online database at the DMRC Headley Court and linked to Imperial College and King's College London. An anonymised data base was constructed using a unique identification number for each participant. Baseline and anthropometric data were transferred to the data base. The study cohorts were awarded codes: 0 for controls, 1 for CRTI-NA and 2 for CRTI-A for application in data interrogation.

Data access

For the conduct of this thesis key data from the Clinical Report Forms (CRFs) were entered onto a secure personal excel database. This data included anthropometric data, time after injury or deployment, laboratory analysis, injury severity, family history, medical and smoking history. This database was then integrated with the electronic database created by the Vicorder device by means of corresponding participant numbers.

Ability to access the data was made possible by entering into an honorary contract as a researcher with Kings College and the Defence Medical Services. Datasets were stored on an encrypted device only and are to be destroyed on completion of the research work along with other related documents.

I visited the rehabilitation and training facilities to help me understand the various processes the CRTI-A encountered and had to deal with on the rehabilitation journey after losing limbs.

I familiarised myself with all the data capturing processes in order to be able to find possible weak points in the protocols and standard operating procedures that could affect data accuracy and helped staff with retraining where appropriate. I familiarised myself with the Vicorder operating software and the outputs expected for data analysis.

3.5 Description of protocol elements

3.5.1 Recruitment

The Department of Defence Statistics (Health) within the UK Ministry of Defence identified and matched injured with non-injured groups for recruiting using deployment records. Participation was voluntary and all participants received information sheets >24 hours before detailed written consent was obtained. Ethical approval was obtained from the Ministry of Defence Research and Medical Ethics Committee (MODREC:357PPE12) and the study is conducted according to the standards of the declaration of Helsinki.

3.5.2 Capturing data

Participants were required to fast and refrain from caffeine and alcohol for at least eight hours leading up to the examination. The haemodynamic data and blood samples were first priority on the examination day to comply with both the Vicorder and fasting tests requirements.

The participants completed baseline questionnaires during a clinical interview with a research nurse, which enquired into medical and family history as well as smoking status. Fasting blood glucose, HbA1c, renal function, lipid levels, Hs-CRP, and full blood counts were measured using venous blood, which was taken at the same visit and processed by the local NHS laboratory. Results were written up in paper records.

The recorded data was transcribed for this study at the rehabilitation centre where the data is stored from the hard copy into digital format and combined with digital data captured from the Vicorder technology to form a comprehensive digital database suitable for interrogation.

3.5.2.1 Anthropometric data

Anthropometric variables including age, weight, height, hip circumference (HC) and abdominal (waist) circumference (WC) were recorded using standard procedures.

(a) Weight

For weight, the Seca 704 wireless column scale with a high capacity of up to 250 kilograms (Class III Approved) was used. The Seca 956 Chair scales were used for weighing while seated when required for CRTI-A.

Figure 3-2: Seca 704 wireless column scale

Figure 3-3: Seca 956 Chair scales

(b) Waist, hip and height measurements

The waist was measured with a non-stretchable tape midpoint between the lowest costal ridge and the upper border of the iliac crest and recorded as abdominal waist circumference (AWC) in cm. The hip measurement was taken at the point yielding the maximum circumference between the knee and the waist and was recorded as hip circumference (HC) in cm.

The Seca 213 Height Measuring device (Leicester) was used to determine height in cm. The participant stood upright in socs and the sliding scale was touched on the top of the head to determine height. Previous height was recorded in the case of bilateral CRTI-A.

(c) Calculations and ratios

Waist to height ratio (WHtR)

Waist to height ratio was calculated as (waist circumference (cm)/height (cm)) x 100 and was used to assess the fat content in the human body.

Conicity Index (CoI)

Conicity Index is a novel indicator of CVD risk reflecting on waist circumference and obesity. It is described by Motamed and colleagues and Segura-Fragoso as a superior CVD risk indicator compared to BMI and was calculated using weight (kg), height (m) and AWC (m) as follows: (Motamed et al., 2015, Segura-Fragoso et al., 2019).

$$C\ index = \frac{\text{Waist Circumference (m)}}{0.109x\sqrt{\frac{\text{Body Weight (kg)}}{\text{Height (m)}}}}$$

Body mass index (BMI)

BMI was determined as weight (kg) divided by height (m^2). We disregarded BMI as it would technically be inaccurate when applied to CRTI-A. The physical nature of the participants, who in most cases were fit and muscular, would also lead to inaccurate assumptions resulting in classifying them as clinically obese according to BMI results, which reflects on body weight irrespective whether it is due to muscular weight or abdominal fat.

3.5.2.2 Laboratory analysis

Venous blood was taken and sent to the local NHS laboratory for analysis of full blood counts, fasting glucose, HbA1c, renal function, creatinine, lipid profile and Hs-CRP (with lower detection limit 0.10 mg/l).

Applied laboratory criteria

The Cockcroft-Gault equation ([140-age] x [weight in kg] / (72 x serum creatinine [µmol/l]) was applied to calculate Creatinine Clearance (Cockcroft and Gault, 1976).

3.5.3 Injury severity

The New Injury Severity Score (NISS) was applied for injury severity quantification (Osler et al., 1997). The scores were provided by the UK Joint Theatre Trauma Registry (JTTR), which comprises a prospectively collected trauma database of every casualty admitted to a

deployed UK medical facility or killed during deployed operations (Smith et al., 2007). This database is securely held and maintained by the Academic Department of Military Emergency Medicine (ADMEM). Access to examine the data for research purposes is restricted and only provided by the Medical Director (Defence Medical Services). NISS was applied to the injured cohort to evaluate and confirm the severity of injuries.

3.5.4 The amputee cohort

The range of amputations assessed in this study are illustrated in Figure 4-1.

(a) Lower limb CRTI-A subgroups

Lower limb distal: Unilateral (n=30); Bilateral (n=7)

Lower limb proximal: Unilateral (n=12); Bilateral (n=23)

Lower limb distal and proximal (n=8)

Knee disarticulation (n=4)

Both knees disarticulated (n=1)

Lower limb distal amp plus knee disarticulation (n=2)

Lower limb proximal amp plus knee (n=4)

Lower limb proximal bilateral plus hand (n=3)

Lower limb proximal bilateral plus elbow disarticulation (n=1)

Lower limb proximal bilateral plus trans radial (n=3)

Lower limb distal subgroups plus proximal, unilateral plus hand (n=1)

Lower limb distal plus proximal, unilateral plus trans humeral (n=1)

Both knees plus one disarticulated (n=1)

(b) Upper limb amputee subgroups

Trans humeral (n=1)

Hand (n=1)

3.5.5 Haemodynamic characteristics and blood pressure

Arterial compliance and central BP were measured with the Vicorder device (Skidmore Medical, UK)(Milan et al., 2019, Pucci et al., 2013, Shahin et al., 2013). The Vicorder uses an oscillometric technique to acquire the pulse waveform. All Vicorder measurements were undertaken by trained medical research nurses in a temperature-controlled room following stringent standard operating procedures. The participant was made comfortable and rested for

Medical, UK) (Milan et al., 2019, Pucci et al., 2013, Shahin et al., 2013). The Vicorder uses an oscillometric technique to acquire the pulse waveform. All Vicorder measurements were undertaken by trained medical research nurses in an average ambient temperature-controlled room following stringent standard operating procedures. The participant was made comfortable and rested for at least five minutes prior to examining blood pressure and arterial compliance. For the Vicorder readings the participants were required to lie comfortably and supine on a medical bed with the head raised at a 30o angle.

Inflatable cuffs (11x85cm Hokanson® SC10TM) were placed around the left thigh (as high as possible) and around the left upper arm. A smaller cuff was placed around the neck below the Adam's apple. The cuff's design is such that both carotid arteries and trachea was not compressed at the same time. The head is tilted slightly back to relax the skin and muscles during the measurement. The distance between the suprasternal notch and the middle of the thigh cough were measured with a non-stretchable flexible tape measure and used as the path length in the calculated pulse wave velocity. The value was entered into the Vicorder software together with the participant's details. Brachial systolic and diastolic blood pressures, augmented pressure, central augmentation index, brachial augmentation index, stroke volume and the Subendocardial Viability Ratio were measured, calculated and recorded by the Vicorder in the proprietary software (SEVR) (Pereira et al., 2015, Pucci et al., 2013, Shahin et al., 2013).

Figure 3-4: PWV; Vicorder cuff application (SMT medical technology GmbH & Co. KG)

(a) Pulse wave velocity

PWV was calculated using the arterial transit time, which is the time delay due to the propagation of the pulse wave along the arterial tree between the upstrokes of two simultaneously measured waves at the femoral and carotid arteries (figure 3-5). PWV is the linear ratio between the measured distance and the time (Pereira et al., 2015).

Carotid-femoral PWV is considered the gold standard for arterial stiffness assessment and underpins quantification of arterial ageing (Weber et al., 2015).

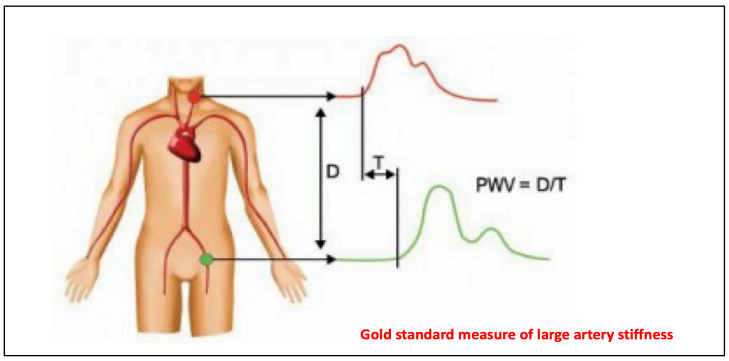


Figure 3-5: Measurement and calculation of pulse wave velocity

(b) Systolic blood pressures

The brachial BP was measured to calibrate the device whereupon the brachial arterial waveform analysis is applied at diastolic pressure to estimate central systolic and brachial systolic BP.

(c) Augmentation Index

Augmentation index is an indicator of subclinical atherosclerotic vascular damage. Several studies have shown AIx to be predictive of adverse cardiovascular events (Chirinos et al., 2005, Weber et al., 2005, Ueda et al., 2004, London et al., 2004).

Early atherosclerosis is associated with reduced arterial elasticity and future cardiovascular events (Kotsis et al., 2017). Cardiovascular risk factors such as DM or elevated fasting glucose, obesity, higher heart rate, hypertension and lipid disorders (particularly higher triglycerides and lower HDL) are associated with a stiffer aorta (Mitchell, 2009).

Augmentation index is expressed in both central aortic AIx and peripheral or brachial augmentation index (pAI). Stiffness of the arteries affects the magnitude of the reflected wave generated in the arterial tree at bifurcations or narrowing of the arteries and the resulting effect on the function of the heart (Mitchell, 2009). In fact, the pressure waveform at any point is a composite of the forward-going and reflected wave (Wilkinson et al., 2000).

Central and brachial augmentation indexes are calculated in different ways:
Central AIx is defined for a central pressure waveform as the ratio of Augmentation Pressure (cAP) to Pulse Pressure (cPP): AIx = cAP / cPP (Figure 3-6) (Wilkinson et al., 2000).

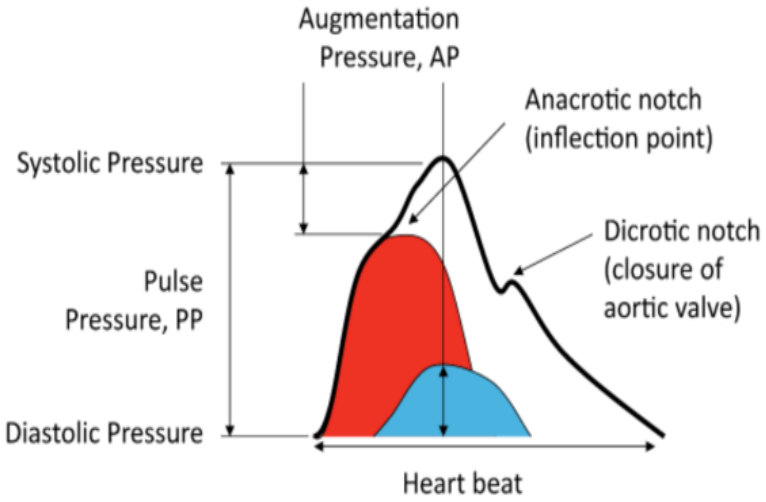


Figure 3-6: Central Augmentation Index (AIx)

Peripheral augmentation index (pAI) on the other hand is defined (as a %) as the ratio of late systolic pressure (P2) to early systolic pressure (P1): pAI = P2 / P1 (Figure 3-7) (Wilkinson et al., 2000).

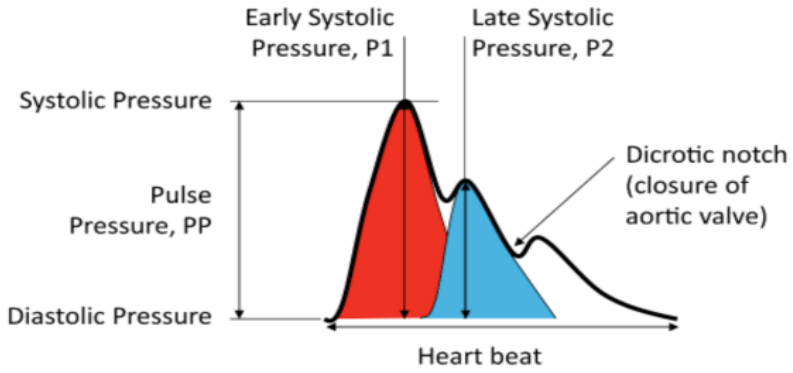


Figure 3-7: Peripheral augmentation index (pAI)

The central augmentation pressure represents the additional systolic BP created by the reflected waves at the aorta expressed as a percentage. The central AIx, was therefore calculated as the percentage proportion of the augmentation pressure to the central pulse pressure (Wilkinson et al., 2000).

There is a linear association between heartbeat and AIx, which needs consideration in studies. For example, an increase in heartbeat from 60 to 110 beats per minute, is associated with a rise in peripheral BP and central diastolic pressure, but no change in central systolic BP. Over the same change in pace a linear reduction in AIx was recorded. These effects are explained by considering wave reflection and therefore pulse wave analysis should be applied to assess systemic arterial stiffness (Wilkinson et al., 2000).

(d) Subendocardial Viability ratio (SEVR)

SEVR was calculated as the ratio of the pressure time integral (mmHg x s) for diastole divided by that for systole and represented as a percentage. SEVR reflects relative myocardial oxygen supply versus demand and is strongly associated with coronary blood flow reserve (Tsiachris et al., 2012).

SEVR is a useful tool for reflecting the balance between myocardial perfusion and left ventricular afterload (Tagawa et al., 2018). SEVR is reflective of myocardial oxygen supply versus demand with a strong correlation to coronary blood flow reserve (Tsiachris et al., 2012). The implications of increased arterial stiffness on the brachial pulse wave with resulting increase in systole time (need for oxygen) and decreased coronary perfusion time (diastole) is explained by means of the curve moving to the right following increase in arterial stiffness (Figure 3-8) (Salvi et al., 2008).

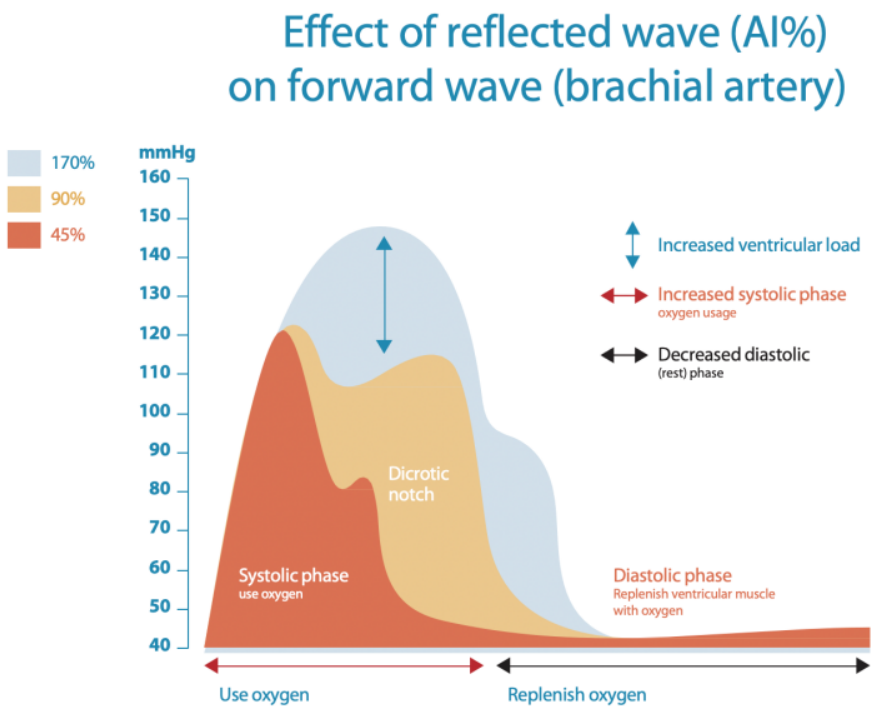


Figure 3-8: The effect of increase in Augmentation Index on the pulse wave: 45%-170% (Salvi et al., 2008)

3.6 Statistical considerations

3.6.1 Sample size/power calculations

Sample size calculations were performed for the primary outcomes using GraphPad StatMate version 2.00 for Windows (GraphPad Software).

We have also calculated sample size for the other key primary endpoint of PWV. Published data on health controls of similar ages to our study population would suggest an average pulse wave velocity of 6.0 – 6.5 m/s with a within group standard deviation of 0.8-1.2 m/s (Doonan et al., 2011) (Reference Values for Arterial Stiffness' Collaboration., 2010). There is a general paucity of data on pulse wave velocity among CRTI-A and multiple trauma patients. However, again, using modelling of our average patient demographic and pilot data along with published data amongst sedentary controls, age and sex matched controls with subclinical CVD to simulate the disease group and allowing for the natural increase in PWV with age, it would be anticipated that trauma patients would be expected to have a PWV of >0.4m/s higher than the healthy control group (Lazdam et al., 2012).

Hence, a sample size of at least 200 in each group (battlefield trauma exposed versus non exposed) would have a 95% power to detect a difference in PWV of $\geq$0.36 m/s at a significance

level (alpha) of 0.05 (two-tailed) assuming a within-group standard deviation of 1.0m/s (Kingwell et al., 1997). Even if the within-group standard deviation were higher than anticipated at up to 1.4 ms/s a minimal sample size of 280 subjects would still have 90% power to detect a >0.4 m/s (>7%) difference in PWV between the battlefield trauma exposed group and the non-exposed group.

3.6.2 Descriptive statistics

All statistical analyses were performed by Graphpad Prism 8 for MacOS version 8.1.1 (1994-2019 Graphpad Software, Inc. San Diego, California 92108). Graphpad Prism was applied for the presentation of all graphical figures. Included continuous variables are presented as means ± standard deviation.

Data inspection and the D'Agostino-Pearson omnibus test (K2) were undertaken to determine whether the data fitted (or not) a Gaussian distribution in order to decide on the appropriate use of parametric or non-parametric tests. Each column of data was scrutinised for outliers applying the outlier search function in Prism 8 before statistical analysis. Spurious data due to erroneous input were removed. Outlier data relating to the data in the column was included in the analysis.

The level and the patterns of missing data were assessed. For questions which elicit high levels of missing data (for example greater than 20%) we were cautious about any analyses based on these data.

The frequency of distribution was plotted on histograms to visually assess and help assume normality to assist in deciding on parametric or non-parametric testing. Data was presented as means ± standard deviation (SD). Unpaired data was examined using an unpaired t-test for parametric testing with Welch's correction where equal standard deviation was not assumed. For non-parametric testing, the Mann-Whitney test was applied to compare ranks. Three or more groups of continuous data were compared using a one-way Analysis of Variance (ANOVA) for parametric data and the Tukey post-hoc test to illustrate differences between the columns. For non-parametric data, the Kruskal-Wallis test with Dunn's post-hoc test was applied. Qualitive data was examined with the Fisher's exact test and Chi-square test. Correlations for parametric data were examined with the Pearson test (±95% confidence interval) and for non-parametric data the Spearman rank test was applied. A two-tailed P value < 0.05 was considered statistically significant for all comparisons.

Chapter 4
Results

Data of 699 military or ex-military personnel were examined with healthy controls (n=394) and CRTI (n=305). The response rate was similar at 41.5% and 37.3% (p=0.268) respectively. In the injured cohort n=200 suffered combat-related traumatic injuries without amputations (CRTI-NA) and n=105 had limb amputations (CRTI-A) (Table 4-1). A slight variation in the sample size for some statistical analysis were noted where data in the columns were unavailable.

Amputations included isolated proximal upper limb amputations (n=3), isolated proximal upper limb amputations (n=2) and all other proximal and/or distal, unilateral and/or bilateral, lower upper limb amputations. Holm-Sidak's multiple comparison tests with a single pooled variance were applied to measure the level of significant difference between the groups for each parameter investigated. Figure 4-1 illustrates the range and proportions of amputations represented in the study cohort.

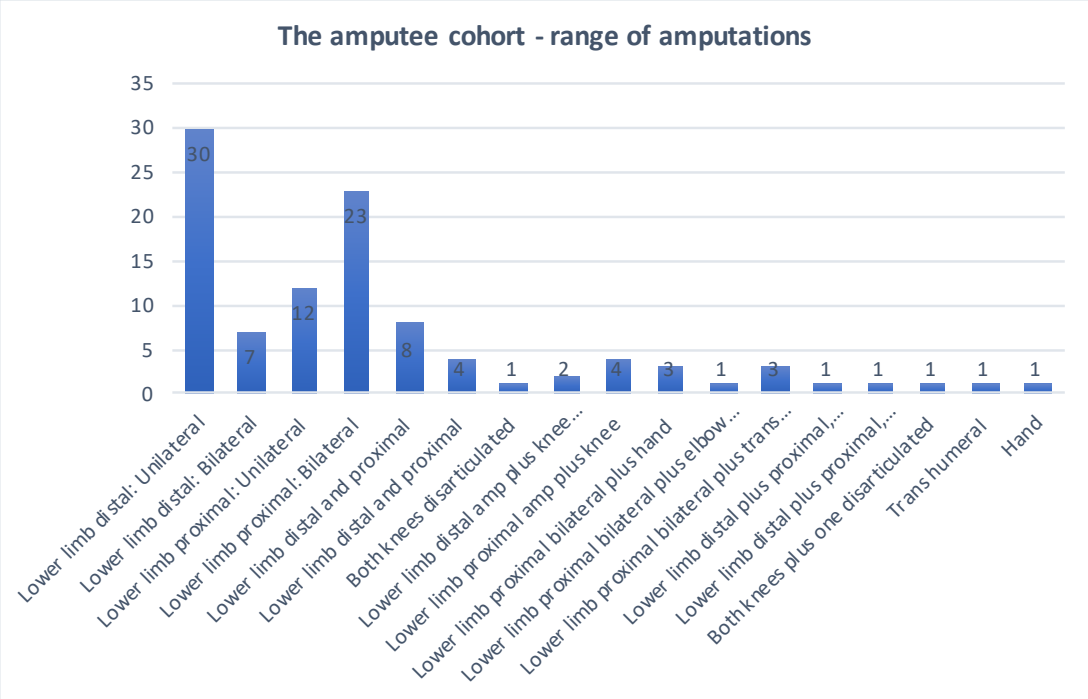


Figure 4-1: Range of amputations in groups with number of participants indicated per group

4.1 Baseline Demographics

A total of 90% of the healthy controls were still serving at the time of examination compared to 36% of the CRTI-NA and 12% of the amputee groups; p<0.001 (Table 4-3).

4.1.1 Age of the participants

The average age for the cohort was 33.9years (± 5.40) with the range for the three groups statistically similar with ages ranging from 23 to 60 overall (Table 4-3). Ages recorded were healthy control, 34.08 years (± 5.34) [23-60] vs CRTI-NA, 34.57years (± 5.95) [24-53] vs amputee group, 33.07 years (± 4.91) [23-52]; p=0.20.

4.1.2 The period from deployment/injury to examination

The period from deployment/injury to examination were significantly longer for the healthy control group, 101.40 months (± 21.49) vs the amputee group, 81.06 months (± 20.29); p<0.001 and CRTI-NA group 88.83 months (± 1.18); p<0.001. The difference between the CRTI-NA and amputee group was significant (Fig 4-2).

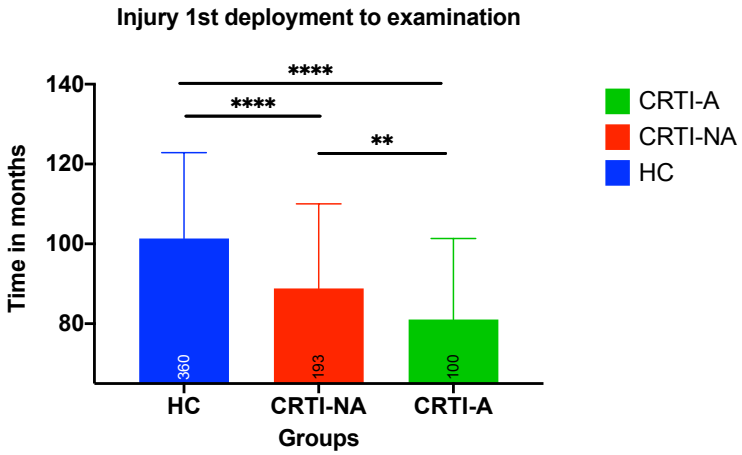


Figure 4-2: The time in months elapsed from 1st deployment or injury to examination compared between the groups.
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=360, Injured (CRTI-NA) n=193, amputees (CRTI-A) n= 100

4.2 Family history of CVD

4.2.1 Reported close family history of CVD (father, mother, brother and sister)

The results showed that family history differences between the three groups were not statistically significant; p=0.25.

4.2.2 Smoking history

Smoking history between the groups was examined applying the chi-squared test to compare smokers and never smoked. The test revealed a difference (however not significant), between the smokers (current plus ex-smokers) in control (total 183/54.8%) vs CRTI-A groups (total 52/52%); p=0.043 (Fig 4-3).

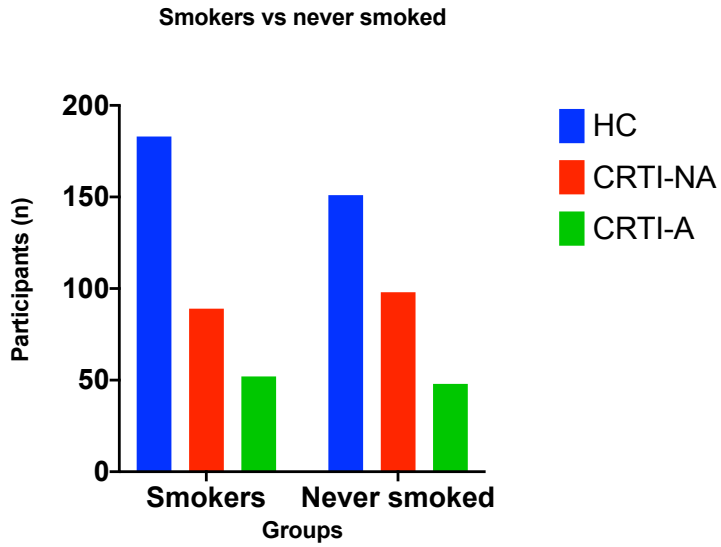


Figure 4-3: Smoking characteristics of the participants compared within the groups

4.2.3 Injury severity score (NISS)

The injury severity scores were significantly higher among the CRTI-A, 36.75 (± 18.01) compared to the CRTI-NA group, 20.11 (± 17.99); p<0.0001 (Table 4-3, Fig 4-4).

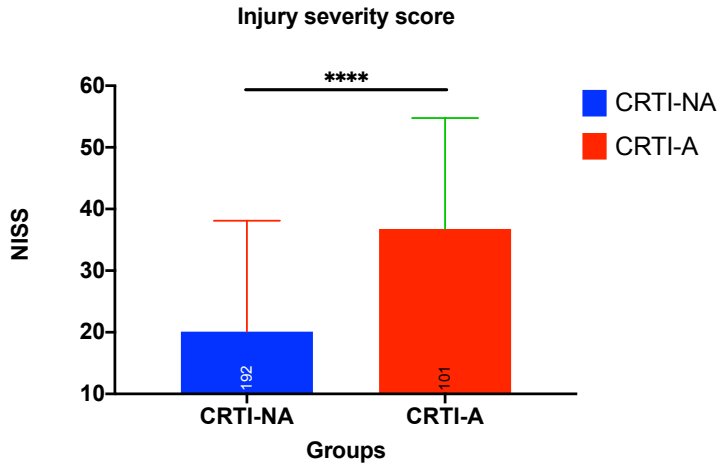


Figure 4-4: Injury severity score (NISS) compared between amputee and CRTI-NA groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=394, Injured (CRTI-NA) n=192, amputees (CRTI-A) n= 101

4.3 Haemodynamic characteristics including arterial stiffness

4.3.1 Resting heart rate

Resting heart rate (beats/min) was significantly higher amongst CRTI-A, 63.67 m^{-1} (± 12.53 m^{-1}) vs CRTI-NA group, 58.35 m^{-1} (± 9.4 m^{-1}); $p<0.001$ and healthy control, 56.39 m^{-1} (± 9.24 m^{-1}); $p<0.0001$. (Table 4-1, Fig 4-5)

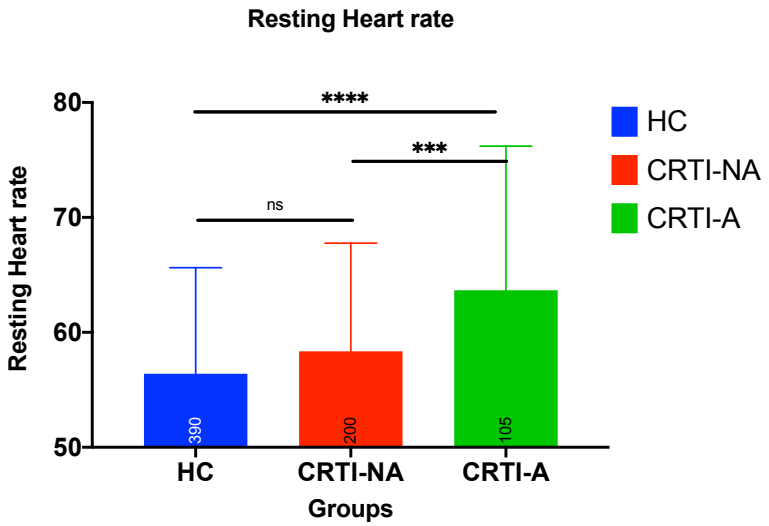


Figure 4-5: Resting heart rate as a CVD risk marker compared between the three groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=390, Injured (CRTI-NA) n=200, amputees (CRTI-A) n= 105

4.3.2 Systolic and diastolic blood pressure

There were no statistically significant differences between aortic and brachial systolic pressures or diastolic pressures across the three groups (Table 4-1).

4.3.3 Pulse pressure

(a) Aortic pulse pressure

The aortic pulse pressure in the CRTI-A, 51.67mmHg (± 6.90 mmHg) group was statistically lower than in the healthy control groups, 54.24mmHg (± 7.84 mmHg); p=0.007. (Table 4-1. Fig 4-6)

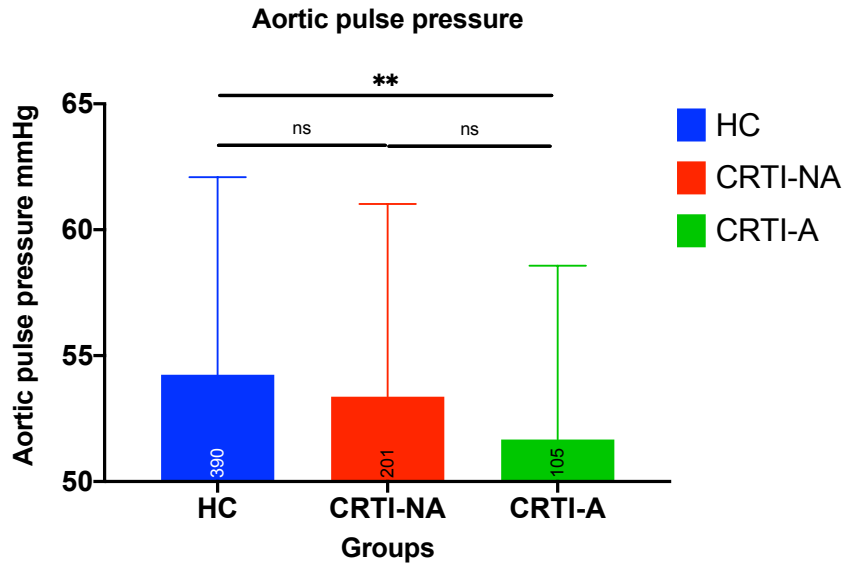


Figure 4-6: Aortic pulse pressure mmHg as a CVD risk marker compared between the groups

Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=390, Injured (CRTI-NA) n=201, amputees (CRTI-A) n= 105

(b) Peripheral pulse pressure

The brachial pulse pressures were not significantly different between the groups (Table 4-1).

4.3.4 Subendocardial viability ratio (SEVR)

Subendocardial viability ratio was significantly lower in the CRTI-A group, 194.0 (± 43.32), compared to the healthy control group, 206.3 (± 46.48); P=0.01. (Table 4-1, Fig 4-7)

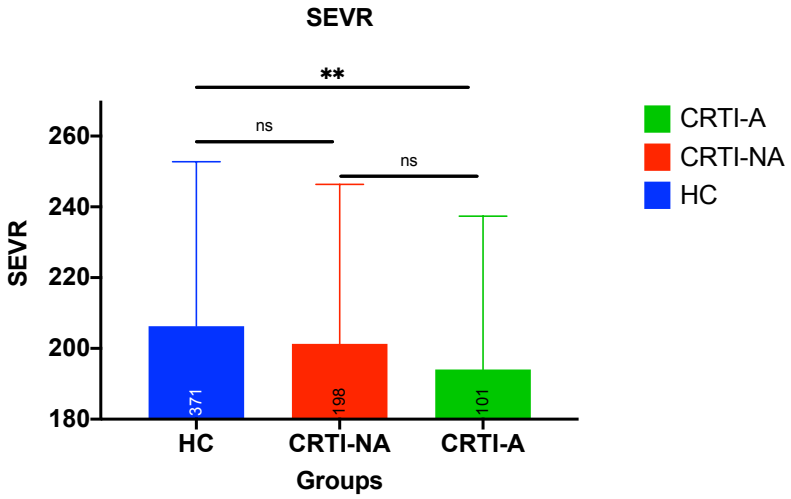


Figure 4-7: Comparison of Subendocardial Viability Ratio (SEVR) between the groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=371, Injured (CRTI-NA) n=198, amputees (CRTI-A) n= 101

4.3.5 Stroke volume

The stroke volume was significantly lower in the CRTI-A group, 88.77ml (± 14.3 ml) compared to the CRTI-NA group, 93.03ml (± 16.09 ml) and the healthy control group, 95.02ml (± 13.77 ml); p<0.001. (Table 4-1, Fig 4-8)

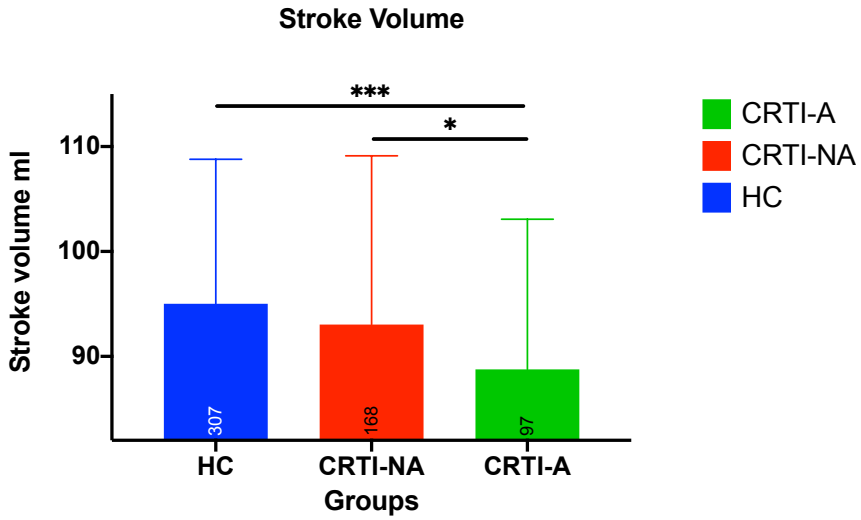


Figure 4-8: Stroke volume (ml) was compared between the groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=307, Injured (CRTI-NA) n=168, amputees (CRTI-A) n= 97

4.3.6 Pulse wave velocity

A significantly lower PWV was recorded in the CRTI-A group, 7.61 ms^{-1} (± 1.51 ms^{-1}) vs the CRTI-NA group, 8.03 ms^{-1} (± 1.64 ms^{-1}); p=0.03. There was no statistically significant difference in PWV between the CRTI-A and healthy control groups. (Table 4-1, Fig 4-9)

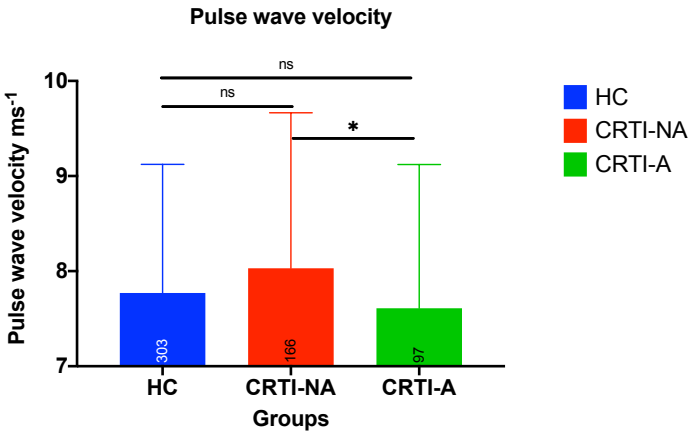


Figure 4-9: Pulse wave velocity (ms^{-1}) was compared between the three groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control (HC) n=303, Injured (CRTI-NA) n=166, amputees (CRTI-A) n= 97

4.3.7 Augmentation index

There were no significant differences in central and peripheral augmentation indexes between all the groups. (Table 4-1, Fig 4-10)

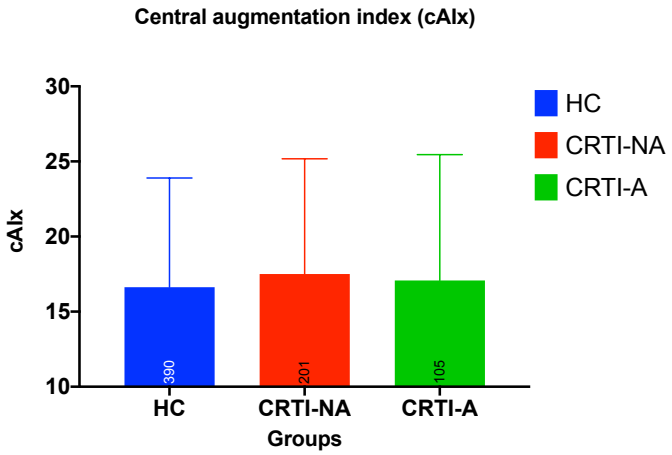


Figure 4-10: Central augmentation Index (cAIx)

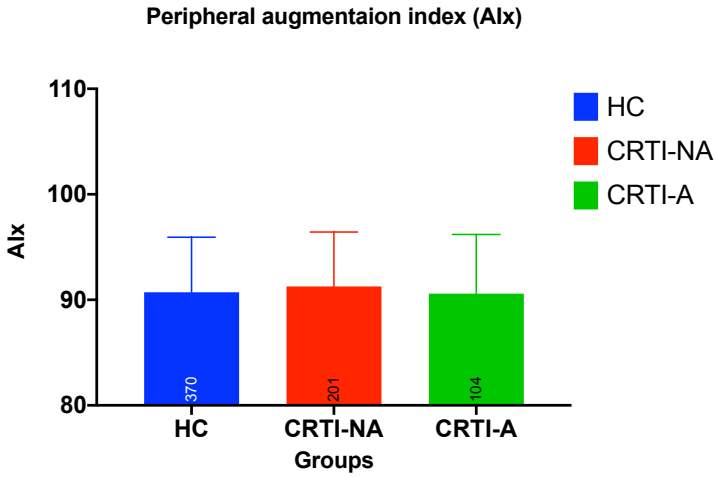


Figure 4-11: Peripheral augmentation index (AIx)

Table 4-1: Comparative baseline haemodynamic characteristics including arterial stiffness (mean ± SD) of the healthy control versus those with traumatic injuries without amputations (CRTI-NA) and those with amputations (CRTI-A)

		Combat related traumatic injury		
Variables	Healthy control Mean ± SD	Non-amputee (CRTI-NA) Mean ± SD	Amputees CRTI-A Mean ± SD	**Kruskal-Wallis p**
Cohort (n)	394	200	105	
Resting heart rate, minute [1]	56.39 ± 9.24	58.35 ± 9.41	63.67 ± 12.53	<0.001 a[ns] b**** c***
Central augmentation index, %	16.63 ± 7.27	17.5 ± 7.67	17.08 ± 8.38	0.456
Peripheral augmentation index, %	90.72 ± 5.22	91.27 ± 5.17	90.59 ± 5.61	0.449
Aortic systolic pressure, mmHg	125.40 ± 10.86	125.70 ± 10.89	124.1 ± 11.18	0.464
Brachial systolic blood pressure, mmHg	71.15 ± 8.05	72.32 ± 8.06	72.48 ± 8.42	0.531
Brachial diastolic blood pressure, mmHg	71.15 ± 8.05	72.32 ± 8.06	72.48 ± 8.42	0.194
Aortic pulse pressure, mmHg	54.24 ± 7.84	53.37 ± 7.65	51.67 ± 6.90	0.007 b**
Peripheral pulse pressure, mmHg	59.76 ± 8.12	58.49 ± 7.98	57.2 ± 7.78	0.008 b*
Subendocardial viability ratio (SEVR)	206.43 ± 46.48	201.30 ± 45.07	194.00± 43.32	0.010 b**
Stroke volume, ml	95.02 ± 13.77	93.03 ± 16.09	88.77 ± 14.3	<0.001 b***
Stroke volume Index ml/m2	45.77 ± 7.12	44.99 ± 9.03	44.58 ± 8.25	0.904
Cardiac index, l/m2	3.90 ± 8.89	3.01 ± 0.65	4.25 ± 7.15	0.017 b*
Pulse Wave Velocity, ms-1	7.77 ± 1.35	8.03 ± 1.64	7.61 ± 1.51	0.031 c*
Tukey's or Dunn's post hoc test: significant difference between groups: a, CRTI-NA vs HC; b, Amputee vs HC; c, Amputee vs CRTI-NA (* = degree of significance)				

4.4 Biochemical characteristics

4.4.1 Serum lipid-profiles

(a) Total cholesterol and LDL cholesterol

Total cholesterol and LDL cholesterol were lower in the CRTI-A group compared to the healthy control and CRTI-NA groups. However, the differences were not statistically significant; p=0.22; p=0.51 respectively. (Table 4-2)

(b) HDL Cholesterol

HDL cholesterol (mmol/l) was significantly lower in the CRTI-A group, 1.12 mmol/l (± 0.27 mmol/l) vs the CRTI-NA, 1.25 mmol/l (± 0.33 mmol/l) vs the control group, 1.25 mmol/l (± 0.27 mmol/l) respectively; p<0.0001. (Table 4-2; Fig 4-12)

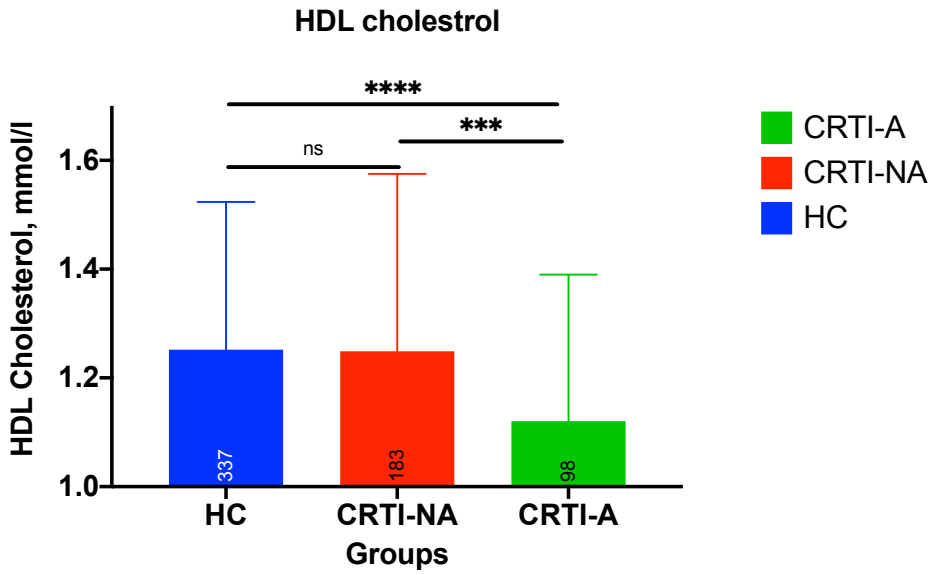


Figure 4-12: HDL cholesterol mmol/l compared between the three groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=337, Injured (CRTI-NA) n=183, amputees (CRTI-A) n= 98

(c) The total cholesterol/HDL ratio

The total cholesterol/HDL ratio was significantly higher in the CRTI-A group, 4.49 (± 1.247) vs the CRTI-NA group, 4.31 (± 1.82) vs control, 4.19 (± 1.48); p=0.01. (Table 4-2, Fig 4-13)

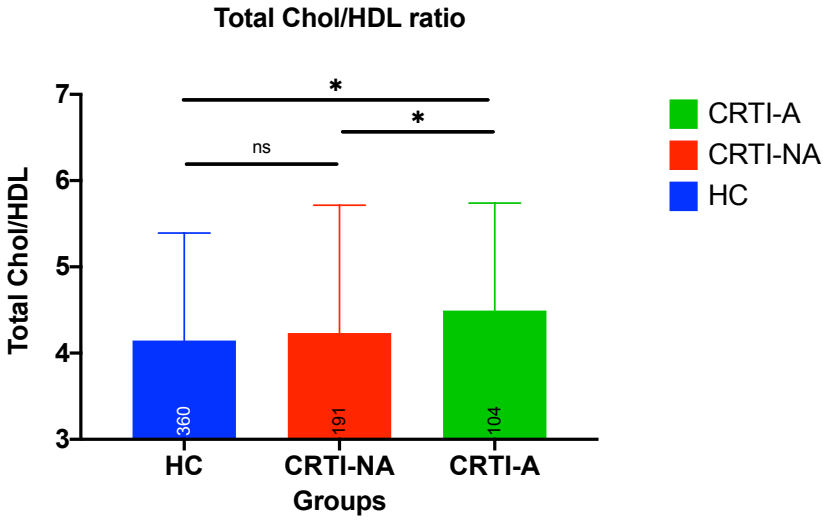


Figure 4-13: Total Cholesterol/HDL ratio compared between the three groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=360, Injured (CRTI-NA) n=191, amputees (CRTI-A) n= 104

(d) Triglycerides

Triglyceride values were not significantly different between the three groups. (Table 4-2)

4.4.2 Glycaemic Index

Glucose and HbA1C values were not statistically different between all the groups (Table 4-2).

4.4.3 Inflammatory markers

(a) Hs-CRP

Hs-CRP levels were significantly higher in the CRTI-A, 2.60mg/l (± 3.81 mg/l) vs control, 1.44mg/l (± 1.69 mg/l); p=0.02. CRTI-A vs CRTI-NA and CRTI-NA vs control did not differ significantly. (Table 4-2, Fig 4-14)

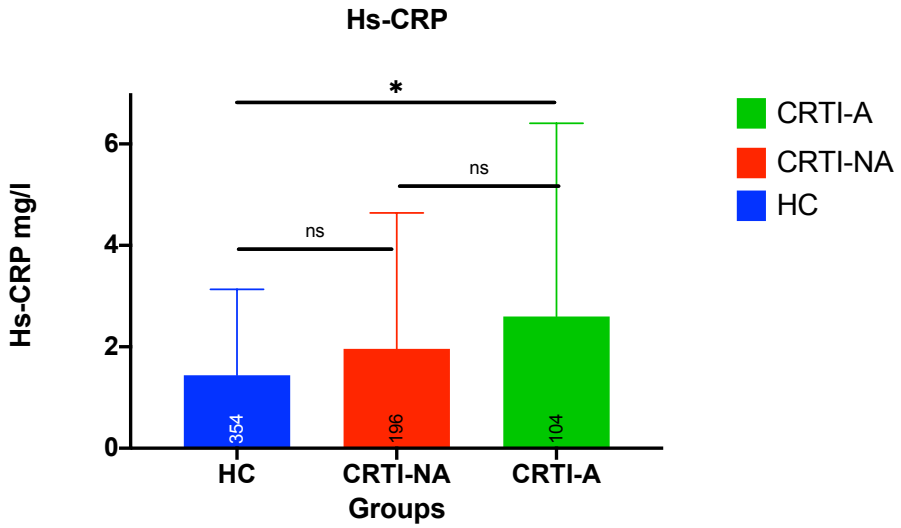


Figure 4-14: Hs-CRP (mg/l) compared between the three groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=354, Injured (CRTI-NA) n=196, amputees (CRTI-A) n= 104

(b) White Cell Count

The white cell count was significantly higher with the CRTI-A, 6.02 ml^{-3} (± 2.30 ml^{-3}) vs control, 5.50 ml^{-3} (± 1.31 ml^{-3}); p=0.02 with no significant difference compared to the CRTI-NA group. (Table 4-2)

4.4.4 Other biochemical markers

(a) Haemoglobin

Haemoglobin levels were not significantly different between the groups. (Table 4-2)

(b) Creatinine

Creatinine values were significantly lower in the CRTI-A, 76.80µmol/l (± 12.02µmol/l) vs the CRTI-NA group, 85.19µmol/l (± 12.66µmol/l) and CRTI-A vs control, 86.78 µmol/l (± 10.87 µmol/l); p<0.0001. (Table 4-2, Fig 4-15)

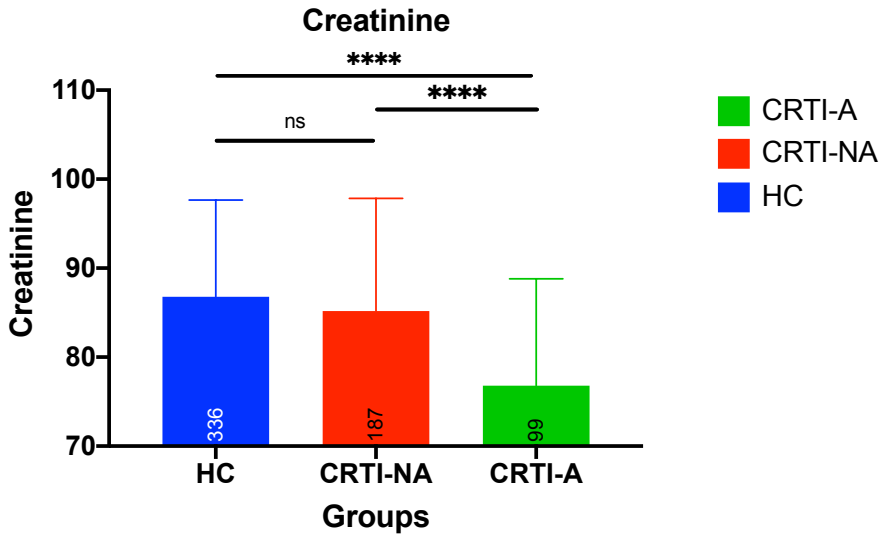


Figure 4-15: Creatinine (µmol/l) compared between the three groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=336, Injured (CRTI-NA) n=187, amputees (CRTI-A) n= 99

Table 4-2: Comparative baseline Biochemical characteristics (mean ± SD) of the healthy control versus those with traumatic injuries without amputations (CRTI-NA) and those with amputations (CRTI-A)

		Combat related traumatic injury		
Variables	Healthy control (HC) Mean ± SD	Non-amputee (CRTI-NA) Mean ± SD	Amputees CRTI-A Mean ± SD	**Kruskal-Wallis p**
Cohort (n)	394	200	105	
Total Cholesterol, mmol/l	5.07 ± 1.06	5.02 ± 0.99	4.82 ± 0.98	0.22
LDL Cholesterol, mmol/l	3.17 ± 0.83	3.11 ± 0.86	3.05 ± 0.80	0.51
HDL Cholesterol, mmol/l	1.25 ± 0.27	1.25 ± 0.32	1.12 ± 0.27	<0.0001 b*** c***
Total Cholesterol/HDL ratio	4.15 ± 1.25	4.23 ± 1.48	4.49 ± 1.25	0.01 b * c *
Triglycerides, mmol/l	1.37 ± 1.26	1.42 ± 0.94	1.55 ± 1.23	0.03
Glucose, mmol/l, fasting	4.93 ± 0.67	4.98 ± 1.06	4.89 ± 0.55	0.54
HbA1c	34.88 ± 4.01	34.94 ± 4.81	34.11 ± 4.06	0.06
Hs-CRP, mg/l	1.44 ± 1.69	1.96 ± 2.68	2.60 ± 3.81	0.02 b *
White Cell Count, x 10^9/l	5.50 ± 1.31	5.59 ± 1.76	6.02 ± 2.30	0.02 b *
Haemoglobin, g/l	152.6 ± 8.70	152.2 ± 10.44	154 ± 9.00	0.75
Creatinine, µmol/l	86.78 ± 10.87	85.19 ± 12.66	76.80 ± 12.02	<0.001 b**** c****
Creatinine clearance, ml/minute	134.10±24.05	138.90±30.22	140.80±27.08	0.04 b**
Tukey's or Dunn's post hoc test: significant difference between groups: a, HC vs CRTI-NA; b, HC vs Amputee; c, CRTI-NA vs Amputee (*-**** = degree of significance) LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, haemoglobin A1c; HsCRP, high-sensitivity C-reactive protein				

4.5 Anthropometric and demographic characteristics

4.5.1 Height

There were no significant differences in the height of the three groups; p=0.24. (Table 4-3)

4.5.2 Weight

The mean weight of the CRTI-A group, 81.05 kg (± 13.73 kg) was significantly lower than both the CRTI-NA, 88.58 kg (± 14.36 kg) and control groups, 87.51 kg (± 12.00 kg); p<0.0001. Weight was not adjusted for loss of limb. (Table 4-3, Fig 4-16)

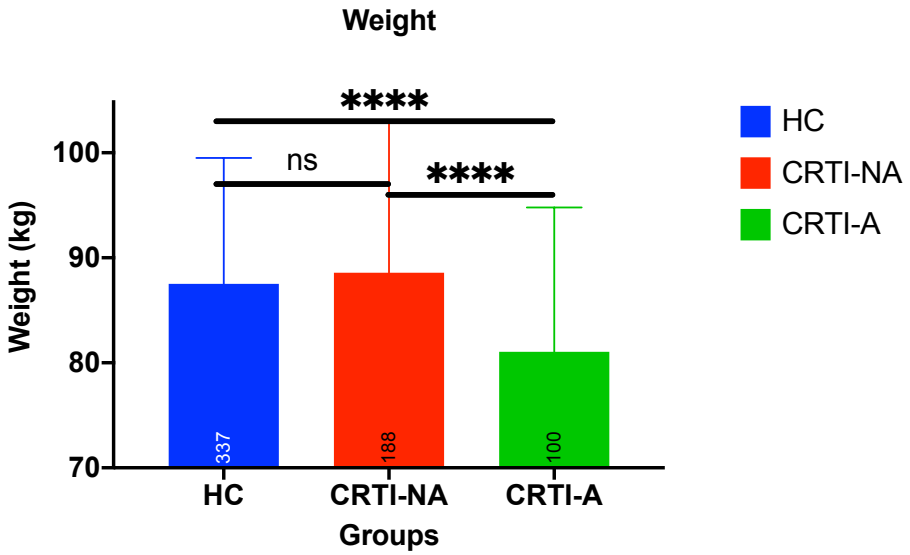


Figure 4-16: Weight comparison between the three cohorts
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=337, Injured (CRTI-NA) n=188, amputees (CRTI-A) n= 100

4.5.3 Hip circumference

With respect to hip circumferences, higher values were recorded with the CRTI-A group, 105.3 cm (± 9.05 cm) comparative to control, 103.2 cm (± 7.06 cm); p=0.05. (Table 4-3)

4.5.4 Abdominal waist circumference (cm) (AWC)

The AWC in the CRTI-A group, 96.18cm (± 11.07cm), were significantly higher than the control group, 92.95cm (± 8.96cm); p=0.01. (Table 4-3, Fig 4-17)

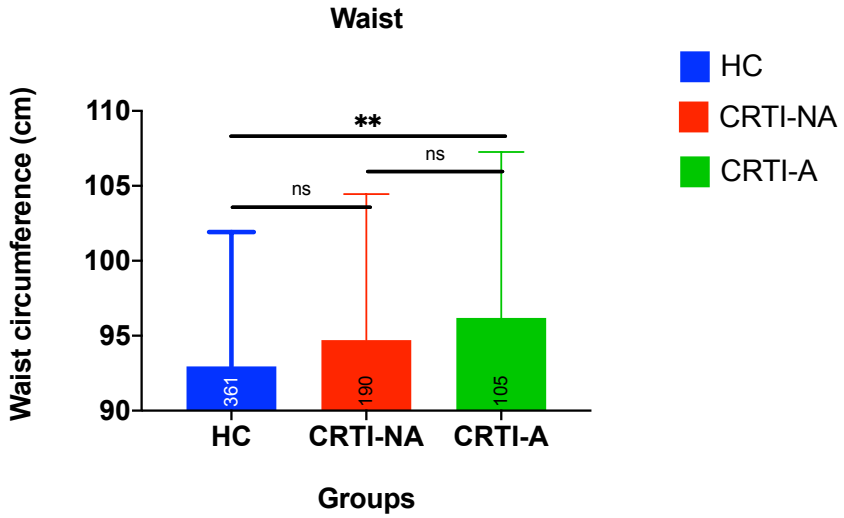


Figure 4-17: Comparison of waist circumference between the groups.
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=361, Injured (CRTI-NA) n=190, amputees (CRTI-A) n= 105

4.5.5 Waist/height ratio (WHtR)

The waist/height ratio in the CRTI-A group, 0.54 (± 0.06), was significantly higher compared to the control group, 0.52 (± 0.05); p=0.03. (Table 4-3, Fig 4-18)

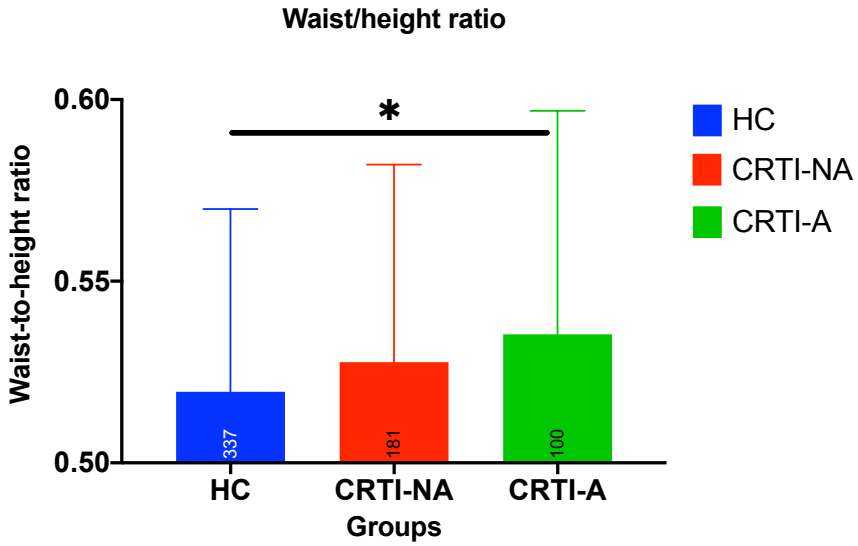


Figure 4-18: Comparison of Waist/height ratio between the groups as a novel CVD risk indicator
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=337 Injured (CRTI-NA) n=181, amputees (CRTI-A) n= 100

4.5.6 **BMI**

BMI, with no adjustment for loss of limb, was significantly lower with the CRTI-A group, 25.07 kg/m^2 (± 4.17kg/m^2) vs control, 27.2 kg/m^2 (± 3.28kg/m^2) and CRTI-NA groups, 27.61 kg/m^2 (± 3.72kg/m^2); p<0.0001. (Table 4-3, Fig 4-19)

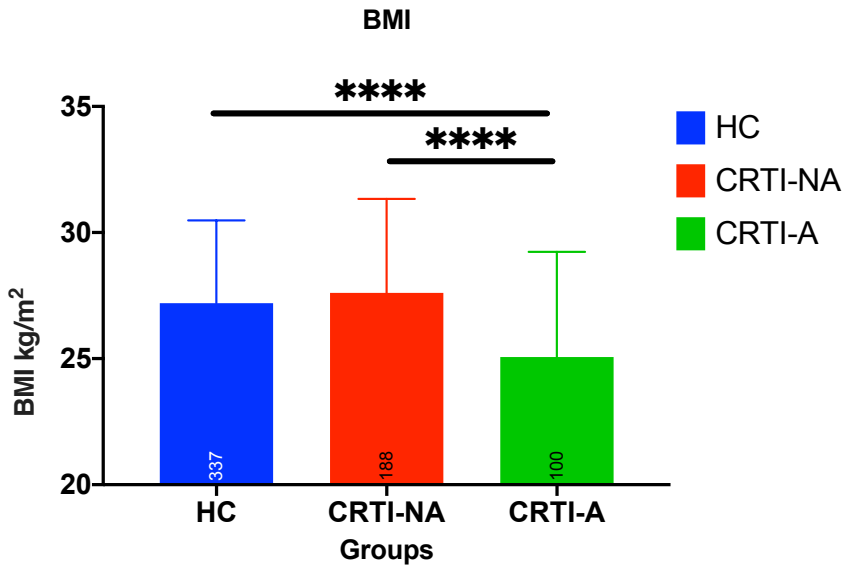


Figure 4-19: Comparison of BMI between the groups
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=337, Injured (CRTI-NA) n=188, amputees (CRTI-A) n= 100

4.5.7 Conicity Index (CoI)

The CRTI-A group was characterized with a significantly higher mean CoI, 1.32 (± 0.09) than the control, 1.23 (± 0.06) and CRTI-NA groups, 1.23 (± 0.07); p<0.0001.
(Table 4-3, Fig 4-20)

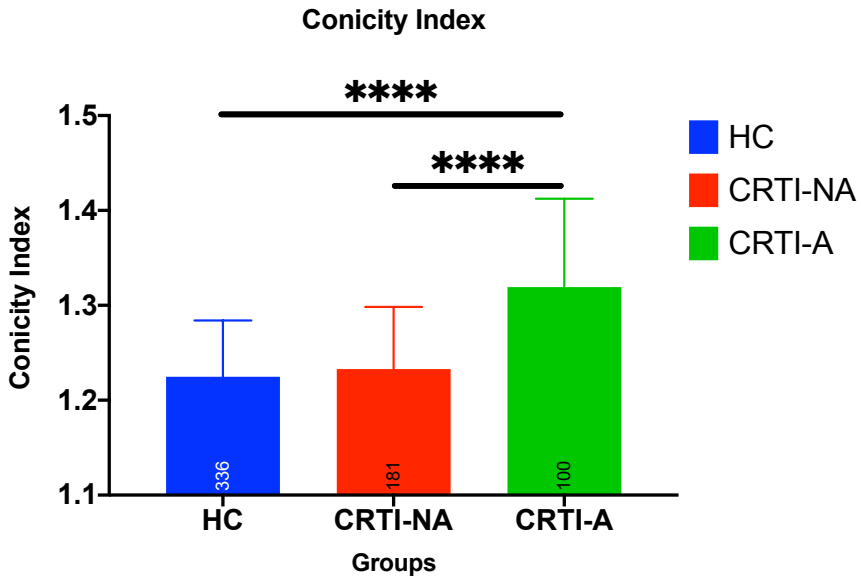


Figure 4-20: Comparison of mean Conicity Index between the study cohorts
Dunn's multiple comparison test: significance **** = p<0.0001; *** = p<0.001; *** = p<0.01; * = p<0.1); ns = non-significant
Healthy control n=336, Injured (CRTI-NA) n=181, amputees (CRTI-A) n= 100

Table 4-3: Comparative baseline Anthropometric and demographic characteristics (mean ± SD) of the healthy control versus those with traumatic injuries without amputations (CRTI-NA) and those with amputations (CRTI-A)

		Combat related traumatic injury		
Variables	Healthy control (HC) Mean & SD	Non-amputee (CRTI-NA) Mean & SD	Amputees (CRTI-A) Mean & SD	**Kruskal-Wallis p**
Cohort (n)	394	200	105	
Age, years Range	34.08 ± 5.34 23-60	34.57 ± 5.95 24 -53	33.07 ± 4.92 23-52	0.20
Height (cm)	179.3 ± 6.12	178.9 ± 7.19	180.3 ± 7.86	0.24
Weight (kg)	87.51 ± 12.00	88.58 ± 14.36	81.05 ± 13.73	0.02
Hip circumference (cm)	103.2 ± 7.07	103.7 ± 8.52	105.3 ± 9.05	0.05
Abdominal waist circumference(cm)	92.95 ± 8.96	94.48 ± 10.23	96.18 ± 11.07	0.01 b**
Waist/height ratio	0.52 ± 0.05	0.53 ± 0.05	0.54 ± 0.06	0.03 b*
BMI	27.20 ± 3.28	27.61 ± 3.73	25.07 ± 4.17	<0.0001 b**** c****
Conicity Index	1.23 ± 0.06	1.23 ± 0.07	1.32 ± 0.09	<0.0001 b**** c***
Injury Severity Score Median (Range)		20.11 ± 17.99	36.75 ± 18.01	<0.0001 c****
Time – last deployment to examination (months)	81.81 ±18.50	88.83 ± 21.18	81.06 ± 20.29	0.0001 a**** b**** c**
Still currently serving n, (%)	301/335 (90%)	67/184 (36%)	12/99 (12%)	<0.001
Ethnicity, n (%) Caucasian Non-Caucasian	 314 (93.2%) 23 (6.8%)	 169 (90.9%) 17 (9.1%)	 84 (89.4%) 10 (10.6%)	 0.49
Family history of CVD #	124/331 37.5%	80/185 43.2%	43/97 44.3%	0.25
Smoking history n, (%) Current Ex Never	 75/334 (22.5%) 108/334 (32.3%) 151/334 (45.2%)	 39/187 (20.9%) 50/187 (26.7%) 98/187 (52.4%)	 15/100 (15.0%) 37/100 (37.0%) 48/100 (48.0%)	 0.04 b*

Tukey's or Dunn's post hoc test: significant difference between groups: a, CRTI-NA vs HC;
b, Amputee vs HC; c, Amputee vs CRTI-NA (*-**** = degree of significance)
history of confirmed CVD in first degree relative

4.6 The impact of injury severity on CVD risk

We were keen to assess if the quantity and the severity (proximal or distal, uni- or bi-lateral) of amputations had an impact on prevalence of CVD risk markers. Descriptive statistics with mean (± SD), Kruskal-Wallis 'p' and Dunn's multiple comparison tests were applied to identify the level of significance of differences between the groups and were compared with HC and CRTI-NA (Table 4-5). The number of subjects evaluated in the tests may vary in the subgroups due to availability of data in the various columns. A Kruskal-Wallis test was performed comparing the mean rank of each column with the mean rank of the control column (Fig 4-22 to 4-27). Results (Mean ± SD) are listed in Table 4-4.

4.6.1 Injury severity scores (NISS)

There was a statistically significant difference in NISS between the CRTI-A and the CRTI-NA groups: (36.75 ± 18.01) and (20.11 ± 17.99); $p<0.0001$, respectively confirming the measure of human trauma (Osler et al., 1997).

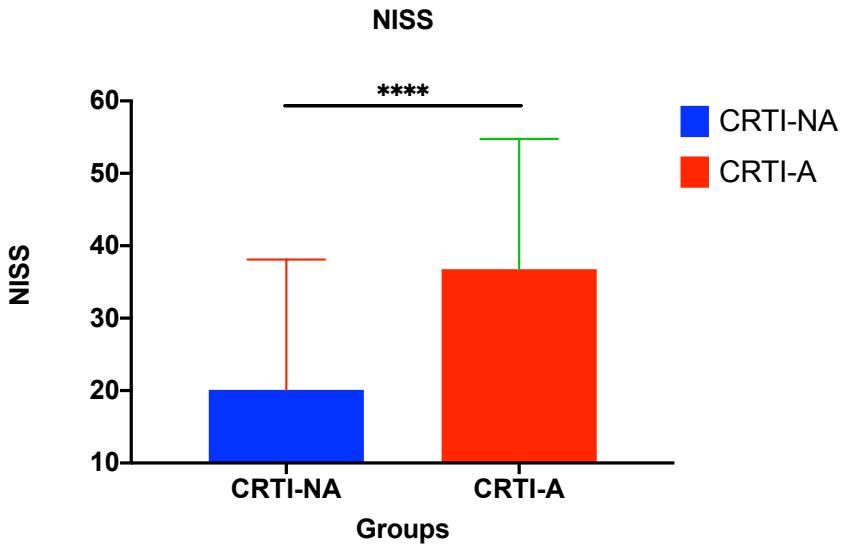


Figure 4-21: New Injury severity score (NISS)

4.6.2 HDL cholesterol

HDL cholesterol was significantly affected by amputations compared to HC and CRTI-NA, p<0.0001. Statistically the most significant affects were noted in the proximal amputations group, 1.08 (± 0.29); p <0.0001. The quantity of distal and proximal CRTI-A affected HDL cholesterol equally albeit weakly significantly; p<0.1. HDL cholesterol is protective against CVD and has a strong inverse relationship with CVD (Cooney et al., 2009). The statistically significant lower HDL value relative to injury severity in our results confirms the increased effect of injury severity on CVD risk. (Table 4-4, Fig 4-22)

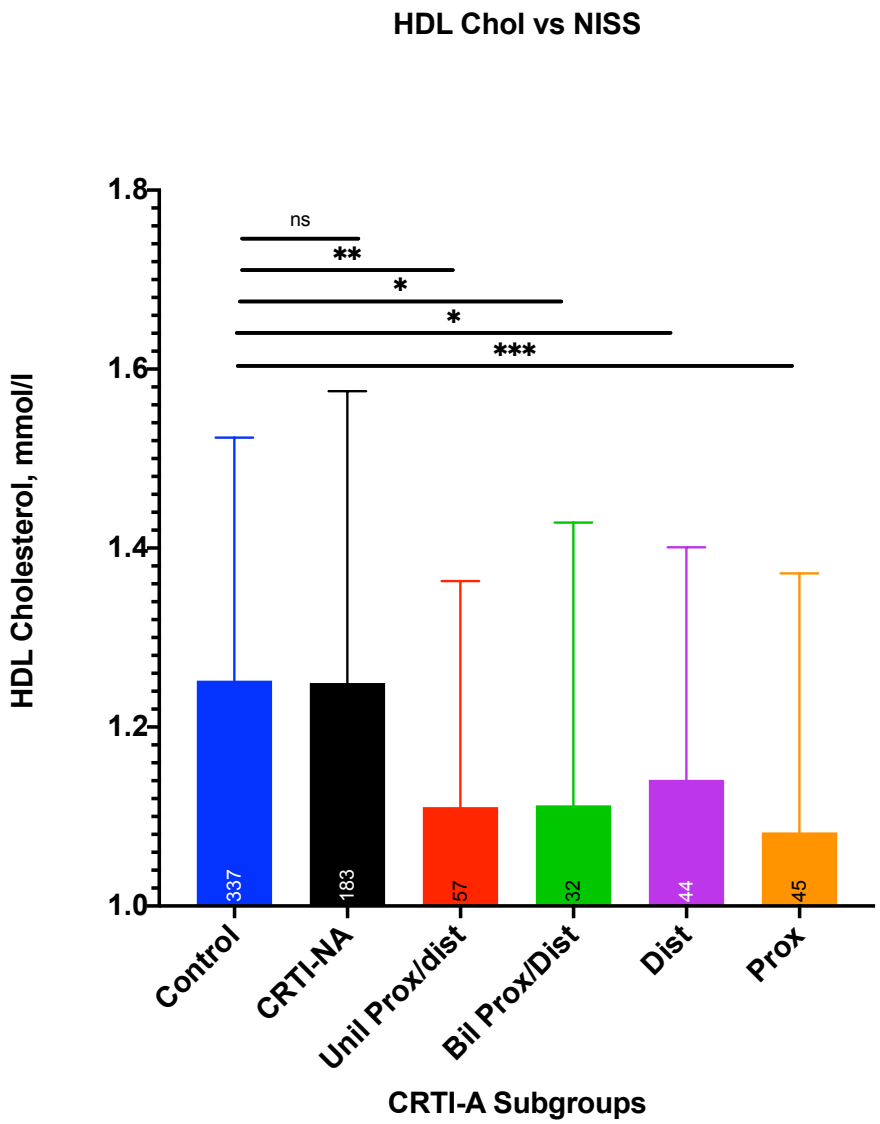


Figure 4-22: Assessing HDL cholesterol (mmol/l) levels affected by the severity of amputation

Healthy Control (n=337), CRTI-NA (Injured) (n=183), Bilateral Proximal/Distal CRTI-A (n=57), Bilateral Proximal/Distal (n=32), Total Distal CRTI-A (n=44), Total Proximal CRTI-A (n=45).

Kruskal-Wallis test significance: **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

4.6.3 Hs-CRP

Hs-CRP levels as a measure of inflammation were recorded, and it was found that Hs-CRP levels were significantly affected by the quantity of amputations compared to HC, 3.82mg/l (± 4.5 mg/l) and 1.43mg/l (± 1.68 mg/l) respectively. This finding confirms the increased effect of multiple amputations on systemic inflammation. Increased Hs-CRP, as a non-traditional CVD risk marker, was found to independently predict risk of all-cause CV mortality (Li et al., 2017) and is directly related to an increase in injury severity (Li et al., 2017, Wilson et al., 2006). (Table 4-4, Fig 4-23)

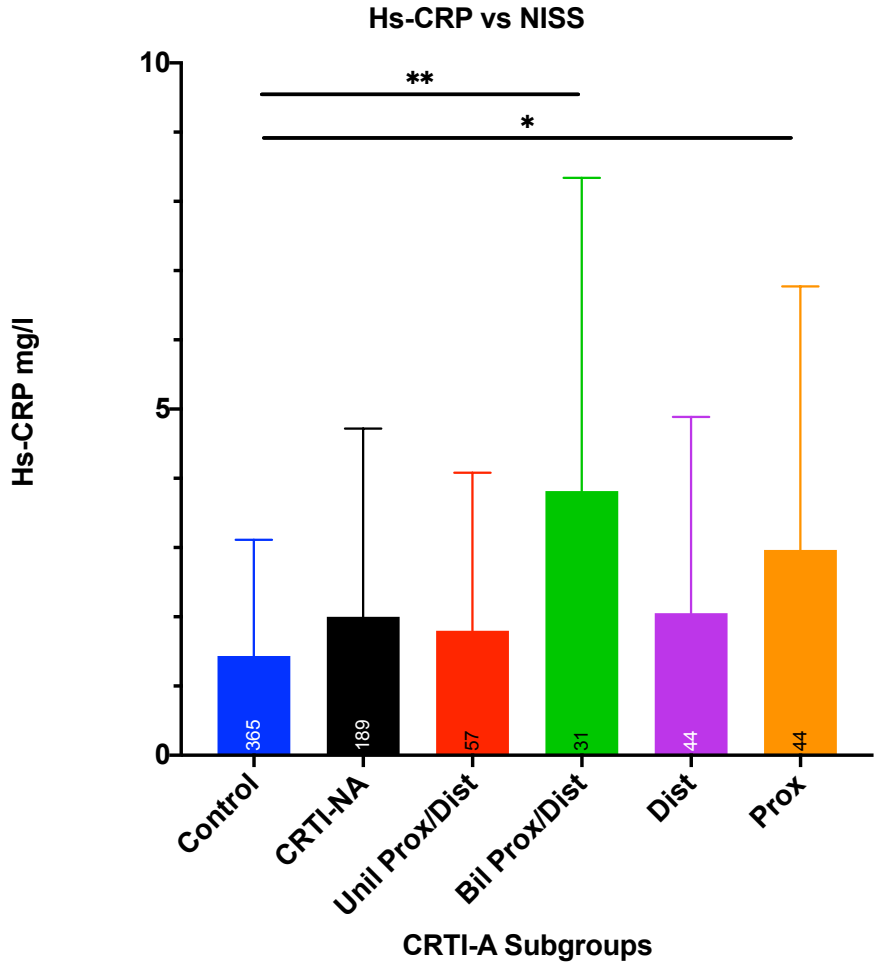


Figure 4-23: Assessment of Hs-CRP (mg/l) affected by the severity of amputation
Healthy Control (n=365), CRTI-NA (Injured) (n=189), Bilateral Proximal/Distal CRTI-A (n=57), Bilateral Proximal/Distal (n=31), Total Distal CRTI-A (n=44), Total Proximal CRTI-A (n=44).
Kruskal-Wallis test significance: **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

4.6.4 Creatinine

Amputations in general had a statistically significant effect on creatinine level compared to HC; p<0.0001. There was no significant effect in the CRTI-NA group. These results are consistent with previous studies alluding that patients with traumatic amputation have significantly lower serum creatinine values compared to the general population due to muscle mass loss (Im et al., 2012, Thurlow et al., 2014). (Table 4-4, Fig 4-24)

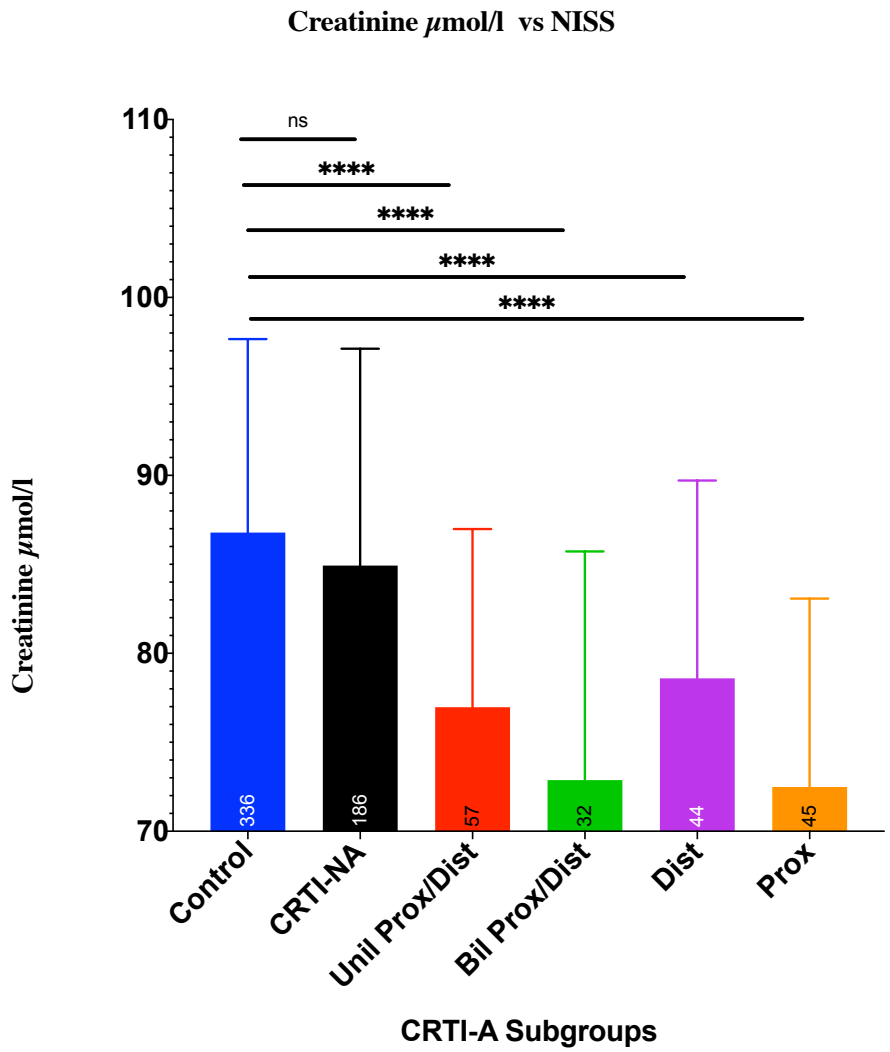


Figure 4-24: Assessing the effect of the severity of amputation on Creatinine µmol/l
Healthy Control (n=336), CRTI-NA (Injured) (n=186), Bilateral Proximal/Distal CRTI-A (n=57), Bilateral Proximal/Distal (n=32), Total Distal CRTI-A (n=44), Total Proximal CRTI-A (n=45).
Kruskal-Wallis test significance: **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

4.6.5 Resting heart rate

Resting heart rate was statistically significantly more affected by both the severity of amputation and the quantity of amputation compared to HC, 64.51^{-1}min (± 11.34^{-1}min), 65.73 $^{-1}$min (± 11.31^{-1}min) and 56.43^{-1}min (± 8.62^{-1}min) respectively; p<0.0001. The effect of CRTI-NA on resting heart rate was not significantly affected compared to HC. An increased resting heart rate is associated with death and CV complications (Hillis et al., 2012) confirming increased CVD risk associated with an increased level of injury severity.
(Table 4-4, Fig 4-25)

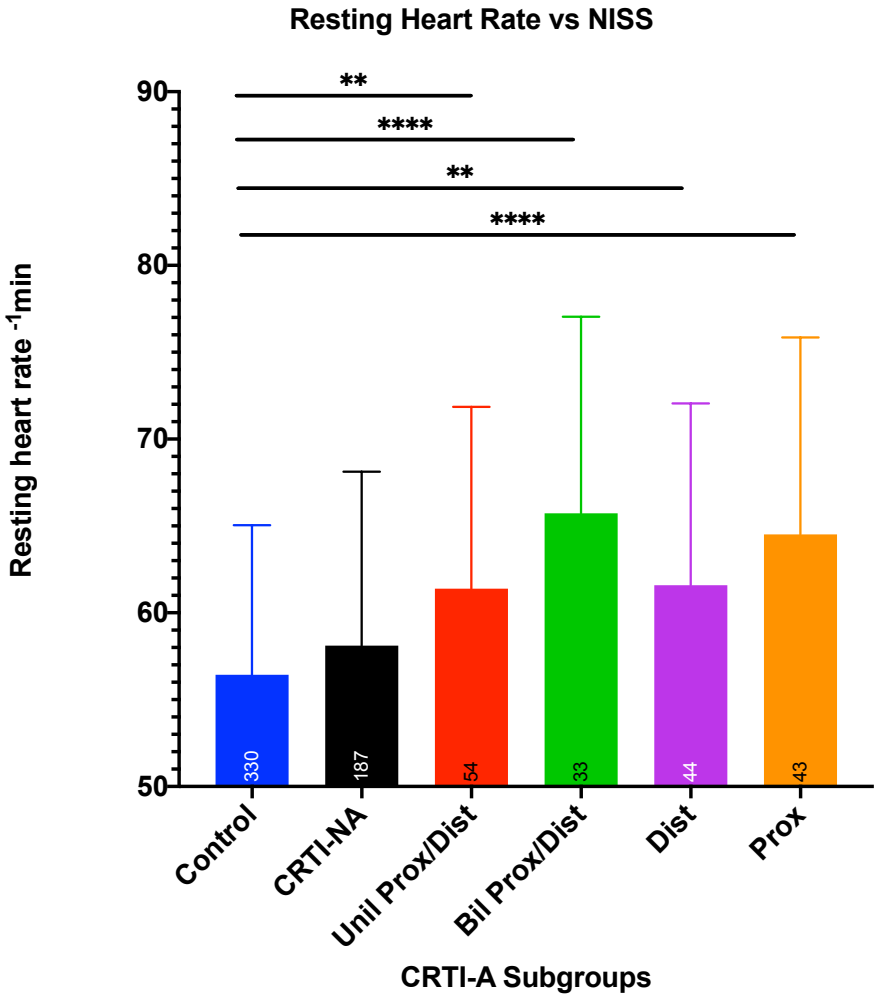


Figure 4-25: Assessing how the severity of amputation affects resting heart rate (/min)
Healthy Control (n=330), CRTI-NA (Injured) (n=187), Bilateral Proximal/Distal CRTI-A (n=54), Bilateral Proximal/Distal (n=33), Total Distal CRTI-A (n=44), Total Proximal CRTI-A (n=43).
Kruskal-Wallis test significance: **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

4.6.6 SEVR

Injury severity did not significantly affect SEVR; however, lower values for SEVR were recorded in each injury group compared to the control group, with the lowest value in the bilateral proximal/distal CRTI-A group. Therefore, the amount of amputations has an effect on SEVR. This finding is consistent with previous studies, alluding there is an inverse association of SEVR with injury severity and CVD risk as well as other CVD risk markers such as Hs-CRP (Sandoo et al., 2012). (Table 4-4, Fig 4-26)

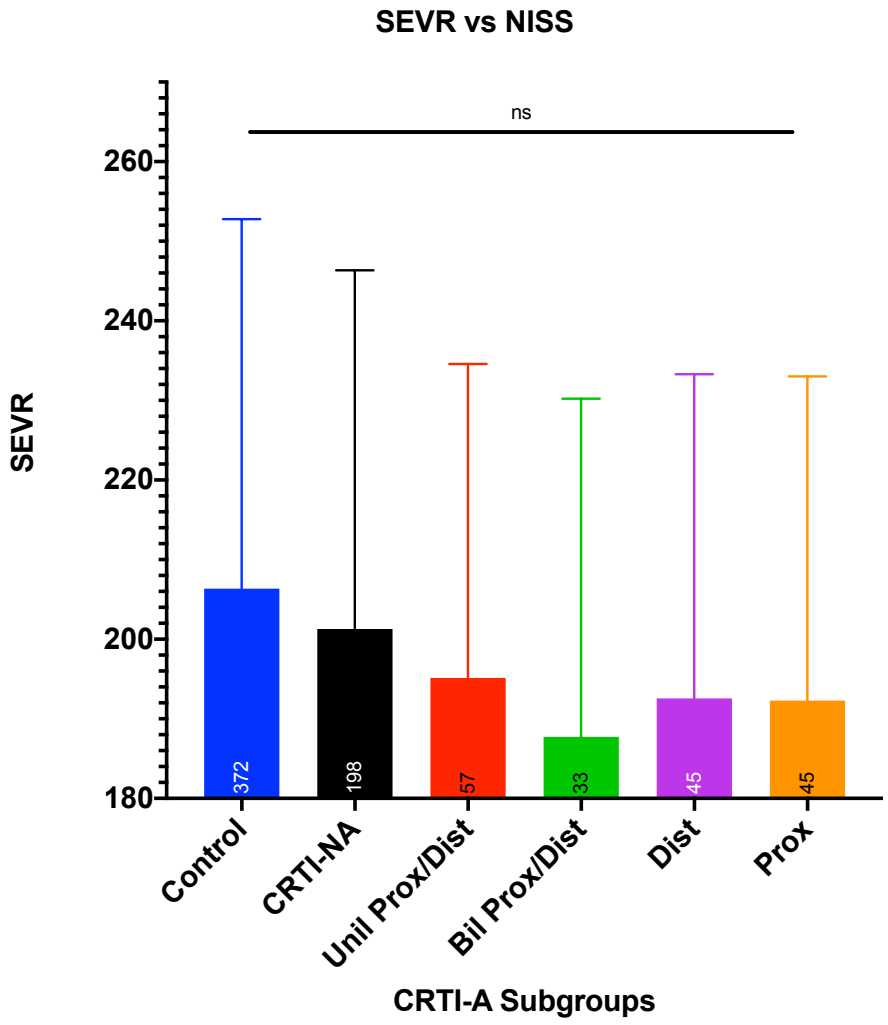


Figure 4-26: CRTI-A comparison groups vs SEVR
Healthy Control (n=372), CRTI-NA (Injured) (n=198), Bilateral Proximal/Distal CRTI-A (n=57), Bilateral Proximal/Distal (n=33), Total Distal CRTI-A (n=44), Total Proximal CRTI-A (n=45).
Kruskal-Wallis test significance: **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

4.6.7 Stroke volume

Stroke volume is significantly affected by the level of amputation (proximal) and the quantity of amputations (bilateral proximal plus distal) compared to HC; 85.80 ml (± 11.78), 85.12 ml (± 11.97) and 95.02 ml (± 13.77), respectively; $p<0.0001$. These values are consistent with an increase in CVD risk due to vascular remodeling and impairment associated with injury severity (Lonnebakken et al., 2011). (Table 4-4, Fig 4-27)

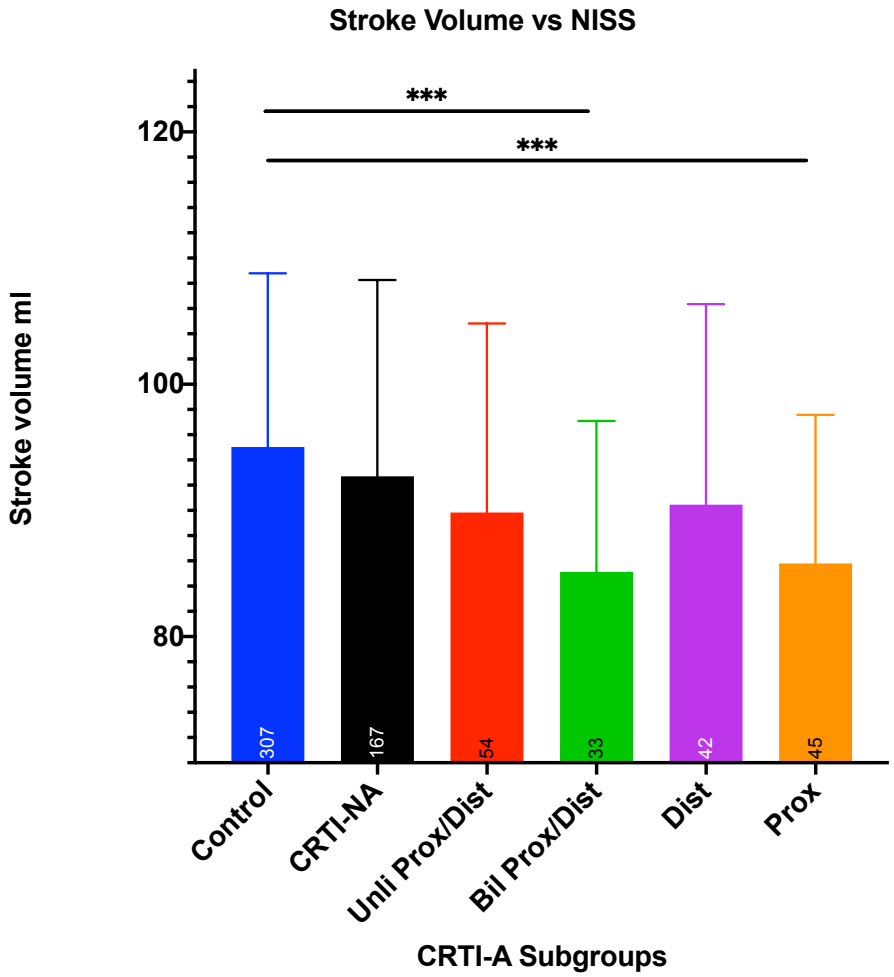


Figure 4-27: Assessing the effect of severity of amputation on stroke volume (ml)
Healthy Control (n=307), CRTI-NA (Injured) (n=167), Bilateral Proximal/Distal CRTI-A (n=54), Bilateral Proximal/Distal (n=33), Total Distal CRTI-A (n=42), Total Proximal CRTI-A (n=45).
Kruskal-Wallis test significance: **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

Table 4-4: Summary of CVD risk variables affected by CRTI-NA and CRTI-A types (NISS subgroups reflect injury severity relating to quantity and severity of amputation).

Groups (Mean ± SD)							**p**
Variables	Healthy control	CRTI-NA (Injured)	Unilateral proximal/Distal CRTI-A	Bilateral proximal/Distal CRTI-A	Distal CRTI-A	Proximal CRTI-A	Kruskal-Wallis p
HDL mmol/l	1.25 ± 0.27	1.25 ± 0.33	1.11 ± 0.25	1.11 ± 0.32	1.14 ± 0.26	1.08 ± 0.29	<0.0001
Hs-CRP mg/ml	1.43 ± 1.68	2.00 ± 2.72	1.80 ± 2.28	3.82 ± 4.5	2.05 ± 2.8	2.97 ± 3.81	0.01
Creatinine	86.78 ± 10.87	84.93 ± 12.19	76.98 ± 10.02	72.88 ± 12.85	78.59 ± 11.12	72.49 ± 10.59	<0.0001
Resting heart rate min^{1}	56.43 ± 8.621	58.11 ± 10.01	61.39 ± 10.47	65.73 ± 11.31	61.59 ± 10.46	64.51 ± 11.34	<0.0001
SEVR	206.3 ± 46.44	201.3 ± 45.07	195.1 ± 39.44	187.7 ± 42.49	192.6 ± 40.74	192.3 ± 40.72	0.01
Stroke volume ml	95.02 ± 13.77	92.7 ± 15.56	89.83 ± 14.99	85.12 ± 11.97	90.45 ± 15.90	85.80 ± 11.78	<0.0001

4.7 Correlation studies

We examined whether there were any correlations between the haemodynamic CVD risk markers and the biochemical or anthropometric CVD risk markers "investigated in this study" between the control and the CRTI-A groups or whether the risk factors had associations with other external factors. The aim was to determine whether amputations could be linked to statistically more significant correlations than in the control group. Spearman correlations between the various CVD risk indices in the control (n=394) and CRTI-A (n=105) groups were examined and listed in Table 4-5; p, two-tailed.

4.7.1 Arterial stiffness markers and inflammatory markers

The association of Hs-CRP values between the HC and CRTI-A groups were assessed and listed in Table 4-5.

(a) Pulse wave velocity and Hs-CRP.

There was practically no correlation between PWV and Hs-CRP in the CRTI-A group compared to the control group. (Fig 4-28 and Fig 4-29)

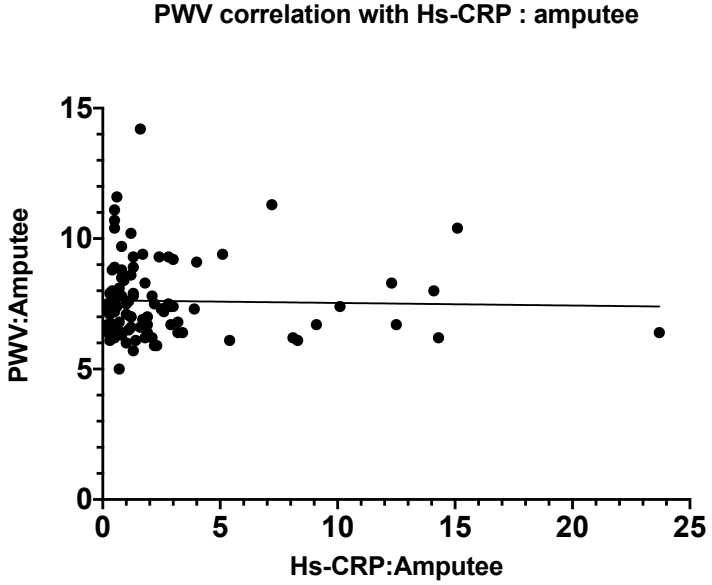


Figure 4-28: Pulse wave velocity correlation with Hs-CRP (mg/l) in the CRTI-A group (r = -0.028, CI -0.23 to 0.18; p = 0.79)

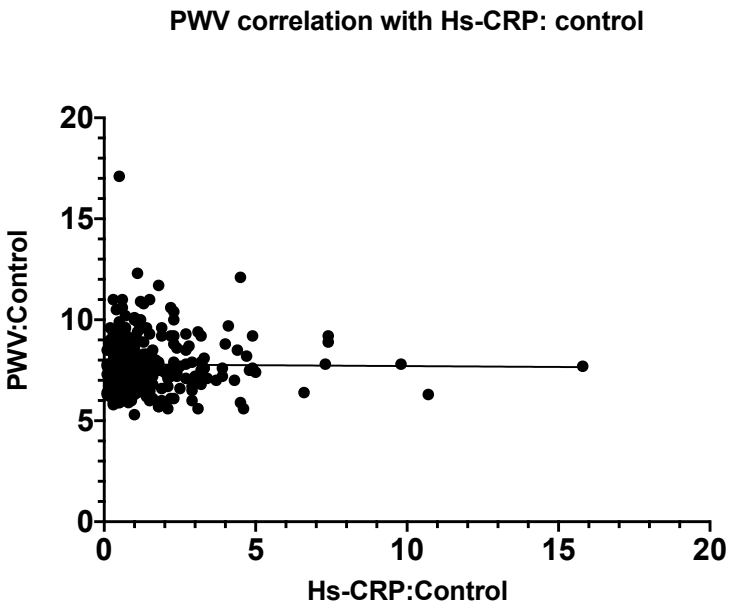


Figure 4-29: Pulse wave velocity correlation with Hs-CRP in the control group (r = 0.005, CI -0.11 to 0.12; p = 0.93)

(b) Central augmentation index and Hs-CRP

Central augmentation index reveals a weak positive correlation with Hs-CRP in the CRTI-A and, the control group, both of which are statistically significant. (Fig 4-30 and Fig 4-31)

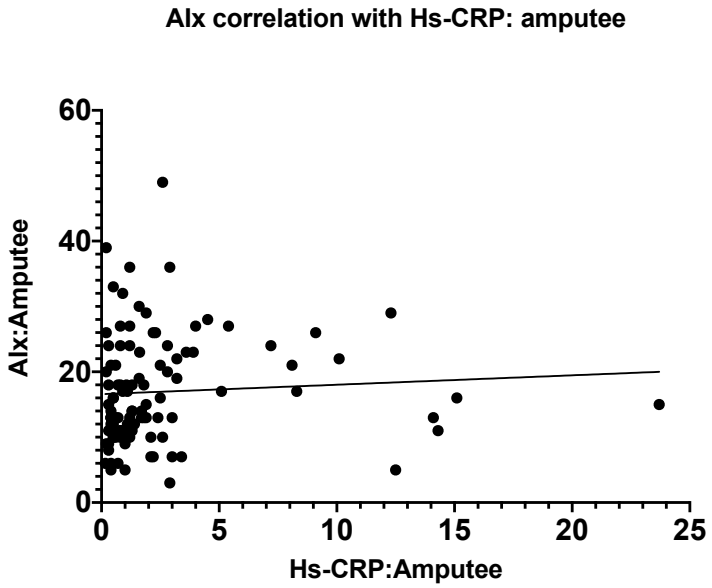


Figure 4-30: Central augmentation index correlating with Hs-CRP (mg/l) in the CRTI-A group (r = 0.23, CI 0.03 to 0.41; p= 0.02; n=105)

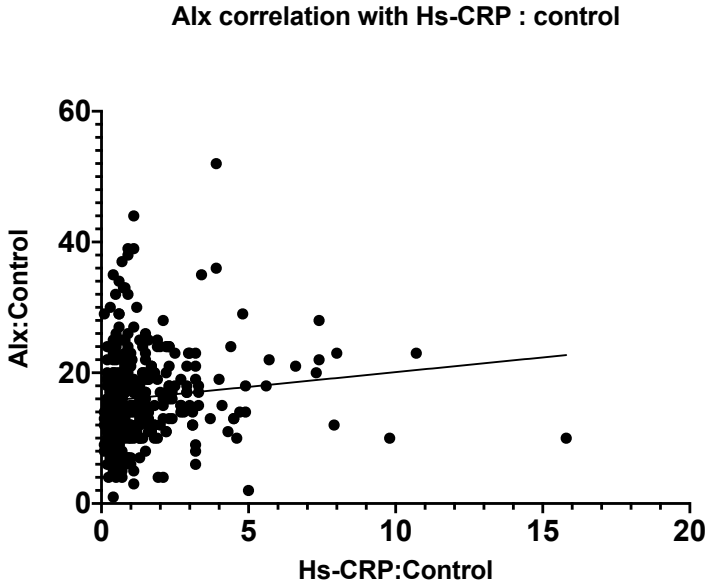


Figure 4-31: Central augmentation index correlating with Hs-CRP (mg/l) in the control group (r = 0.20, CI 0.09 to 0.30; p <0.001; n=394)

(c) Central augmentation index (AIx) and total cholesterol/HDL ratio

Central augmentation index (AIx) revealed a similar weak positive correlation with total cholesterol/HDL ratio in the CRTI-A and control groups, which is statistically significant. (Fig 4-32 and Fig 4-33)

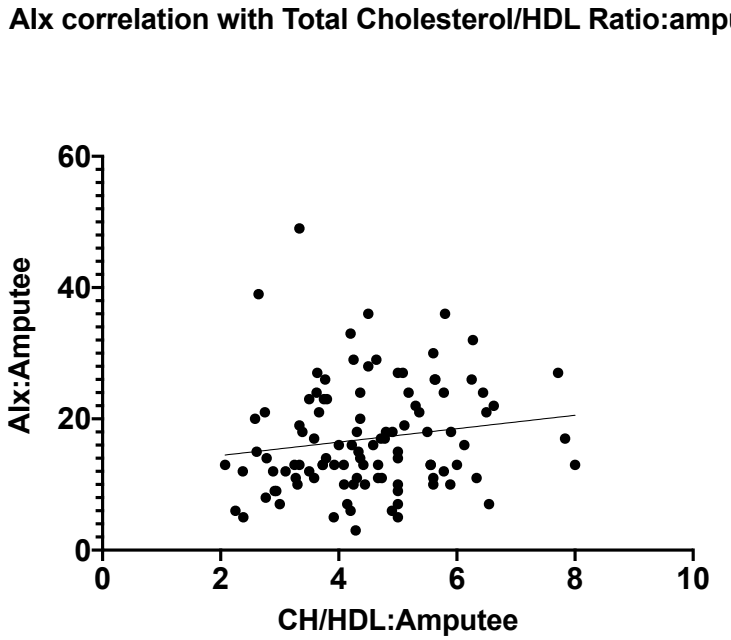


Figure 4-32: Central augmentation index correlating with Total Cholesterol/HDL Ratio in the CRTI-A group (r = 0.21, CI 0.01 to 0.39; p= 0.04)

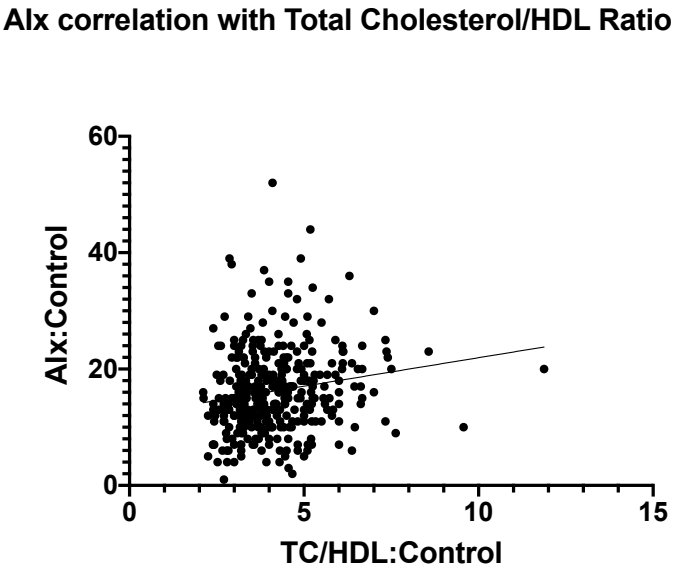


Figure 4-33: Central augmentation index correlating with Total Cholesterol/HDL Ratio in the control group (r = 0.21, CI 0.10 to 0.30; p <0.001)

4.7.2 **Haemodynamic characteristics**

(a) SEVR and Resting heart rate (RHR)

There is an inverse correlation between SEVR and Resting Heart Rate and statistically more significant in the control group compared to the CRTI-A group (r = -0.68 and r = -0.60 respectively); p<0.001. (Table 4-5, Fig 4-34 and Fig 4-35)

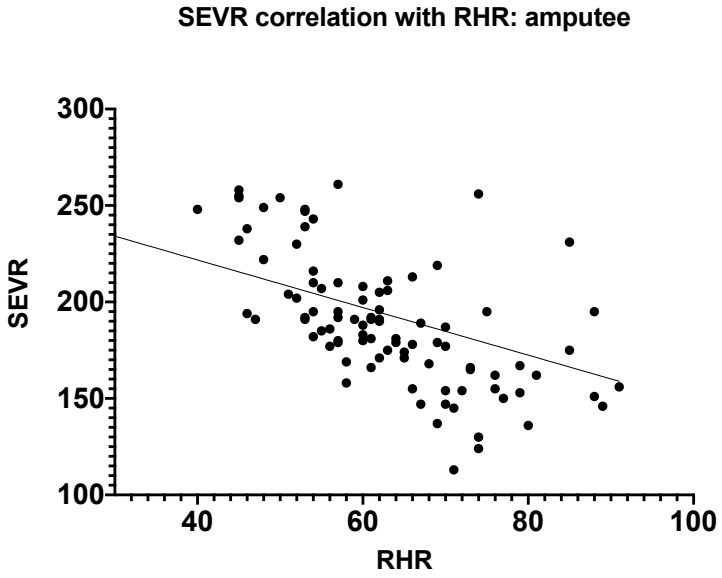


Figure 4-34: Correlation between SEVR and Resting heart rate in the CRTI-A group (r = -0.60, CI -0.72 to -0.46; p<0.001)

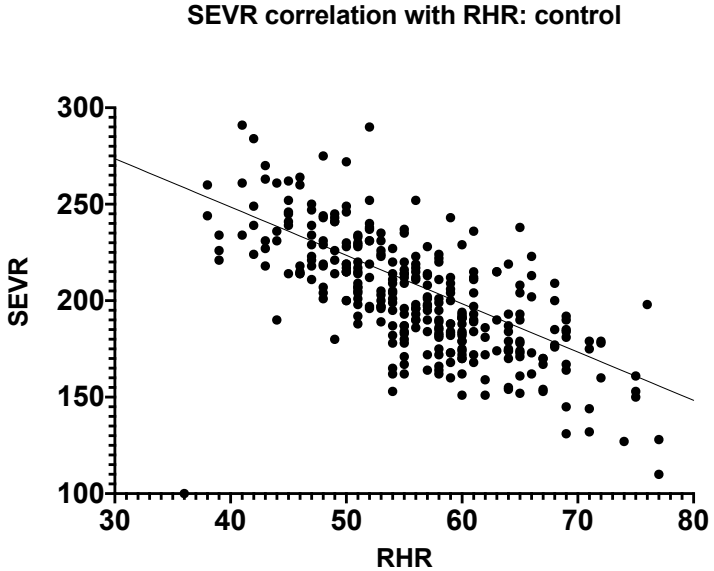


Figure 4-35: Correlation between SEVR and Resting heart rate in the control group (r = -0.68, CI -0.74 to -0.62; p<0.001)

4.7.3 **Arterial stiffness indices and anthropometric CVD risk markers**

(a) AIx and weight-to-height ratio (WHtR)

There is a positive and statistically significant correlation between peripheral augmentation index (AI) and Waist-to-height ratio (WHtR) (Fig4-36) with a stronger correlation revealed in the CRTI-A group compared to control (Fig 4-37 and Fig 4-37).

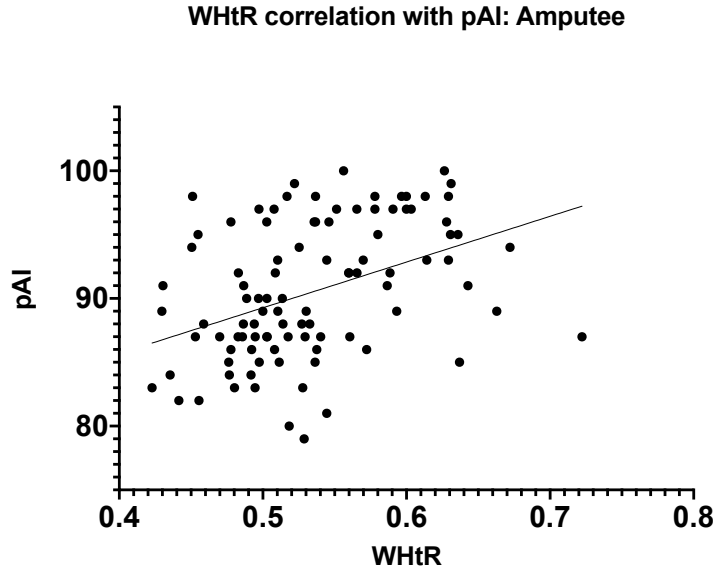


Figure 4-36: Correlation between AIx and WHtR in the CRTI-A group (r= 0.4, CI 0.22 to 0.56; p<0.001)

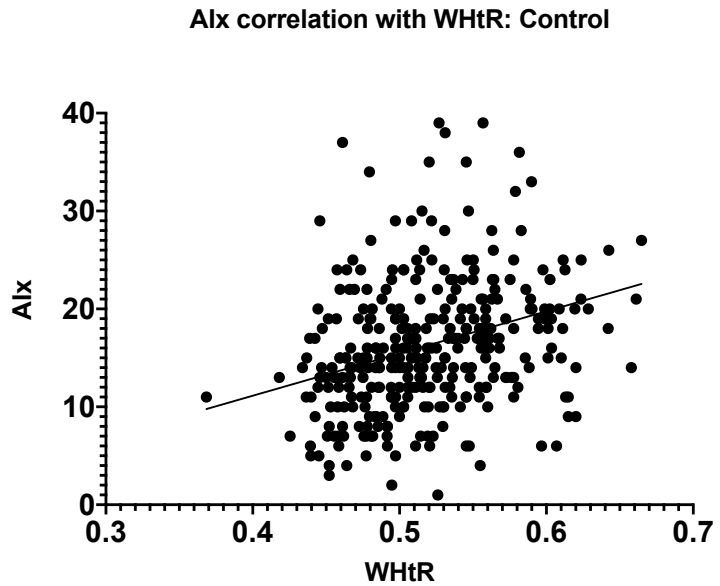


Figure 4-37: Correlation between AIx and WHtR in the control group (r= 0.34, CI 0.23 to 0.43); p<0.001

(b) PWV and WHtR

There is a weak positive correlation between PWV and WHtR in the CRTI-A group, which is more positive than in the control group (r = 0.15 and r = -0.007 respectively).

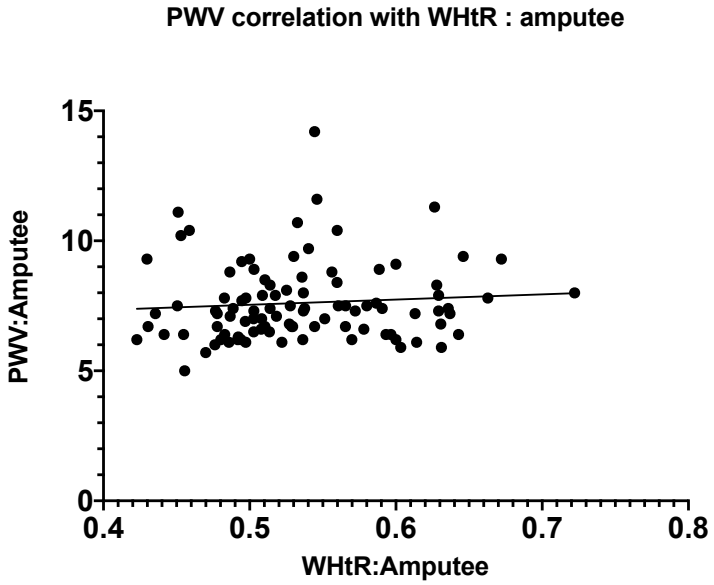


Figure 4-38: PWV correlation with WHtR (r = 0.15, CI -0.06 to 0.34); p = 0.15

(c) AIx and Conicity Index

There is a weak positive correlation between AIx and Conicity Index. This correlation is statistically significant (p<0.001). (Fig 4-39 and Fig 4-40)

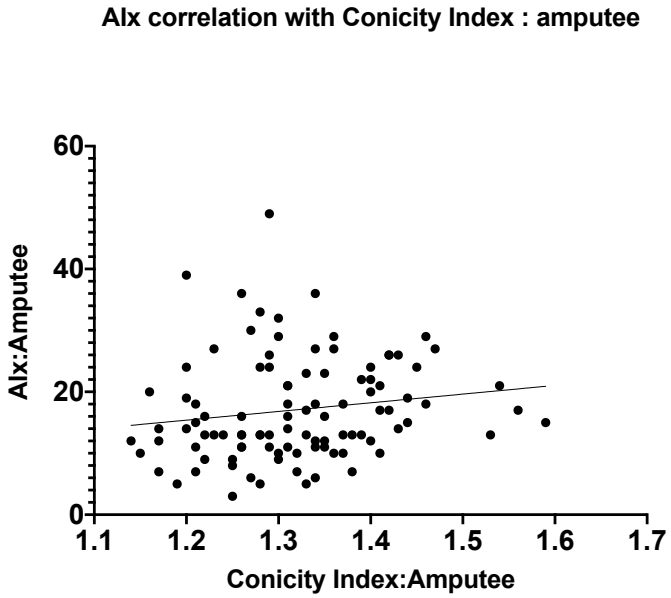


Figure 4-39: Correlation between AIx and Conicity Index in the CRTI-A group (r= 0.24, CI 0.04 to 0.42); p<0.001

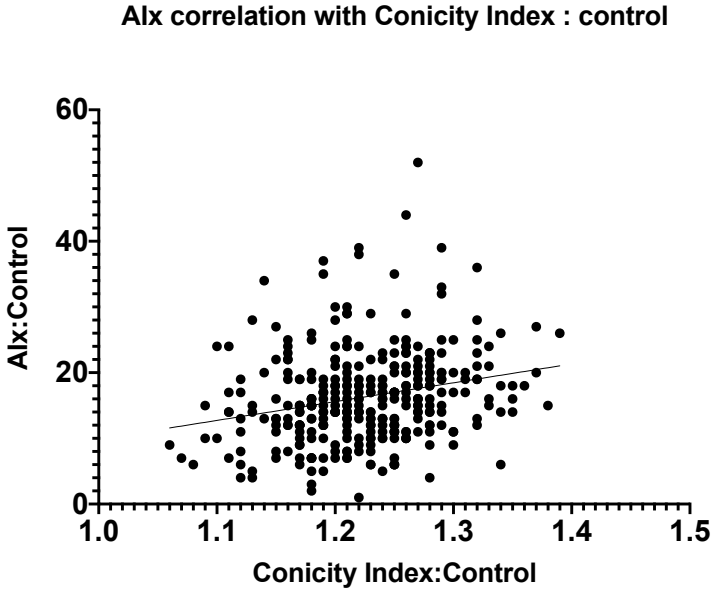


Figure 4-40: Correlation between AIx and Conicity Index in the control group (r= 0.26, CI 0.15 to 0.36); p<0.001

4.7.4 Other correlations

(a) Time elapsed between injury and examination

There were no significant correlations between the period in time from injury to examination and any of the examined parameters.

(b) NISS

There was a moderate correlation between NISS, weight, time to examination and resting heart rate (Fig 4-42).

Spearman correlation analysis was applied to determine the strength of the relationships between the CVD risk factors in relation to the study cohorts. The results were listed in Table 4-5 and the correlation studies were illustrated in Figure 4-41.

Table 4-5: Correlation analysis within the groups

Correlation study		**Control** xy pairs 394	**CRTI-NA** xy pairs 200	**CRTI-A** xy pairs 105
PWV/Hs-CRP	r	0.01	-0.09	-0.03
	CI (95%)	-0.11 to 0.12	-0.25 to 0.07	-0.23 to 0.18
	p	0.93	0.23	0.79
	Significance	ns	ns	ns
AIx/Hs-CRP	r	0.20	0.15	0.23
	CI (95%)	0.09 to 0.30	-0.0004 to 0.29	0.03 to 0.41
	p	<0.001	.04	0.02
	Significance	***	*	*
RHR / SEVR	r	-0.68	-0.73	-0.60
	CI (95%)	-0.74	-0.79 to -0.66	-0.72 to -0.46
	p	<0.001	<0.001	<0.001
	Significance	***	***	***
WHtR/PWV	r	-0.007	-0.09	0.15
	CI (95%)	-0.012 to 0.11	-0.25 to 0.07	-0.06 to 0.34
	p	0.91	0.25	0.15
	Significance	ns	ns	ns
WHtR / pAI	r	0.36	0.42	0.43
	CI (95%)	0.26 to 0.45	0.29 to 0.54	0.25 to 0.58
	p	<0.001	<0.001	<0.001
	Significance	***	***	***
WHtR / AIx	r	0.34	0.41	0.40
	CI (95%)	0.23 to 0.43	0.28 to 0.53	0.23 to 0.56
	p	<0.001	<0.001	<0.001
	Significance	***	***	***
WHtR / TC/HDL	r	0.42	0.40	0.41
	CI (95%)	0.32 to 0.50	0.26 to 0.52	0.22 to 0.56
	p	<0.001	<0.001	<0.001
	Significance	***	***	***
WHtR / TG	r	0.43	0.33	0.41
	CI (95%)	0.34 to 0.52	0.19 to 0.46	0.22 to 0.56
	p	<0.001	<0.001	<0.001
	Significance	***	***	***
WHtR / Hs-CRP	r	0.39	0.40	0.52
	CI (95%)	0.30 to 0.48	0.26 to 0.52	0.36 to 0.66
	p	<0.001	<0.001	<0.001
	Significance	***	***	***

P value summary (significance): **** = p<0.0001; *** = p<0.001; ** = p<0.01; * = p<0.1; ns = non-significant

Significant correlations between the CVD risk factors are grouped together for comparison in the groups (Spearman coefficient "r" is added for comparison)

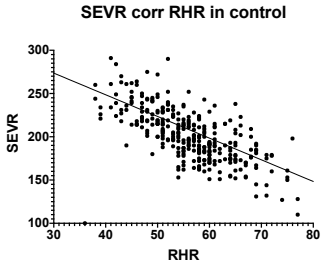
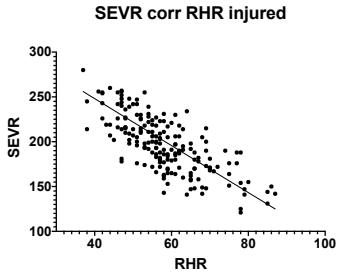
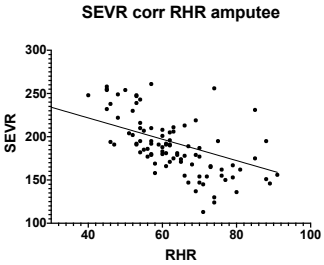
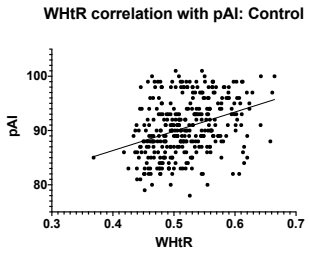
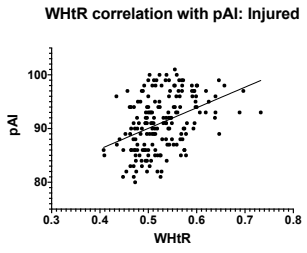
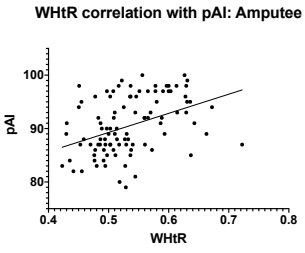
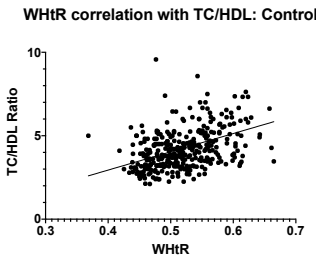
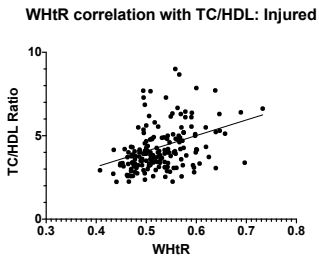
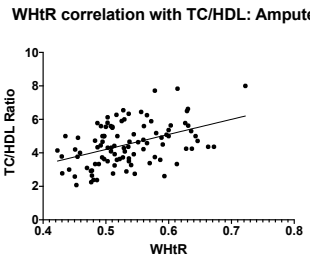
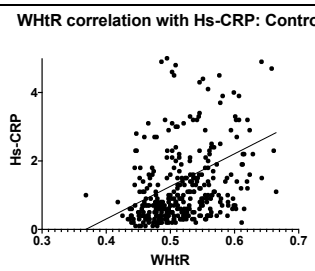
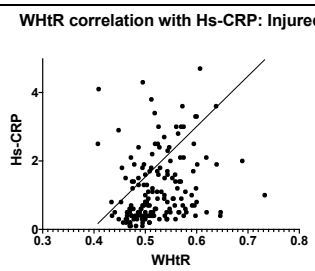
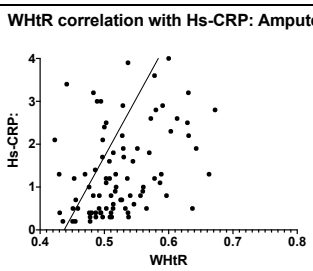
		
r = -0.6835	r = -0.7320	r = -0.6044
		
r = 0.3573	r = 0.4188	r = 0.4315
		
r = 0.4166	r = 0.3948	r = 0.4050
		
r = 0.3928	r = 0.3975	r = 0.5218

Figure 4-41: Correlation analysis within groups

	W	WHtR	LDL	TG	TC/HDL	HSCRP	aPP	aSBP	aDBP	AIx	CO	SEVR	AI	CI
H	0.46	-0.19	-0.05	-0.06	-0.04	-0.05	-0.03	0.01	0.01	-0.06	0.01	-0.03	-0.07	0.15
HC	0.76	0.67	0.13	0.25	0.29	0.3	0.16	0.21	0.24	0.28	0.06	-0.12	0.28	0.72
WHR	0.36	0.67	0.16	0.29	0.28	0.23	0.12	0.22	0.25	0.23	0.08	-0.13	0.24	0.68
W		0.6	0.11	0.25	0.26	0.25	0.17	0.25	0.25	0.22	0.06	-0.1	0.23	0.62
WHtR			0.21	0.4	0.42	0.42	0.2	0.3	0.34	0.38	0.09	-0.16	0.39	0.9
T1			0.13	-0.02	0.02	-0.04	0.1	0.11	0.09	0.06	-0.06	0.04	0.07	-0.01
HB			0.03	0.21	0.13	-0.04	0.02	0.09	0.12	0.02	0.11	-0.14	0.01	0.15
WBC			-0.06	0.23	0.16	0.31	0.14	0.17	0.13	0.11	0.26	-0.24	0.1	0.26
TC			0.93	0.45	0.58	-0.00322	0.03	0.15	0.22	0.13	-0.02	-0.07	0.13	0.18
HDL			-0.09	-0.37	-0.72	-0.23	-0.05	-0.02	-0.07	-0.17	-0.07	0.1	-0.16	-0.31
LDL				0.3	0.69	0.01	0.03	0.12	0.17	0.12	-0.02	-0.04	0.12	0.19
TG					0.61	0.14	0.05	0.18	0.27	0.21	0.09	-0.15	0.2	0.35
TC/HDL						0.2	0.04	0.11	0.21	0.22	0.05	-0.13	0.2	0.39
GL						0.06	0.13	0.16	0.17	0.2	0.06	-0.11	0.22	0.23
HBA1C						0.13	0.03	0.07	0.11	0.13	-0.03	-0.01	0.13	0.08
CREAT						-0.08	0.03	0.000149	-0.04	0.05	-0.15	0.17	0.03	-0.08
HSCRP							0.12	0.14	0.14	0.18	0.11	-0.13	0.18	0.39
RHR							0.02	0.15	0.21	0.1	0.46	-0.69	0.09	0.28
PP							0.9	0.63	-0.11	-0.08	0.62	-0.11	-0.07	0.02
aPP								0.63	-0.02	0.32	0.55	-0.09	0.34	0.15
aSBP									0.66	0.09	0.45	-0.13	0.11	0.26
aDBP										0.19	0.01	-0.06	0.22	0.33
AIx											-0.07	0.01	0.95	0.33
CO												-0.67	-0.05	0.09
PWV												-0.01	0.07	0.06
SEVR													0.02	-0.19
AI														0.35
HR														0.000866
NISS														0.17
CI														

H: height, **HC:** hip circumference, **WHR:** waist to hip ratio, **W:** weight, **WHtR:** waist to height ratio, **HB:** haemoglobin, **WBC:** white blood cell count, **TC:** total cholesterol, **HDL:** high density lipoprotein cholesterol, **LDL:** low density lipoprotein cholesterol, **TG:** triglycerides, **TC/HDL:** TC/HDL ratio, **GL:** glucose, **HbA1C:** haemoglobin A1C test, **CR:** creatinine, **HsCRP:** high-sensitivity C-reactive protein, **RHR:** resting heart rate, **PP:** pulse pressure, APP; aortic pulse pressure, aSBP: aortic systolic blood pressure, **aDBP:** aortic diastolic blood pressure, **Aix:** central augmentation index, **CO:** cardiac output, **PWV:** pulse wave velocity, **SEVR:** subendocardial viability ratio, **AI:** peripheral augmentation index, **HR:** heart rate, **NISS:** new injury severity score, **CI:** conicity index (* $p < 0.05$)

Significant | Moderate | Negative

Figure 4-42: Correlation matrix with Spearman's Rank Correlation Coefficient Rs and Probability (p, two-tailed) Value Calculator

Chapter 5
Discussion

ADVANCE is the first observational prospective study aimed at examining the effects of combat related traumatic injuries sustained by military personnel on medium and long-term cardiovascular health. A growing amount of evidence suggests that there is a substantial reduction in cardiovascular related morbidity and mortality in the general population attributed to improved treatment of cardiac risk factors and CVD (Scrinivasan, 2017). The aim of this thesis was to analyse the baseline data and specifically assess indices such as PWV and augmentation index reflecting on arterial stiffness. We compared a profile of traditional CVD risk factors for prediction of cardiovascular risk in a cohort of combat-related traumatically injured military personnel of which some have suffered amputations. Our hypotheses are that traumatic limb amputation is associated with a greater burden of cardiovascular risk factors than in a frequency matched population of non-injured groups, exposed to the same role in the same operational environment at the same time.

During my visits to Headley Court, I had the opportunity to meet some of the injured soldiers where they shared experiences with me. I learned how they coped with life after the traumatic injuries and how they went about rehabilitation. It helped me create a holistic view of issues they face and factors affecting their health and wellbeing. I wanted to learn how CVD risk profiles could be affected.

5.1 Haemodynamic indices including augmentation index and pulse wave velocity

5.1.1 Pulse wave velocity (PWV)

World Health Organisations estimated in 2010 that CVDs are the leading contributions to mortality in developed countries and the developing world (Shirwany and Zou, 2010). A growing number of studies have demonstrated the association between arterial stiffness and stroke (Tuttolomondo et al., 2016, Yoon et al., 2013). As conventional risk factors cannot fully account for the pathogenesis, it is essential to detect biomarkers of artery injury for an appropriate intervention. We were investigating whether an amputation would alter the compliance and distensibility of affected arteries thereby contributing to CVD risk (Shirwany

and Zou, 2010). To our knowledge, this is the first study to compare measures of arterial stiffness amongst traumatic amputees with frequency matched non-injured military personnel.

As for any hydrodynamic circuit, waves generated by the intermittent function of the heart travels down the aorta, arteries, etc. are reflected at reflection sites, generating waves that travel back to the heart (Salvi et al., 2008). Under normal circumstances, reflection sites would be bifurcations or narrowing of the arteries. The close circuit characteristic of the arterial system, the small size of arteries and the fast speed of pressure waves (4-30m/s) causes the backward wave to superimpose on the same forward wave (Salvi et al., 2008). As a consequence, arterial blood pressure results from the sum of the forward and backward pressure waves. Therefore, we expected that an amputated artery would amplify the reflected wave more so than a bifurcation or narrowing of the artery diameter. It was further expected that a lower limb amputation would affect the distensibility of arteries resulting in a change in PWV indicative of a change in arterial stiffness also expressed as augmentation index (Mattace-Raso, 2010, Shirwany and Zou, 2010).

The association between stiffening of arteries in the larger central arterial system, such as the aortic tree, and CVD is that it significantly contributes to cardiovascular diseases and is positively associated with systolic hypertension, coronary artery disease, stroke, heart failure and atrial fibrillation (Shirwany and Zou, 2010).

The simplest, most robust, reproducible and non-invasive method of detecting regional arterial stiffness is by measuring pulse wave velocity (PWV), also known as pressure wave velocity. The fundamental principal of mechanism is that the pressure wave travels faster in a stiffer artery (Chen et al., 2017).

In this study there were no statistically significant differences in arterial stiffness as measured by PWV (Table 4-1, Fig 4-9) between the three groups. This finding does not support the increase in arterial stiffness Malgalhães and colleagues reported when they investigated arterial stiffness with male veterans from the Angolan war (n=146) with lower limb amputations and compared them with age-matched civilian non-amputees (Magalhaes et al., 2011). It was anticipated that the CRTI-A group would have higher PWV and hence relatively greater arterial stiffness than the CRTI-NA and control groups (Magalhaes et al., 2011, Labouret et al., 1983).

An explanation for the lack of increased PWV following proximal amputation lies in a study by Baksi et al who confirmed in a recent study that reflected waves formed at occlusion of an artery are dissipated and barely discernible >40cm from the occlusion site in healthy large

arteries (Baksi et al., 2016). Our results confirm therefore that traumatic limb amputations are not at this early stage associated with an increase in large arterial stiffness as measured by PWV.

Our results, contradictory to previous studies, could be attributed to the relatively young age 33.07 years (± 4.91 years); p=0.20 of the CRTI-A group and relatively recent injuries (6.5-7 years; time last deployment to examination; (Table 4-1) (Mitchell et al., 2004, Edwards and Lang, 2005). Magalhaes et al compared CRTI-A, older subjects than our cohorts, 48.1 years (± 6.3 years) with a control where an increase in PWV was recorded with control 9.9 m/sec (± 1.8 m/sec) vs CRTI-A 10.8 m/sec (± 1.9 m/sec); p=0.01.

5.1.2 Augmentation Index (AIx)

Upon cardiac systole, a forward propagated wave travels down the aorta forming a reflected wave when mismatched impedance is encountered (Shirwany and Zou, 2010). The reflected wave summates with the forward wave, increasing pulse pressure. This increase in pressure, also known as augmented pressure and expressed as augmentation index (AIx) is associated with the physical state and the distensibility of the arterial system (Shirwany and Zou, 2010). Mitigating arterial stiffness may lower the burden of target organ damage and, in turn, clinical CVD (Vasan et al., 2019).

AIx is affected by the magnitude of the reflected wave rather than the velocity (Shirwany and Zou, 2010). The vascular structure, function and BP are the major components in arterial stiffness. Arterial stiffness is also induced short-term or long-term by inflammation, oxidative stress, renin-angiotensin-aldosterone system and genetic factors (Lacolley et al., 2009, Liao et al., 2015). Diastolic pressure and the distance from the site of reflection to the heart influences the AIx indirectly (Laurent et al., 2006).

An expected resulting increased reflected wave at the point of femoral arterial flow interruption lies in the confirmation of the theory linking an increased reflected wave with increased augmentation index (Naschitz and Lenger, 2008). The results did however not support this theory. There were no statistically significant differences between the peripheral and central augmentation indexes between the three groups (Table 4-1, Figures 4-6,7).

An alternative explanation for the lack of variance in AIx between the groups could be attributed to the level of fitness of military personnel and the resulting effect on arterial stiffness due to the known effect of physical exercise induced availability of nitric oxide (NO).

Physical exercise and specifically aerobic training has been noted to be an effective intervention in the treatment and prevention of hypertension and CVD due to increased arterial stiffness via reduction in oxidative stress of the endothelium and endothelial NO production (Korsager Larsen and Matchkov, 2016, Li et al., 2014).

However, Volmar et al observed an asymmetrical flow pattern after leg amputations at the aortic bifurcation developing damage to the aorta at a later stage resulting in aneurysms (Vollmar et al., 1989). This observation confirms a change in reflected wave flow possibly linked to later stage increase in arterial stiffness and needs further investigation at later stages following traumatic injuries.

5.1.3 Resting heart rate

Additional features of increased cardiovascular risk profile included higher resting heart rates with CRTI-A significantly higher than control and CRTI-NA; $p<0.0001$, (Table 4-1, Fig 4-5). A resting heart rate of > 65 beats/min has been shown to be a strong independent predictor of premature mortality and stroke, but a lesser effect on CVD (Woodward et al., 2014, Hillis et al., 2012).

5.1.4 Subendocardial viability ratio (SEVR)

Parameters characterising SEVR are the systolic pressure-time index (SPTI: reflecting the cardiac workload) and the diastolic pressure-time index (DPTI: reflecting the subendocardial flow supply). DTPI:SPTI ratio represents the subendocardial oxygen supply-demand ratio. An increase in arterial stiffness generates an increase in ventricular afterload and a reduction in oxygen supply to the myocardium, which explains the association between aortic stiffness cardiovascular mortality and morbidity (Salvi et al., 2008).

SEVR, (Table 4-1, Fig4-7), an index of myocardial oxygen supply and demand calculated through pulse wave analysis and associated with vascular compliance, was significantly lower with CRTI-A compared to healthy controls and supports earlier study outcomes (Tsiachris et al., 2012, Sandoo et al., 2012).

Although statistically non-significant (possibly due to our sample size), a lower SEVR observed with the bilateral distal 181.20 (± 36.27) and bilateral proximal 189.20 (± 44.23) CRTI-A groups compared to the control group 206.34 (± 46.44) constitutes a novel finding relative to injury severity (Table 4-5, Fig 4-23). SEVR is a new and useful CVD risk marker as it is an indirect marker of coronary flow reserve (Tsiachris et al., 2012). Reduced SEVR

has been linked to an increased risk of adverse CVD outcomes (Ekart et al., 2016, Tagawa et al., 2018). SEVR is potentially an early cardiovascular risk marker as it can be affected by even short-term functional changes in coronary microcirculation (Doonan et al., 2011).

Coronary flow reserve and SEVR are reduced by hypertension, poor diastolic function, chronic inflammation, increased left ventricular mass, endothelial dysfunction and the presence of CVD and its associated risk factors such as diabetes and metabolic syndrome (Pirat et al., 2008, Sandoo et al., 2012).

Possible reasons for lower SEVR are supported by our comparative data reflecting on injury severity, which include increased HS-CRP levels with Bilateral proximal amputations (Table 4-5, Fig 4-20) and increased resting heart rate (Table 4-5, Fig 4-22) reflecting on systemic inflammation and an increased risk of arterial sheer stress (Sandoo et al., 2012, Tsiachris et al., 2012). The statistically significant correlation between SEVR and resting heart rate ($r = -0.60$; $p<0.001$, Fig 4-34) confirms the significance of SEVR as an early marker for CVD risk.

5.1.5 Brachial and Central BP, pulse pressure

Brachial Systolic and diastolic pressures did not vary significantly between the groups (Table 4-1). However, aortic pulse pressure and stroke volume were significantly lower with the CRTI-A group compared to the control; $p<0.01$, (Table 4-1, Fig 4-6). Reduced pulse pressure and stroke volume may be associated with poorer heart function, an outcome which is further supported by the reported reduced SEVR (Fig 4-7) and related cardiac perfusion in the CRTI-A group. Aortic pulse pressure is a marginally better predictor of CVD than brachial pulse pressure and is associated with multiple adverse cardiovascular outcomes and provides a prognostic utility beyond that of mean arterial pressure (Aznaouridis et al., 2016, Selvaraj et al., 2016). A raised aortic pulse pressure is normally associated with atherosclerosis and increased risk of CVD, however a lower aortic pulse pressure on the other hand is not necessarily reassuring and indicative of poor cardiac perfusion, low stroke volume and heart failure (Selvaraj et al., 2016).

5.2 Biochemical indices

5.2.1 Hs-CRP

C-reactive protein (CRP) is a non-specific biomarker of inflammation, which is an important step in the generation of atherosclerosis and the development of unstable plaque (Wilson et al., 2006). Application of high sensitivity assays (Hs-CRP) can demonstrate subclinical inflammatory states, which may indicate vascular inflammation. CRP, found in human atherosclerotic plaque, has been shown to cause endothelial cell dysfunction, oxidative stress and intimal hypertrophy in experimental models (Wilson et al., 2006). Hs-CRP is regarded as a non-traditional risk factor which may be included to improve the performance of traditional multivariable risk assessments for CVD (Lin et al., 2018). Clinical studies have shown that elevated Hs-CRP levels in healthy populations predict vascular events such as myocardial infarction (MI), stroke and diabetes development. In patients with acute coronary syndromes, higher Hs-CRP levels are associated with adverse outcomes and subsequent vascular events (Wilson et al., 2006). In vitro studies suggested an association between CRP, inhibition of endothelial nitric oxide synthase and impaired vasoreactivity (Jialal et al., 2009). We would therefore investigate if there is a statistically significant association between Hs-CRP and AIx in our study cohorts.

A systematic review in 2018 concluded that there were insufficient adequately powered clinical trials which evaluated the incremental effect of Hs-CRP in CVD risk assessment to initiate preventative therapy (Lin et al., 2018). However, Hs-CRP levels were measured to assess the association between Hs-CRP and inflammation linked with traumatic injury and amputations.

Hs-CRP levels were significantly higher in the CRTI-A group compared to control (Table 4-2, Fig 4-14), supporting the concept of elevated Hs-CRP with low grade systemic inflammation and greater risk of arterial sheer stress (Park et al., 2019) and independent prediction of CV mortality (Li et al., 2017). Elevated Hs-CRP levels associated with the severity of injury as found in the comparison proximal vs distal CRTI-A vs the control group are consistent with findings by Ejtahed et al observing increased chronic inflammation in the bilateral lower limb CRTI-A group (Table 4-5, Fig 4-23) (Ejtahed et al., 2017).

5.2.2 The effect of Hs-CRP on NO Synthase

Hs-CRP has been found to inhibit endothelial nitric oxide synthase (eNOS) activity and bioactivity (Jialal et al., 2009). The importance of this clinical implication is that inflammation linked to traumatic injury and amputation increases CRP production, which inhibits nitric oxide (NO) formation, inducing endothelial dysfunction (Wilson et al., 2006). NO inhibition increases arterial stiffness, which increases the risk of hypertension and affects SEVR (Jialal et al., 2009).

5.2.3 Creatinine

An increase in creatine (or decrease in GFR) is associated with reduced kidney function and increased CVD risk, even larger than that associated with traditional risk predictors (Smith et al., 2003, Fácila et al., 2006). This marker is immediately and readily obtained, and available to for example all hospitals, should it be required for a CVD risk profile. However patients with amputations have significantly lower serum creatinine values compared to the general population (Im et al., 2012).

A study evaluating the effect and the extent of amputations on creatinine concluded that clinicians should interpret creatinine levels for CRTI-A with caution (Thurlow et al., 2014). Our findings confirm this conclusion, and it is notable that creatinine levels were lower among the CRTI-A (Table 4-2, Fig 4-15; $p<0.0001$). Serum Cystatin C levels were found to be more useful in estimating GFR than serum Creatinine following amputations (Thurlow et al., 2014). The falsely lower creatinine is likely explained by less production in creatinine by lower body mass in the CRTI-A following limb loss (Im et al., 2012). This is a novel finding that has received little attention in the literature, but has been described following polytrauma (Minville V, 2011). The mechanisms to explain this observation are uncertain and those cited include enhanced urinary creatinine excretion and chronic inflammation (Minville V, 2011). Conclusion: Creatinine-based estimators of GFR may overestimate renal function in the setting of traumatic amputation (Im et al., 2012).

5.2.4 Total Chol/HDL ratio

Total Chol/HDL ratio was notably higher (Table 4-2, Fig 4-13; $p = 0.01$) with the CRTI-A group compared to the CRTI-NA and control groups and HDL cholesterol was significantly lower in the CRTI-A group (Table 4-2, Fig 4-12; $p<0.0001$), adding to the increased CVD risk profile.

Dyslipidemia is the elevation of cholesterol, triglycerides (TC), or both, or a low HDL cholesterol level that contributes to the development of atherosclerosis (Lemieux et al., 2001). Dyslipidemias are caused by primary (genetic) or secondary causes such as lifestyle and other factors (Goldberg, 2018). The most important secondary causes are a sedentary lifestyle, excessive dietary intake of saturated fat, cholesterol and trans fats. Other secondary causes are diabetes mellitus, alcohol overuse, chronic kidney disease, hypothyroidism, primary biliary cirrhosis and drugs such as thiazides, beta-blockers, retinoids, cyclosporine, etc. (Goldberg, 2018).

Patients with hypercholesterolaemia have a higher central pulse pressure and stiffer blood vessels than matched controls, despite similar peripheral blood pressures (Wilkinson and Cockcroft, 2007). These haemodynamic changes may contribute to the increased risk of cardiovascular disease associated with hypercholesterolaemia (Wilkinson and Cockcroft, 2007). Dyslipidemia itself usually causes no symptoms but can lead to symptomatic vascular disease, including coronary artery disease, stroke, and peripheral arterial disease (Goldberg, 2018). The total cholesterol value is divided by the HDL number to determine the ratio. According to the Framingham Heart Study, a cholesterol ratio of 5 indicates an average risk of heart disease for men with risk doubled when the ratio reaches 9.6 or the risk is halved with a ratio of 3.4 (Lemieux et al., 2001). This study concluded that a variation in the TC/HDL ratio may be more associated with a predictive ischemic heart disease risk due to substantial alterations in metabolic indices and insulin resistance than a variation in the LDL/HDL ratio (Lemieux et al., 2001).

Another systematic review challenged the validity of the hypothesis that cholesterol is atherogenic and found that elderly patients with high LDL cholesterol live as long or longer than those with low LDL cholesterol (Ravnskov et al., 2016). This finding challenges the use of lipid profiles as a CVD risk indicator. In our study there was no statistically significant difference in the LDL values between the three groups.

5.2.5 Glycaemic index

Markers of insulin resistance are waist circumference and fasting insulin levels. Increased basal insulin resistance beyond a threshold, strongly predicts adverse cardiac events at 1 year in type 2 diabetes mellitus (Scrinivasan, 2017). Those below the threshold, seems to be at similar risk to non-diabetics (Scrinivasan, 2017). Glucose ($p=0.54$) and HbA1C ($p=0.06$) values were not statistically different between all the groups (Table 4-2). Insulin levels were not measured in this study due to practical implications at times of data gathering. It has to be noted for future consideration that increased insulin resistance is strongly related to chronic

vascular inflammation (Vlachopoulos et al., 2010, Dregan, 2018) and has been described as an important aetiological factor explaining the increased CVD risk found with lower limb CRTI-A (Peles et al., 1995, Rose et al., 1986, Naschitz and Lenger, 2008). Peles et al observed higher mean fasting plasma insulin levels during an oral glucose tolerance test with traumatic amputees (n=53) compared with matched controls (n=53), while body mass index, blood pressure and lipid profiles were similar (Peles et al., 1995). Rose et al observed increased insulin levels and blood pressure among above knee CRTI-A (n=9) versus below-elbow normotensive (n=12) CRTI-A (Rose et al., 1987).

5.3 Anthropometric indices affecting the CVD risk profile

Obesity

Obesity is among the leading causes of elevated cardiovascular disease (CVD) mortality and morbidity (Akil and Ahmad, 2011, Motamed et al., 2015).

5.3.1 Visceral fat

The results of this study showed that an increase in visceral fat, as measured by weight, waist circumference, WHtR, BMI and conicity index, was significantly associated with the CRTI-A group compared to control.

5.3.2 Weight increase

The significant association of weight increase with amputation underpins the suggestion that CRTI-A has an impact on the CVD risk profile.

Weight gain, represented by WHtR, correlates significantly with aortic systolic and diastolic BP, triglycerides, lipid profile, Hs-CRP, AI, resting heart rate and SEVR (Table 4-5) confirming the importance of visceral fat in the development of CVD risk factors. BMI is not appropriate to be applied in this context of CVD risk as weight is reduced due to limb loss and the calculation is not corrected as such. The healthy physical nature of most of the participants with increased muscle weight adds to the inappropriateness of applying BMI in our observations.

Visceral fat is related to arterial stiffness independent of traditional risk factors and indicative of cardiovascular risk (Corrigan et al., 2017, Shahriar et al., 2009). There was a statistically strong significant difference in Conicity Index (CI) between the CRTI-A group in comparison to the healthy control and CRTI-NA groups ($P<0.0001$, Table 4-3). Waist circumference

(P=0.01, Table 4-3) and WHtR (p=0.03, Table 4-3) were significantly different but not statistically as strong as CI. However, in correlation studies, WHtR demonstrated stronger correlations than CI with CVD risk indices. These findings are consistent with the literature with risk factors including hyperglycaemia 13.1%, systolic hypertension 18.9%, diastolic hypertension 25.6%, abdominal obesity 82.5%, high total cholesterol 36.7%, low HDL 25.9%, high LDL 24.7%, high triglycerides 32.1%, and smoking 31.8%. The most common risk factor was abdominal obesity (Shahriar et al., 2009). However, Shariar and colleagues conducted their research 22.3years (± 3.9 years) after amputation while the study by Rose et al made their observations 14-15 years after amputation compared to the seven years for our study cohorts (Shahriar et al., 2009, Rose et al., 1987). Development of metabolic syndrome as CVD risk, which includes lipid profile, was observed by Ejtahed et al 31.5 years after amputation (Ejtahed et al., 2017). Relatively early observation of the development of CVD risk factors in our study cohorts confirms the findings of the presence of these risk factors in earlier studies.

5.4 The importance of injury severity on the CVD risk profile

The New Injury Severity Score (NISS) (Osler et al., 1997) as provided by the UK Joint Theatre Trauma Registry (JTTR) reflects a statistically significant greater value for the CRTI-A group 36.75 (± 18.01) in comparison to the CRTI-NA (CRTI-NA) group 20.11 (± 17.99); p<0.0001 (Smith et al., 2007), (Table 4-3, Fig 4-21).

The proximal lower limb CRTI-A group consistently demonstrated an increased cardiovascular risk profile in comparison to the distal CRTI-A (bilateral more so than unilateral), the CRTI-NA and the healthy control group, thereby revealing a consistent correlation between increased subclinical risk profiles and level of injury severity. (Table 4-5, Fig 4-22 to 4-27). This finding is consistent with literature reporting that each 5-point increase in NISS was associated with a 6% increase in the incidence of hypertension, 13% increase in coronary artery disease, 13% increase in diabetes mellitus and 15% increase in chronic kidney disease (Stewart et al., 2015). Table 4-5 lists the significant differences between the level of amputation (injury severity) and the healthy control group. HDL cholesterol (Fig 4-22) had significantly lower values for proximal amputations compared to the control group (p<0.01). Hs-CRP values (Fig 4-23) were weakly significantly higher in the bilateral proximal CRTI-A group compared to the control group (p<0.1) indicating increased inflammation. Creatinine values (Fig 4-24) were strongly significantly lower in the proximal CRTI-A groups compared to the control group, consistent with the loss of muscle mass (P<0.0001). Resting heart rate (Fig 4-25) was significantly higher in the bilateral proximal group, consistent with the increase premature mortality and stroke risk (p<0.001) (Woodward

et al., 2014). SEVR values (Fig 4-26) were lower but not statistically significant compared to the control group (ns). Stroke volume values (Fig 4-27) were significantly lower in the distal and proximal CRTI-A groups compared to control ($p<0.01$). These results confirm the increased CVD risk associated with severity of injury and particularly in the case of proximal amputations.

5.5 Correlation studies

We applied Spearman analysis (p, two tailed) to analyse associations between the CVD risk parameters (Correlation matrix: Fig 4-42) The most significant correlations were illustrated in Fig 4-41.

The statistically most significant correlations involved SEVR and waist-to-height ratio.

We expected PWV to have a significant relationship with Hs-CRP due to the NO inhibiting role of CRP. There was however no significant association between Hs-CRP and PWV in the CRTI-A.

Augmentation index (AIx) showed a weak positive correlation with Hs-CRP in the CRTI-A group more so than the control group. This finding confirms the NO inhibitive effect of CRP and confirms that AIx is a more sensitive parameter than PWV as CVD risk indicator as far as arterial stiffness is concerned.

AIx showed a weak positive correlation with TC/HDL ratio in the CRTI-A group. There was however no difference in correlation compared with the control group. The increase in CVD risk associated with TC/HDL ratio correlates with the increase in CVD risk associated with AIx, however the correlation in the CRTI-A group was not more significant than in the control group.

The linear association between SEVR and resting heart rate showed the strongest, albeit negative significant relationship in the CRTI-A group compared to the control group. This finding confirms the increased levels of CVD risk as reflected by both SEVR and resting heart rate respectively measured in the CRTI-A group and compared with the control group.

Obesity or increase in visceral fat showed the strongest correlation with haemodynamic CVD risk markers relating to arterial stiffness. Of the anthropometric risk markers, we have reviewed, WHtR had the strongest correlations with other CVD risk markers. WHtR had a

non-significant relationship with PWV although the correlation was marginally stronger in the CRTI-A group compared to the control group. There is a stronger positive correlation between WHtR and AIx in the CRTI-A group compared to the control group. Conicity Index showed a weaker correlation with AIx in the CRTI-A group compared to the control group.

WHtR had a positive correlation with lipid profiles, however there were no statistically significant differences between the three groups for correlations with TC/HDL ratio and TG (Table 4-5).

WHtR and Hs-CRP had a positive correlation with the stronger correlation observed in the CRTI-A group compared to the control group ($r = 0.52$ and $r = 0.43$; $p<0.001$), (Table 4-5 respectively). This finding confirms the increased risk due to vascular inflammation found with obesity.

5.6 Confounding factors affecting CVD risk profile

The time period for traumatic injury or deployment to examination is longer for the controls than for CRTI-A and CRTI-NA groups, which could indicate potential bias in recruitment. It could be argued that the unequal time periods are allowing for unequal development of CVD risk factors. However, we are evaluating CVD risk factors developing over the previous seven-year period and not clinical outcomes, which will be examined during the ongoing longitudinal ADVANCE Study. We cannot therefore determine or predict whether the CVD risk factors will develop further or be maintained over time. Given the seven-year period we would have anticipated development of general CVD risk factors as determined in the systematic review of publications.

The majority of the control group is exposed to different circumstances than most of the CRTI-NA and CRTI-A groups. A total of 90% of the healthy control cohort were still serving and therefore exposed to physical military exercise and organized military life compared to 36% of the CRTI-NA cohort and 12% of the CRTI-A cohort still being enlisted. A regular fitness regime while serving in the military promotes endothelial functionality and nitric oxide (NO) promotion during variable pace or aerobic training. On the other hand, CRTI-NA personnel and CRTI-A who have been discharged into the NHS and civilian life, do not receive the same benefits of physical training and organized military support. Endothelial dysfunction and lack of NO due to physical inactivity does have an adverse effect on the correlation between oxidative stress and hypertension (Korsager Larsen and Matchkov, 2016).

The negative effects of sustained physical training in the healthy control group, has also been noted, which could affect results comparing healthy controls with the CRTI-NA or CRTI-A groups. Intense aerobic exercise can also injure the endothelial muscles resulting in collagen formation in the endothelium (with calcification as a result) due to mechanical strain on the muscles in the arterial walls, which causes stiffening of the arteries (Korsager Larsen and Matchkov, 2016). This stiffening of the arterial walls increases systolic blood pressure and is reflected in increased augmentation index and pulse wave velocity. This increase in CVD risk factors are then recorded for the healthy control group and might mask the milder increase in arterial stiffness recorded in the CRTI-NA or CRTI-A groups leading to an insignificant false statistical result. The exercise patterns post injury should therefore be analysed to rule out the confounding effects of excess exercise on vascular health and ultimately on CVD risk, which could as a matter of fact be prevalent in all the groups. Consideration for factors contributing to the production of NO as an endogenous vasodilator and inhibitor of vascular smooth muscle contraction should be applied in evaluating arterial stiffness (Zhou et al., 2004).

5.7 Strengths and limitations

Our considerable sample size, which by far exceeds previous cross-sectional study cohorts, underpins findings of previous cardiovascular risk assessments amongst CRTI-A (Rose et al., 1987, Vollmar et al., 1989, Yekutiel et al., 1989). A sample size of at least 100 in each of the subgroups would have more than 80% power to detect a 3% difference in the central AIx and a 1.2 mg/l difference in Hs-CRP levels at a significance level of 0.05 (two-tailed).

Further characteristics lending gravitas to the study are the frequency matching of the groups by age, height, operational exposure and time of deployment. The NISS reference data confirms the severity of injuries sustained by recruited participants in the subgroups (Osler et al., 1997).

There are limitations to the study.

Significant variation in time from injury to examination (Fig 4-2) could suggest potential recruitment bias. The impact of psychological factors such as PTSD has not been considered in this study and will be subject to future examinations.

The Vicorder measures regional pulse wave velocity, which is affected by protocols determining the distance between the test points as well as by obesity. Patients are required to stay in the supine position, which could have limitations due to injuries or amputations. The accuracy of the measurement of the distance from the carotid to the femoral artery is

questionable especially in patients with abdominal obesity (Van Bortel et al., 2002, Laurent et al., 2006). The large heterogeneity of arterial structure at different sites, constitutes further limitations (Pereira et al., 2015).

The healthy controls were more likely to be still serving and serving military personnel are more likely to conform to structured health and fitness regimes, meals and medical care than discharged personnel (CRTI-NA and CRTI-A) caring for themselves.

This study did not examine the level of rehabilitation achieved post amputation. Compromised mobility could lead to early deposit of visceral fat, elevating the CVD risk profile. Physical activity associated with an increase in heart rate correlates with nitric oxide (NO) formation in the endothelium. NO antagonises the effect of angiotensin II on vascular tone increasing the CVD risk profile (Zhou et al., 2004, Bataineh and Raij, 1998, Hasegawa et al., 2019).

The presence and level of infection after amputations, which has an effect on CVD, (Khademi et al., 2019), was not recorded in this study although participants with active disease were excluded. Atherosclerosis the underlying cause of CVD and stroke, is an inflammatory (low grade) disease with pathobiological mechanisms, which are common to the host's responses to many chronic infectious diseases (Fong, 2009). With 34% US casualties developing infection after amputation, the impact on the development of CVD could be considerable (Weintrob et al., 2018).

Further limitations of this study are the absence of PTSD, exercise and activity data, which could assist the understanding of mechanisms affecting higher visceral fat levels and the adverse lipid and inflammatory profile.

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5.8 Future directions

Early stages after amputation in this study did not reveal significant increases in arterial stiffness and resulting changes in pulse wave velocity, which could lead to discounting vascular damage following amputations due to lack of presence of changes in haemodynamics.

Therefore, further studies should consider investigation at later stages after traumatic injuries.

Volmar et al observed an asymmetrical flow pattern after leg amputations at the aortic bifurcation developing damage to the aorta at a later stage resulting in aneurysms (Vollmar et al., 1989). This observation confirms a change in reflected wave flow possibly linked to a later stage increase in arterial stiffness.

Regional determination of PWV, as conducted in this study, could lead to discrepancies due to inaccuracy of measuring the distance between the suprasternal notch and the cuff on the thigh. Inaccuracies could be because of an increased waistline, operator error or bias or error between different operators. The distance is only measured once and applied for three measurements of PWV. A local PWV measuring technique is currently under development, which should be preferential for an early and precise assessment of arterial distensibility (Pereira, 2015).

The finding of this study confirms suggestions that AIx might be a more accurate determinant of arterial stiffness and CVD risk marker than PWV (Aminuddin et al., 2014).

The level of physical exercise capabilities after rehabilitation, might have an impact on general fitness thereby affecting arterial stiffness and abdominal fat deposits, which affects CVD risk as found in this study. Quantification of physical capabilities was measured by means of distance walked in a certain time, which measures ability but not necessarily fitness level for comparison. A more appropriate Cardiorespiratory Fitness Test (CRF) such as the Chester Step Test, which lasts for a maximum duration of 10min and requires an individual to step (height is pre-determined by age and physical activity levels) to a metronomic beat. The protocol consists of five stages, each lasting for 2min which increases in speed cumulatively. CRF is calculated by heart rate responses at the end of each stage (Gray et al., 2017).

Future studies need to undertake the daunting task of assessing, evaluating and proving causality between infection after traumatic injuries and amputations with ischaemic heart disease and stroke (Fong, 2009, Weintrob et al., 2018).

The greater prognostic value of non-invasive assessment of central aortic blood pressure as opposed to peripheral blood pressure in the early detection of CVD should be considered in future studies (Zuo et al., 2018, Cziraki et al., 2018, Ryuzaki et al., 2017, Sharman and Laurent, 2013).

5.9 Conclusion

Analysis of the baseline data confirmed that military servicemen who have experienced serious battlefield related traumatic amputations, demonstrate a more adverse cardiovascular risk profile than that of less and non-injured servicemen exposed to the same operational environment at the same time. Our major novel findings were that pulse wave velocity, a measure of arterial stiffness, was not significantly affected by combat-related traumatic injuries and amputations. Our findings confirm results from previous publications that AIx may be a better marker to reflect arterial stiffness and CVD risk in young men than PWV (Aminuddin et al., 2014).

Based on the results of this study and the review of existing literature, it became clear that clinicians caring for combat-related amputees should use easy to apply indices and non-invasive technology to monitor closely for early onset of obesity, increase in augmentation index and hypertension development as prominent risk factors for CVD.

The majority of the risk factors highlighted by this study are modifiable such as resting heart rate, abdominal waist and hip circumference and lipid profile by means of lifestyle changes, supporting the aim of the ADVANCE Study to identify risk factors that could be addressed and mitigated early to prevent progression of subclinical CVD. The importance of the findings in this study will be quantified during the progression of the ADVANCE study as hard clinical endpoints emerge.

Based on the findings of this study, we hypothesise that if a future increase in arterial stiffness and blood pressure is found, it would relate to the composite effects of chronic vascular inflammation with probable causes such as increased insulin resistance due to an increase in abdominal circumference rather than due to haemodynamic effects of the amputation per se on arterial wave reflection.

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Appendix A:

Ministry of Defence – Application for MoDREC Approval; Research Ethics Committees.

Ministry of Defence

Application for MoDREC Approval

Research Ethics Committees

Document Description: Application for MoDREC approval for scientific research.		
Version	Date	Description
1.0	19 Jun 2006	First issue
1.1	07 July 2008	Minor format & content changes
This document will be subject to version control by:		

SIT-ISTA 2 01.K.47 MoD Main Building London SW1A 2HB	Telephone: 0207 218 2512 Fax: 0207 218 9948 Email: George.Papadopoullos754@mod.uk

MoDREC Protocol No: 357/PPE/12

MINISTRY OF DEFENCE

Ministry of Defence Research Ethics Committees (MoDREC)

APPLICATION FOR MoDREC APPROVAL

Please read the notes in the MoDREC Application Form Guide (formerly MoDREC Researcher Guide) before completing this form. Enter text in the grey boxes, which will expand automatically to encompass your text. Please e-mail the completed form and any supporting documents to *ethics@mod.uk*

1. TITLE OF STUDY

ADVANCE Study
ArmeD SerVices TrAuma RehabilitatioN OutComE Study

2. DATE/VERSION

Date 21/01/19**Version** 14

3. NATURE OF PROJECT

Longitudinal cohort study to investigate long-term medical and psychosocial outcomes of physical battlefield trauma casualties.

4. INVESTIGATORS

4a. Principal Investigator
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5. PREFERRED TIMETABLE

5a. Preferred start date: 31.08.2015

5b. Expected date of project's completion: 31.12.2039

6. SPONSOR / OTHER ORGANISATIONS INVOLVED AND FUNDING

6a. Department/Organisation requesting research:
Defence Medical Services

6b. If you are receiving funding for the study please provide details here:
£1.14million from Help for Heroes . Additional funding from MoD for £1.63million has been secured over the first 5-year period. Additional future funding is expected from both sources.

6c. Please declare any competing interests:
None

7. OTHER RESEARCH ETHICS COMMITTEE (REC) APPROVAL

Has the proposed study been submitted to any other reviewing body? If so, please provide details:
N/A.

8. PURPOSE OF THE STUDY

1. Background

The Defence Medical Services (DMS) are in a unique period in their history. For the first time in generations the UK is, and has been, sustaining significant numbers of battlefield casualties, from the conflicts in Afghanistan and previously in Iraq. Many of these casualties have such severe complex injuries that previously they would not have survived. If it were not for the world leading, cutting edge battlefield trauma care provided by the UK DMS in operational theatre these patients would still not be surviving. Rehabilitation of these casualties takes place at DMRC Headley Court often over many months. DMRC Headley Court is therefore ideally placed to investigate the long-term outcomes of this cohort of severely injured UK armed forces battlefield casualties, a critical piece of research that it is essential to complete.

The long-term study of these patients needs to be undertaken to ascertain many aspects of their health and psychosocial outcomes and so the DMS can learn for future conflicts. Previous studies into war veterans have been completed from conflicts such as Vietnam and World War II and have investigated long-term health outcomes but some have been inconclusive, others have focused just on Post Traumatic Stress Disorder (PTSD) patients and most have been retrospective cross-sectional studies rather than the more methodologically robust longitudinal cohort studies (Boscarino, 2006; Bramsen et al., 2007; Kunnas et al., 2011; Modan et al., 1998; Kang & Bullman, 2001; Watanabe & Kang, 1996; Fett, et al., 1987a; Fett, 1987b; Macfarlane et al., 2000). None of the previous studies have been in as severely injured a cohort as the UK DMS are faced with at present. Other studies into long term health related outcomes such as bone health, including osteoarthritis and osteopenia/osteoporosis and fracture, all cause mortality and long term vocational and quality of life outcome are limited and insufficient (Watanabe &

Kang, 1996; Dutta et al., 2008; Fett et al., 1987a; Fett et al., 1987b; Johnson et al., 2010; Kang & Bullman, 2001; Kulkarni, et al., 1998; Macfarlane et al., 2000; Shahriar et al., 2009; Sherk et al., 2008; Vollmar et al., 1989) and generally not transferable to our current uniquely severely injured population of casualties.

There have been some studies investigating cardiovascular disease (CVD) in war veterans (Kunnas et al.; 2011; Modan et al.; 1998; Boehmer et al.; 2004). In a recent retrospective study of 667 war veterans followed up for 28 years from 1980, outcome data was available for the 102/667 who had been injured or wounded in action (Kunnas et al., 2011). Amongst the men who had been injured in action the risk of coronary artery disease (CAD) related death was increased 1.7 fold (28.4% vs 19.6% at 28 years) and there was also a significant increased risk of depression. However, data on baseline risk factors and population age at inclusion were not defined, nor were the relative prevalence of other CVD related events (eg. stroke, peripheral vascular disease). Furthermore, there was no available data on the types of injury sustained in order to allow a meaningful translation to our current UK military battlefield casualties. In another longitudinal study Modan et al (1998) evaluated the 24-year mortality rates of male traumatic lower limb amputees (n = 201) of the Israeli army wounded between 1948 and 1974, compared with a sample representing the general population (n = 1,832). They noted that CVD was twofold higher in amputees than in controls and that crude mortality rates were significantly higher (21.9% vs 12.1%, $p < 0.001$) in amputees than in controls due mainly to increased CVD-related mortality (8.9% vs 3.8%, $p < 0.001$). However, the mechanism for this increase was poorly defined as only selected risk factors for CVD were available on the 101 surviving amputees (aged 50 to 65 years) and a sample of the controls (n = 96) matched by age and ethnic origin. This limited data suggests that the increased mortality may be explained by an increased risk of CVD risk factors among the trauma victims. However, this cohort is >24 years from injury, the defined risk factors were limited and only available for the survivors and not for those who subsequently died. Furthermore the time from injury to population sampling is not defined making any applicability extremely difficult. Consequently, there is the need for a prospective longitudinal cohort study to more accurately determine the changes in CVD risk factors and other health related quality of life (QoL) components and their effects on clinical outcomes including CVD-related endpoints.

In addition to trauma care, physical rehabilitation and assessment and prevention of medical comorbidities, health care challenges for this patient group include minimizing the impact of amputation or other severe injury on work, social function and mental health, quality of life and well-being. On top of the physical trauma, this patient group is faced with social and psychological challenges; including high rates of depression and anxiety disorders including, post-traumatic stress disorder (PTSD), body-image anxiety and reduced social functioning, social discomfort and negative sense of self and identity (Melcer et al., 2010; Melcer et al., 2012; Sandweiss et al., 2011). This is an area of research where work has been limited and the majority of the literature is from general population samples (Horgan & MacLachlan., 2004).
Our collaborators at Kings College Mental Health Research (KCMHR) have great experience in military mental health studies and are the leading civilian UK centre of excellence for military health research. KCMHR carries out interdisciplinary

research and combines professionals with a wide variety of backgrounds (epidemiology, psychiatry, psychology, public health and sociology). Since its inception in 1996, KCMHR has conducted several large cohort studies of UK military personnel (Fear et al., 2010; Hotopf et al., 2006; Mulligan et al., 2012; Rona et al., 2006; Unwin et al., 1999) and the experience of the KCMHR team will be essential in this broader and longer medico psychosocial cohort study.

Therefore, although some research has been performed into medical outcomes of war veterans the conclusions are generally drawn from less than ideal methodologically designed studies and are not transferable to our current unique population. The long-term outcomes of our casualties are therefore unknown and they are vital to investigate, as these casualties must not be forgotten. Unless a comprehensive and rigorous longitudinal cohort study is established the long-term outcome of these patients will be unknown as they are discharged out of the Armed Forces to different regions of the country and the NHS.

2. Study Objectives

To investigate the long-term cardiovascular, musculoskeletal and other health and psychosocial related outcomes of UK armed services physical battlefield trauma patients.

3. Study Hypotheses

Long-term outcomes of physical battlefield trauma casualties, in comparison to the "non exposed" group, will demonstrate:

- Increased risk of cardiovascular disease
- Increased cardiovascular disease related events
- Increased rates of osteoarthritis
- Different mental health outcomes
- Different long term quality of life outcomes
- Different occupational outcomes

9. STUDY DESIGN, METHODOLOGY AND DATA ANALYSIS

1. Study Design

Cohort study with the exposure being physical battlefield trauma.

Group 1:"Exposed Group": n = 600 battlefield trauma casualties

Group 2: "Non-Exposed Group": n = 600
Frequency matched for age, sex, service, rank, deployment and combat role.

2. Outcomes

Primary Outcomes

a. **Cardiovascular risk**- as determined by pulse wave velocity at 20 years.
b. **Major Adverse Cardiovascular Endpoint (MACE) -** Composite Cardiovascular Disease (CVD) endpoint of cardiovascular death, non fatal

myocardial infarction, stroke, transient ischaemic attack (TIA), arterial revascularisation (coronary artery bypass grafting, percutaneous coronary intervention, carotid endarterectomy or stenting and peripheral arterial stenting or bypass) at 20yrs.

c. **Osteoarthritis of the hip and knee-** as determined by patient reported outcomes and radiographic assessment at 20yrs.

Secondary Outcomes

a. Cardiovascular risk as determined by pulse wave velocity, augmentation index and central blood pressure
b. Cardiovascular risk as determined by more traditional cardiovascular risk factors (eg. blood pressure and diagnosis of hypertension, lipid profile, blood glucose/Diabetes mellitus, smoking history, HsCRP and abdominal waist circumference)
c. Cardiovascular disease as determined by individual components of the primary composite CVD score and peripheral vascular disease and other CAD (angina).
d. Musculoskeletal disease (osteoarthritis and osteoporosis),
e. All cause mortality
f. Pain - back and stump/phantom (if applicable)
g. Mental health
h. Functional status
i. Quality of life
j. Employment
k. Relationship status and sexual function at :
    I. "Baseline"
    II. 3yrs
    III. 5yrs
    IV. 10yrs
    V. 15yrs
    VI. 20yrs

3. Trial Subject Selection

Eligibility Criteria : Group 1 : Exposed Group

Inclusion Criteria

a. UK Armed services personnel
b. Male
c. Sustaining physical battlefield trauma, while on deployment, requiring aeromedical evacuation and direct UK hospital admission
d. Injured during 2003 or after.

Exclusion Criteria

a. Females
b. Patients who are unwilling or unable to give informed consent
c. Patients with established CVD (previous stroke or transient ischaemic attack [TIA], ischaemic heart disease [IHD], peripheral vascular disease)
d. Past medical history of Diabetes
e. Past medical history of renal or liver disease
f. Aged <18 and >50 years

g. Active acute infection with systemic features of sepsis, at the time of recruitment, as defined below. Potential participant with active acute infection will be considered for recruitment once the acute illness is treated and resolved.

(American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. 1992):
2 of 3 of:
i. Temperature >38ºC or <36ºC
ii. Heart rate>90beats/min
iii. Respiratory rate >20 breaths/min

Eligibility Criteria : Group 2 : Non-Exposed Group
Frequency matched for age, sex, service, rank, deployment and combat role.

Inclusion Criteria
a. UK Armed services personnel
b. Male
c. Previously deployed.
d. No battlefield trauma, as defined in the inclusion criteria.

Exclusion Criteria
h. Females
i. Patients who are unwilling or unable to give informed consent
j. Patients with established CVD (previous stroke or transient ischaemic attack [TIA], ischaemic heart disease [IHD], peripheral vascular disease)
k. Past medical history of Diabetes
l. Past medical history of renal or liver disease
m. Aged <18 years and >50 years
n. Active acute infection with systemic features of sepsis, at the time of recruitment, as defined below. Potential participant with active acute infection will be considered for recruitment once the acute illness is treated and resolved.

(American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. 1992) :
2 of 3 of :
i. Temperature >38ºC or <36ºC
ii. Heart rate>90beats/min
iii. Respiratory rate >20 breaths/min

4. Recruitment, Consent and Randomisation Processes

Recruitment
Both battlefield trauma exposed volunteers and non-exposed volunteers will be recruited. Potential battlefield trauma exposed participants and matched non-exposed controls will be identified through Defence Statistics using the following data sources: Initial NOTICAS, Defence Patient Tracking System, DMICP, DMRC Complex Trauma Database, DRMC Prosthetic database, the Joint Theatre Trauma

Registry and Joint Personnel Administration (JPA) (including move and track), as per the inclusion and exclusion criteria as detailed above. These potential participant details will then be transferred to the King's Centre for Military Health Research (KCMHR) at King's College London and stored on a secure database. Potential participants will then be approached by a member of the research team. Those potential participants still in the services will be contacted via their military contact details recorded on JPA or through their GP. Those potential participants who have left the services will be contacted either via their final contact details on leaving the armed forces or via their NHS number and NHS GP (NHS REC will be applied for) or through other common forms of communication such as social media. A verbal explanation of the trial and patient information sheet will be provided by the authorised trial clinician for the participant to consider. This will include detailed information about the rationale, design and personal implications of the study and include a participant invitation form; participant information sheet and participant consent form (Appendix A-C). Following information provision, participants will have at least 24 hours to consider participation and will be given the opportunity to discuss the trial with their family and healthcare professionals before they are asked whether they would be willing to take part in the trial. This process will be clearly documented into the participant's medical notes.

Non-exposed volunteers will be recruited from the same military units as the battlefield exposed volunteers. Non-exposed volunteers will be approached via letter, e-mail, telephone or social media. Once matched non-exposed volunteers are identified the same recruitment proceeds will be followed as for the patients mentioned above.

Consent
Assenting participants will then be formally assessed for eligibility and invited to provide informed, written consent. The right of the participant to refuse consent without giving reasons will be respected. Further, the participant will remain free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment. A copy of the consent will be given to the participant, one filed in the Trial Master File, one filed in the hospital. The written consent will be taken by an authorized clinician.

Randomisation
Not applicable

Un-Blinding
Not applicable

5. Study Outcomes

Cardiovascular Risk

Large artery stiffness and pulse wave analysis
Cardiovascular disease is the leading cause of mortality in the western world (Yach et al., 2004). Consequently, there is growing interest in improved methods to accurately predict future cardiovascular risk. Hence, there has been a drive toward the use of a "global risk assessment" summating the effects of individual risk factors on CVD risk

assessment, rather than relying solely on individual conventional cardiovascular risk factors, such as diabetes mellitus (DM), hypertension, elevated cholesterol, and smoking (Boos et al., 2007).

Large artery stiffness leads to increased pulse wave velocity (PWV) and accelerated wave reflections and causes an increase in myocardial demand and central systolic pressure along with a decrease in coronary artery perfusion pressure. This increase in pulse wave velocity as a result of increased arterial stiffness is associated with many of the common cardiovascular risk factors such as age, high blood pressure, smoking, cholesterol levels and obesity, and these lead to a dramatic increase in risk of heart attack, stroke and heart failure (London et al., 2004)

Importantly increased pulse wave velocity has also been shown to be an independent predictor of cardiovascular morbidity and mortality in several population groups, including healthy controls (Shechter et al., 2009; Sutton-Tyrrell et al., 2005; London et al., 2001; Cruickshank et al., 2002; Laurent et al., 2001; Weber et al., 2004)

The Vicorder (Skidmore Medical Limited, Bristol, United Kingdom) simultaneously records the pulse wave from the carotid and femoral site by using the oscillometric method. It has been well validated against the gold standard measures of arterial stiffness (van Leeuwen-Segarceanu et al., 2010). It non-invasively analyses central systolic blood presure, pulse wave velocity and the augmentation index (AI) (non invasive measure of arterial stiffness utilising aspects of the pulse wave reflections which may be influenced by traumatic injury) in one integrated system over five minutes using an upper thigh cuff and a neck transducer (Pucci et al., 2013). Its simulataneous assessment of the carotid and femoral pulse wave generates excellent reproducibility with a within-subject coefficient of variation 2.8% (Hickson et al., 2009). By utilizing central and peripheral measurements of arterial stiffness this allows for accurate quantification of CVD risk (Woodman et al., 2005). Hence this system can still be used in a multiple limb amputee.

Heart Rate Variability (HRV)
HRV refers to the temporal changes in the beat to beat internals in the heart, which are subject to continuous autonomic nervous system and competing sympathetic versus parasympathetic control. HRV is a marker for altered autonomic balance has been linked to adverse clinical conditions such as cardiac death, stroke and ill mental health (Cardoso et al., 2014; Fyfe-Johnson et al., 2016; Kemp et al., 2012; Kemp et al., 2013; Sessa et al., 2018; Thayer et al., 2012; Tucker et al., 2012).

A small (13g) portable Electro-Cardiogram (ECG) device (Mega Motion Faros 180 recorder; Mega, Finland) will be used to record a high quality 5 minute continuous single lead ECG.

Other CV risk factors
Other well recognized cardiovascular risk factors will be recorded to establish overall cardiovascular risk and change in cardiovascular risk with time, using standard cardiovascular risk calculators (Appendix D).

- Blood pressure
- Fasting full lipid profile (including total and HDL cholesterol)
- Current and past smoking history in pack years
- Family history of coronary disease in first degree relatives <60 years old
- Fasting blood glucose/HbA1C
- BMI
- Total body fat as per DEXA scanning
- Abdominal waist circumference
- High sensitive CRP (Boekholdt et al., 2006; Boos & Lip, 2005)
- Resting heart rate (Woodward et al., 2012)

Standard assessment of blood pressure, abdominal waist circumference, family history of CVD and smoking history in pack years will be taken. BMI via traditional methods and total body fat/composition via the gold standard measure of DEXA will be measured as described in more detail below**.** Approximately 50 mL (in total) of fasted blood will be taken via a venous puncture to measure renal function, full blood count, glucose and HbA1c ad lipid profile and in addition males sex hormones will be measured. Approximately 20mL of blood the will be centrifuged to create serum which will be stored long term (-80ºC freezer initially at DMRC then Imperial College London) for batch testing of HsCRP and for testing any future biomarkers of cardiovascular or musculoskeletal disease that are not currently available but may become so in the next 20yrs during the course of the study (eg. homocysteine, apolipoprotein). Approximately 10mL of whole blood/plasma/DNA will be stored at -80ºC for genetic or other analysis at later date. In addition 50mL of urine will be stored for future biomarker and genetic testing. Blood and urine will be taken and stored at baseline and all subsequent visits.

Cardiovascular Disease
Participants will be assessed for new onset of CVD since last study visit:

- Major Adverse Cardiovascular Endpoint (MACE)-composite CVD outcome : cardiovascular death, non fatal myocardial infarction, stroke, TIA, arterial revascularisation (coronary artery bypass grafting, percutaneous coronary intervention, carotid endarterectomy or stenting and peripheral arterial stenting or bypass)
- Individual components of the composite CVD outcome
- Angina
- Peripheral vascular disease
- Hypertension- physician/GP diagnosed

The use of composite cardiovascular endpoints is commonplace, internationally recognized and validated in cardiovascular outcome research (Heart Protection Study Collaborative Group, 2002; Schächinger et al., 2000; AIM-HIGH Investigators et al., 2011; Ridker et al., 2002; Abola et al., 2012; Raiko et al., 2010; Anderson et al., 2011; Sehestedt et al., 2012). Clinical trials often use composite outcomes to provide an overall estimate of the effect of an intervention. Composite outcomes are appropriate under many circumstances such as when no single most important outcome exists, when several endpoints are thought to be important and when two endpoints are clinically related (such as non fatal myocardial infarction and death

from fatal myocardial infarction). Also, because death is an uncommon occurrence very large sample sizes are required to detect differences in deaths among trial groups. Composite outcomes are thought to facilitate the evaluation of infrequent outcomes, such as death in smaller trials.

Musculoskeletal Disease: Osteoarthritis, Osteoporosis, Spondyloarthritis and Body Composition

- Radiographic assessment of osteoarthritis of the hips and knees
- DEXA body composition assessment
- DEXA bone mineral density assessment
- BMI calculation

Small retrospective studies have also indicated an increased risk of osteoarthritis in amputees (Kulkarni et al., 1998) who will form a large proportion of the battlefield trauma group. Participants will be assessed radiographically for osteoarthritis of the knees. Posterior-anterior views with knees in semi flexed position (7-10 degrees) will be used which is the recommended view for the assessment of osteoarthritis (Buckland-Wright et al., 2004; Buckland-Wright et al., 1999) and is used in recent cohort studies (Nevitt et al., 2006; Wesseling et al., 2009) Anterior-lateral views and skyline views (inferior-superior) of the patellofemoral joint with the knees in 30 degrees of flexion will also be taken (Spector et al., 1996; Wesseling et al., 2009). Hips will also be assessed radiographically with an AP pelvis-focal length 100cm, hips internally rotated15 degrees) (Leyland et al., 2012). Radiographs will be scored according to the Kellgren and Lawrence radiographic osteoarthritis scoring method (Kellgren & Lawrence., 1957) for both the hip and the tibiofemoral joint of the knee. The patella femoral joint will be scored using the OARSI scoring method (Altman & Gold, 2007)

In limited populations obesity is a recognized complication of amputation (Shahriar et al., 2009) BMI will be recorded using an adjusted formula for amputees (Tzamaloukas et al.,1994), and also using the gold standard measure of body composition the DEXA scanner (Lohman et al., 2000) which has previously been used in a military population (Nindl et al., 1997; 1996). Measuring body composition and in particular total body fat is an important factor in determining long-term risk of CVD, diabetes mellitus and osteoarthritis.

DEXA scanning will also be used to assess changes in bone mineral density as previous smaller retrospective studies have shown evidence of femoral neck osteopenia in amputees (Kulkarni et al., 1998; Leclercq et al., 2003; Sherk et al., 2008). Incidence of osteoporotic defining hip fracture will be recorded at each follow up visit, as although loss of BMD is suggested there is no evidence to date to suggest increase fracture rate in this population.

The participants will also complete the Manchester-Oxford Foot Questionnaire (Morley et al 2013) (Appendix Z9) to assess pain and functional outcomes related to foot and ankle injury

The study population is similar to the at risk population for spondyloarthritis i.e. young males, and there is evidence to suggest that trauma precipitates

spondyloarthritis/ankylosing spondylitis (Cury et al., 1995; Olivieriet al., 1990; Olivieri et al., 1989; Olivieri et al., 1988; Olivieri et al., 2008). The AP pelvis x-rays will also therefore be scored for sacroiliitis via the modified New York score (van der Lindenet al., 1984). Sacroiliitis is a key feature of spondyloarthritis as is the insidious onset of back pain, a feature that is also present in amputees (Hammarlundet al., 2011). The presence of the gene HLA-B27 which is strongly associated with spondyloarthritis and particularly Ankylosing Spondylitis will therefore be assessed as will inflammatory back pain and spondyloarthritis criteria (Sieper et al., 2009a; Sieper et al., 2009b) so the nature, cause and course of back pain in both populations can be determined.

Mobility, Disability, Function & Pain (amputee specific*)

- Amputee Mobility Predictor Questionnaire (AMPQ)*
- Specialist Interest Group in Amputee Medicine (SIGAM) Mobility Grade*
- Prosthetic satisfaction (Numerical rating scale) *
- Socket comfort score*
- Prosthetic use (time in hours per week) *
- Numerical rating scale (NRS) for stump pain*
- Numerical rating scale for phantom limb pain (intensity/frequency/impact) *
- Six minute walk test (including maximal heart rate)
- Respiratory function (FEV1/FVC)
- Numerical rating scale for back pain
- Inflammatory back pain questionnaire
- Oswestry Disability Index
- Non arthritic hip score (NAHS)
- Knee osteoarthritis outcome score (KOOS)
- Disability arm shoulder hand (DASH) questionnaire

All participants will be assessed for mobility, disability, physical function and pain. Standard outcome measures in amputees will be recorded at baseline and at each visit. These will include the Amputee Mobility Predictor Questionnaire (AMPQ) (Gailey et al., 2002) (Appendix E) (amputees only) to assess the study participants' subjective feeling of functional ability. This assessment is routinely used within amputee mobility research (Hafner, et al., 2007; Miller & Deathe., 2004) and also within the DMRC as a functional assessment tool.

The SIGAM (Specialist Interest Group in Amputee Medicine) mobility grades (Ryall et al., 2003) (Appendix F) will be used as will the six minute walk test to assess overall function (Balke., 1963; Lin & Bose, 2008; Naughton, et al., 1963). Developed in 1963 by Balke to evaluate functional capacity (Balke, 1963) the six-minute walk test (6MWT) measures the distance an individual is able to walk over a total of six minutes on a hard, flat surface and is valid in the able bodied and amputees. The goal is for the individual to walk as far as possible in six minutes. The individual is allowed to self-pace and rest as needed as they traverse back and forth along a marked walkway. The subjects' respiratory function will also be measured to assess basic respiratory capability and its contribution to functional capacity. Prosthetic comfort score will be assessed with the socket comfort score (Appendix G) (Hanspal et al., 2003) and prosthetic satisfaction will be assessed using a numerical rating scale

(Appendix H) and prosthetic use recorded (hours used previous day and previous week) (Appendix I).

Phantom pain (Desmond & MacLachlan, 2010; Kern et al., 2009; Sherman & Sherman, 1983) and stump pain (Byrne, 2011) are common consequences of amputation . The frequency, intensity and impact of phantom limb pain and stump pain will each be measured using numerical rating scales (Appendix J & K).

Back pain has been reported in amputees in small retrospective studies (Hammarlund et al., 2011). Therefore, back pain will be assessed at each study visit in its severity/frequency/impact with a numerical rating scale (NRS)(Appendix L). The nature of back pain will be assessed with an inflammatory back pain assessment and spondyloarthritis assessment (Sieper, et al., 2009b) (Appendix M) and the impact of back pain on function will be assessed with the Oswestry Disability Index (ODI) (Fairbank et al., 1980) (Appendix N).

There is some association between amputation and osteoarthritis of the knees and hips (Kulkarni et al., 1998). Radiographic assessment is planned as noted above. Validated and internationally recognized patient reported outcomes relevant to knee (Knee osteoarthritis outcomes score) (Roos & Lohmander., 2003; Roos & Toksvig-Larsen, 2003) (Appendix O) and hip (Non arthritic hip score) (Christensen et al., 2003) (Appendix P) pain and osteoarthritis will be completed along with numerical rating scales for pain in each of the knee and hip joints (Appendix Q).

Upper limb pain is also a recognized consequence of both upper and lower limb amputation (Davidson, 2004; Ostlie et al., 2011). The disability arm, shoulder hand questionnaire (DASH) (Germannet et al., 1999; Davidson, 2004) (Appendix R) will be used to assess on going upper limb pain and function and a body pain manikin to indicate the sites of pain (Appendix S).

Pain will also be assessed using the Brief Pain Inventory (Tan G et al 2004) (Appendix Z17), characterized using the Neuropathic pain symptom inventory (Bouhassira D et al 2004) (Appendix Z18) the impact of pain will be assessed by the Pain Catastrophizing Scale (Sullivan et al 1995) (Appendix Z19)

Audiograms:
Military personnel are often exposed to loud noise both in combat and in training. The MoD has numerous standard operational procedures in place to minimize the risk of noise induced hearing loss. However, despite this, this is a feature of some serving military personnel and veterans. Those injured participants in the study, many of whom will have been injured by an explosive device, are at even higher risk of noise induced hearing loss. All military personnel are subject to programmed hearing tests throughout their military careers. It is proposed that all participants have their career audiograms reviewed and all participants, once they have exited the armed services, continue with audiograms as part of the 5yr follow-up program allowing for a more definitive assessment of the effect of a military career on hearing levels and a comparison between those involved and not involved in blast injury.

Quality of Life

Quality of life (QoL) will be measured at baseline and at each follow-up visit using a validated QoL outcome measure, the European Quality of life 5 domain (EQ5D) (EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group., 1990; Brooks., 1996; Dorman et al., 1997) (Appendix T).

Mental Health
Participants will have a mental health screen, specifically for anxiety and depression using the Patient Health Questionnaire 9 (PHQ-9) (Spitzer, Kroenke, & Williams, 1999) (Appendix U), the General Anxiety Scale- 7 Questions (GAD-7) (Spitzer et al., 2006) (Appendix U1) and the Post Traumatic Stress Disorder (PTSD) with the National Centre for PTSD Check List (PCL-5) (Blevins C et al 2015) (Appendix V). Current and past alcohol consumption since injury will be assessed with the Alcohol Use Disorders Identification Test (AUDIT) (Berner et al., 2007; Bohn et al., 1995) (Appendix W) and current and past drug use will be assessed using the Drug Use Disorder Identification test (DUDIT) (Berman et al 2007)(Appendix Z1)

Social support has been shown to be an independent etiological and prognostic factor for the development of cardiovascular disease (Hemingway & Marmot, 1999; Barth & von Kanel, 2010) and poor mental health (Lehtinen et al, 2005). The well-validated Multidimensional Perceived Social Support (MSPSS) measure (Zimet et al., 1988) will be used to measure social support in our ADVANCE population (appendix Z5).

Early life experiences have been shown to be linked to adult experiences of both physical and mental health in both the general population (Carr et al., 2013; Coelho et al., 2014) and military populations (Iverson et al., 2007). Significant childhood adversity factors from Iverson's (2007) have been incorporated from the King's Centre for Military Health Research Health and Well-being Study for use with ADVANCE (Appendix Z6).

Post-Traumatic Growth (PTG) is highly prevalent in veterans of Iraq/Afghanistan (Piertraz et al., 2010). PTG is related to lower pain perception, cortisol and less relapse in those who experience both PTG and life threatening illnesses (Barskova & Oesterreich, 2009). The PTG-Inventory (PTG-I) will be used to examine PTG in the ADVANCE population (Appendix Z7).

Mild Traumatic Brain Injury

Occurrence of mild traumatic brain injury, a common injury from the Afghanistan conflict, and subsequent symptoms will be assessed using the Ohio State University Traumatic Brain Injury Identification Method questionnaire (Appendix Z15) (Corrigan et al,2007) and the Rivermead post-concussion questionnaire (Appendix Z16) (King et al 1995) respectively.

Sleep
Quality and duration of sleep, along with insomnia and sleep apnoea, have been linked to poorer physical and mental health (Reid et al, 2006; Cappucio et al., 2013; Alvaro et al., 2013). In particular, sleep duration has been linked to coronary heart disease (Cappuccio et al., 2011). Questions previously investigated by a large scale operational mental health examination ("Operational Mental Health Needs Evaluation", 2011) using an adapted version of the Insomnia Severity Index (ISI)

(Bastien et al., 2001) will be used to examine sleep problems in the ADVANCE study population. The sleep duration section of the Pittsburgh Sleep Quality Index (Buysse et al., 1991) will be used to examine sleep duration. (Appendix Z8).

Relationship Status & Sexual Function
Current relationship status and relationship history since injury will be recorded. Sexual function will be assessed using the Arizona Sexual Experiences Scale (ASEX) (McGahuey, et al., 2000) (Appendix X).

Employment Status & History and Social Outcomes
Current employment/education and history of employment and education since injury will be recorded using an employment history questionnaire (Appendix Y).

Personal reasons for leaving the Armed Forces (Appendix Z10), Highest level of education attainment (Appendix Z11), Veteran Specific Outcomes (Appendix Z12), Who lives with you? (Appendix Z13) and Friends and Family Network Questionnaire (Z14) will also be administered to get in depth information of social outcomes.

Physical Activity
Current (last week) physical activity will be recorded using a valid patient reported questionnaire, the International Physical Activity Questionnaire (IPAQ) (Booth, 2000) (Appendix Z2). This will be useful to monitor participant's physical activity over the 20yr period as this may be related to the particpant cardiovascular, osteoarthritis and mental health outcomes.

Mortality & Cause of Mortality
In the event of death during the 20 year follow-up period cause of mortality will be recorded. The participant will be flagged with the National Health Service Central Register (NHSCR) to provide date of death in the unlikely event of this occurring. This will avoid unnecessary and potential upset by contact from the study team if a patient has died since the previous follow up.

6. Trial Schedule

Visit by Visit Schedule (Approximately 4-5 hrs per visit. Patients will be given as much time as they require and accommodation will offered if required) (see Appendix Z)
At Baseline and all subsequent visits (unless stated otherwise) :

- Patient demographics
  - Age
  - Sex
  - Regiment/unit
  - Job/role
  - Prosthetic components
- All injuries documented
- Date of injury/trauma
- Injury severity score (Baker & O'Neill., 1976; Baker, O'Neill et al., 1974)

- All subsequent trauma, requiring hospital admission, subsequent to commencing the study.
- Past medical history
- Drug History – prescribed and recreational (Appendix Z1)
- Method of discharge from Armed Services:
  - End of engagement/commission
  - Medical discharge
  - Administrative discharge
  - Redundancy
  -Still serving
  -Died in service
- Blood tests (baseline and at each subsequent follow up study visit)
  - Fasting lipids, glucose and HbA1c
  - Full blood count, urea and electrolytes and liver function and gamma GT.
  - Serum to be stored for batched hsCRP testing.
  - HLA-B27 (baseline only)
  - Serum and whole blood/plasma to be stored
  - Testosterone, FSH, LH and SHBG

- Urine: 50mL of urine for storage (baseline and at each subsequent follow up visit)
- Imaging
  - Pulse wave velocity assessment
    This is a simple, non-invasive and painless test that takes <5 minutes to perform. No ionising radiation is involved. The subject is supine and fully rested. A three lead ECG electrode is attached to the patient's chest and a pencil-like sensor is gently held against the subject's wrist to record a blood pressure signal from their pulse. With additional data from a second blood pressure recoding at the brachial artery the Pulse Wave Analysis system will calculate the pressure waveform at the heart to provide a detailed cardiovascular risk profile (eg AI, central blood pressure). Aortic pulse wave velocity (PWV) is calculated from sequentially recorded pressure waveforms of the carotid and femoral artery.
    Heart Rate Variability assessment
    During the Vicorder assessment of arterial stiffness a 5-minute HRV recording will be made. This will be followed by a further 5 minute recording thereafter with the subjects provided with a sample audio-visual breathing prompt to allow controlled respiration at a breathing rate of 5-12 breaths per minute.This assessment is non-invasive and requires a single lead ECG via two skin electrodes over the right shoulder and apex beat respectively.
  - DEXA- body composition and bone mineral density
    The combined investigation of body composition and bone mineral density takes a maximum of 20 minutes. The subject is laying supine on an open couch/table in a comfortable position. There is a minimal amount of radiation such that DEXA operators do not need to take any special precautions.
  - Radiographs hips and knees
    Posterior- anterior views with knees in semi-flexed position (7-10 degrees) will be used performed. Anterior-Lateral views and skyline views (inferior-superior) of the patellofemoral joint with the knees in 30 degrees of flexion will also be taken. Hips will also be assessed radiographically with an AP

pelvis-focal length 100cm, hips internally rotated15 degrees. The total length of time for all radiographs will be 20 minutes.

- Questionnaires
  - Amputee Mobility Predictor Questionnaire (AMPQ)
  - Prosthetic satisfaction (Numerical Rating Scale-NRS)
  - Prosthetic use questionnaire
  - Numerical rating scale- back pain (frequency/intensity/impact)
  - Numerical rating scale- stump pain (frequency/intensity/impact)
  - Numerical rating scale- phantom pain (frequency/intensity /impact)
  - Oswestry Disability Index (ODI)
  - Non Arthritic Hip Score
  - Knee osteoarthritis outcomes score
  - Disability of the arm, shoulder and hand questionnaire
  - Pain manikin
  - European Quality of Life 5 Domains
  - Post Traumatic Stress Disorder Check List (PCL-5)
  - Arizona Sexual Experience Scale (ASEX)
  - Alcohol Use Disorder Identification Test (AUDIT)
  - Patient Health Questionnaire 9
  - Generalized Anxiety and Depression Score-7 (GAD 7)
  - Employment history
  - Insomnia Severity Index (ISI)/ Pittsburgh Sleep Quality Index (PSQI)
  - Post Traumatic Growth Inventory (PTG-I)
  - Multidimensional Scale of Perceived Social Support (MSPSS)
  - Childhood Adversity (CA)
  - Manchester-Oxford Foot Questionnaire (MOXFQ)
  - Drug Use Disorders Identification Test (DUDIT)
  - Personal reason for leaving Armed Forces Question
  - Highest level of education attained question
  - Veteran specific outcome questions
  - Who lives with you question
  - Friends and family network question
  - Ohio State University TBI Identification Method
  - Rivermead Post Concussion Questionnaire
  - Brief Pain Inventory
  - Neuropathic Pain Symptom Inventory
  - Pain Catastrophising Scale
- Assessment
  - Current medication
  - Smoking history in pack years
  - New fracture history
  - BMI, abdominal waist circumference
  - BP and heart rate
  - SIGAM mobility grade
  - Spirometry (FEV1 and FVC)
  - Inflammatory back pain and SpA criteria
  - Audiogram (if participant has left the armed services)

Any significant risk or disease that requires medical action/treatment, as per standard treatment guidelines, that is detected during the study will be reported to the participant's primary care physician with the consent of the participant.

7. Data Collection, Source Data and Confidentiality

General
All information collected during the course of the trial will be kept strictly confidential.

Study data will be held securely on paper and, on an electronic database satisfying MoD security requirements at the DMRC Headley Court and KCMHR (King's College London). Access to the database will be restricted to authenticated, named users logged into either the KCL network or MoD network. The data collected will strictly only be used in this research and other relevant military research.

All staff are required to sign a confidentiality agreement, reminding them of their obligations and the disciplinary action which would result from a breach. The study will comply with MoD standards for handling personal data. No individual will ever be identified in reports or publications. All reports will be on aggregated data.

DMRC Headley Court and ADVANCE Study staff will comply with all aspects of the Data Protection Act 1998 and operationally this will include:

- Consent from patients to record personal details including name, date of birth, address and telephone number, service number, NHS number, GP name and address.
- Appropriate storage, restricted access and disposal arrangements for patient personal and clinical details.
- Consent from patients for access to their military and NHS medical records by responsible individuals from the research staff, the sponsor or from regulatory authorities, where it is relevant to trial participation. (NHS REC approval will be sought)
- Consent from patients for the data collected for the trial to be used to evaluate safety and develop new research.

Archiving
In line with the principles of GCP / UK Clinical Trial Regulations Guidelines, at the end of the trial data will be securely archived at each participating centre for a minimum of 15 years.

Arrangements for confidential destruction will then be made. If a patient withdraws consent for their data to be used, it will be confidentially destroyed immediately. On receipt of a completed questionnaire, all personal identifiers (contact details etc.) will be removed and will be stored in locked metal cabinets within the study area.

Questionnaires will be kept in separate, locked cupboards in a secure room within the study area. Study data will be anonymised, using a unique identification number for each participant and the key to personal identification will be kept separately.

8. Statistical Considerations

Sample Size/Power Calculations
Sample size calculations were performed for all 3 primary outcomes using GraphPad StatMate version 2.00 for Windows (GraphPad Software).

A sample size of 600 in the battlefield trauma exposed group and 600 in the non-exposed group was sufficient even with an estimated 10% drop out every 5 years to power the study to at least 80% for each of the 3 primary outcomes.

Primary Outcome 1 : Cardiovascular Disease - Composite CVD Endpoint
Sample size calculations were based on the primary composite CVD end-point. Published data have shown CVD event (varying endpoints used) hazard ratio of ≥1.70 among those with traumatic injury compared to healthy controls (Hrubec & Ryder, 1980; Kunnas et al., 2011; Modan et al., 1998). Given the age and demographic of our target population event rates are likely to be low. However, the study is using a well defined, published and measurable broad composite CVD primary endpoint and has a prolonged follow-up period which both significantly reduce the sample size needed to maintain statistical power.

The rate of the primary MACE endpoint has been estimated using a number of combined resources and includes :

1. Relevant CVD composite endpoint risk calculators

   i. http://www.cks.nhs.uk/cvd_risk_assessment_and_management/management/detailed_answers/assessment_of_cvd_risk/cvd_risk_calculators
   ii. http://www.cardiosmart.org/cardiosmart/default.aspx?id=298

2. Published data from similarly aged populations (Raiko et al., 2010) (AIM-HIGH Investigators et al., 2011) .

3. Data modelling and applying these to our average patient demographic at inclusion (24 year old male with a normal blood pressure and lipid profile).

We have estimated that we would expect a primary composite CVD event rate of ≥10% at 20 years in the healthy control group with a hazard ration of ≥1.7 in the battlefield trauma group. Based on this assumption we have calculated that a sample size of at least 400 in both the battlefield trauma exposed group and the non-exposed group would provide a >80% power to detect a hazard ratio of ≥1.7 at a two-sided alpha of 0.05 over a 20-year follow up period. The initial recruitment of 600 participants will have a natural drop out rate of approximately 10% every 5 years. This would result in a sample size of approx. 400 at 20 years, and therefore still be sufficient to identify differences in composite CVD endpoints between the groups.

Even if there was a drop out rate of 15%, every 5 years which could leave 313 subjects by 20 years from an initial cohort of 600. This would still be a sufficient sample size to detect a different in MACE at a hazard ratio of ≥1.8 with 80% power.

Primary Outcome 2: Cardiovascular Risk as Determined by Pulse Wave Velocity

We have also calculated sample size for the other key primary endpoint of pulse wave velocity (PWV). Published data on health controls of similar ages to our study population would suggest an average pulse wave velocity of 6.0 – 6.5 m/s with a within group standard deviation of 0.8-1.2 m/s (Doonan et al., 2011) (Reference Values for Arterial Stiffness' Collaboration., 2010). There is a general paucity of data on pulse wave velocity among amputees and multiple trauma patients. However, again using modelling of our average patient demographic and pilot data along with published data amongst sedentary controls, age and sex matched controls with subclinical CVD to simulate the disease group and allowing for the natural increase in PWV with age it would be anticipated that trauma patients would be expected to have a PWV of >0.4m/s higher than the healthy control group (Lazdam et al., 2012) Hence, a sample size of at least 200 in each group (battlefield trauma exposed versus non exposed) would have a 95% power to detect a difference in PWV of ≥0.36 m/s at a significance level (alpha) of 0.05 (two-tailed) assuming a within group standard deviation of 1.0m/s (Kingwell et al., 1997). Even if the within group standard deviation were higher than anticipated at up to 1.4 ms/s, a minimal sample size of 280 subjects would still have 90% power to detect a >0.4 m/s (>7%) difference in PWV between the battlefield trauma exposed group and the non-exposed group. Therefore, an initial sample size of 600 would be sufficient, even with a 17.5% drop out every 5 years which would reduce the cohort to 285, to identify differences in changes in PWV between the groups.

Primary Outcome 3: Hip and Knee Osteoarthritis as Determined by Radiographic Assessment

Based on the mean prevalence of hip osteoarthritis in the 40-50 year old age bracket of the general population being 2.8%, (standard deviation - 7%) (van Saase et al., 1989) (Dagenais et al., 2009) and an expected two-fold increase in hip osteoarthritis in the battlefield trauma group (Kulkarni et al., 1998) a two-sided test with an alpha value of 0.05 and a power of 95% the sample size would need to be 82 to detect a difference between the groups. Therefore, the proposed 600 participants at baseline would be more than sufficient, even with a higher than expected drop out rate.

Similarly, a sample size of 600 even with a significant drop out rate is adequate to detect difference in osteoarthritis of the knee between the 2 groups (mean prevalence in general population aged 40-50 years 8.7%, standard deviation 5.9%, expected prevalence in amputees 16.7%) (van Saase et al., 1989) (Burke et al., 1978) with a two-sided test, an alpha value of 0.05 and a power of 95%.

Statistical Analysis

Sample weights will be generated to take account of the sampling frame and how this relates to the UK Armed Forces as a whole. The sampling weights will compensate for unequal probabilities of selection based on age, rank and combat versus non-combat role on deployment. We will examine the characteristics of non-responders

and also of those who did not participate at follow-up. Predictors of non-response will be examined with logistic regression analysis. If there are significant predictors, we will take these factors into account in the analyses.

The level and the patterns of missing data will be assessed. For questions which elicit high levels of missing data (for example greater than 20%) we would be cautious about any analyses based on these data.

Data inspection and the Kolmogorov-Smirnov test will be used to assess normality of all continuous data. Paired continuous data comparisons will be undertaken using the paired t-test for normally distributed data and the Wilcoxon matched pairs test for non-parametric data respectively. Longitudinal time-dependent comparisons of $\geq$2 time points (baseline, 2, 5 and 10 years etc) will be performed with Repeated Measures ANOVA for normally distributed data, with the Tukey post-test for all significant results. Repeated Measures of non-parametric continuous will be performed using the Friedman test with post-test for all significant results. Cox proportional hazard models will was used to evaluate the effects of previous trauma on the event and event-free survival. A two tailed *P* value <0.05 will be considered statistically significant for all comparisons. Logistic regression will be used to eliminate confounders and identify predictors of outcome.

10. ETHICAL CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding ethical research involving human subjects adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013. Informed written consent will be obtained from the participants prior to registration into the study. The right of a subject to refuse participation without giving reasons will be respected. The participant will remain free to withdraw, and withdraw previously collected data, at any time from the study without giving reasons and without prejudicing his further treatment.
The study will be submitted to and approved by MoDREC prior to entering participants into the study.

11. PARTICIPANTS TO BE STUDIED

Number of participants: 1200

Lower age limit: 18

Upper age limit: 50

Gender: Male

Please provide justification for the sample size:
See Section 9, subsection 8 above.

12. SELECTION CRITERIA

See Section 9, subsection 3 above.

13. RECRUITMENT

13a. Describe how potential participants will be identified:
See Section 9, subsection 4 above.

13b. Describe how potential participants will be approached:
See 13a

13c. Describe how potential participants will be recruited:
See 13a

14. CONSENT

14a. Please describe the process you will use when seeking and obtaining consent:
See Section 9, Paragraph 4 above.

A copy of the participant information sheet and consent form must be attached to this application. For your convenience proformas are provided at the end of this document. These should be filled in, modified where necessary, and attached to the end of your application.

14b. Will the participants be from any of the following groups?

Under 18 : No	**Subordinates : Yes**
Prisoners : No	**Pregnant or nursing mothers : No**
Mental Illness : possibly	**Learning disabilities : Yes, possibly**

How will you ensure that participants in the groups listed above are competent to consent to take part in this study?
The experience at DMRC Headley Court is that very few battlefield trauma casualties have significant traumatic brain injury. Any that do will be very carefully considered for the study and only if the treating team are 100% confident with the mental capacity of that patient will they be approached for the study. If there is any concern what-so-ever regarding the capacity of a volunteer to give consent then a formal assessment of capacity will be performed by the clinical psychologist on the unit.

14c. Are there any special pressures that might make it difficult for people to refuse to take part in the study? How will you address such issues?
No

15. PARTICIPANT INVOLVEMENT: RISKS, REQUIREMENTS AND BENEFITS

15a. What are the potential hazards, risks or adverse effects associated with the study?

Minimal risk from radiation exposure as detailed below

1. Details of other ionising radiation

Procedure	No of procedures	Estimated procedure dose
AP Pelvis X-ray	1 at baseline and 1 at each of following visits – 3 yr, 5 yr, 10 yr, 15 yr, 20 yr	0.4 mSv based on the NDRL of 3 Gy cm^2
X-ray examination of both knees each involving PA, anterior lateral and skyline views	1 at baseline and 1 at each of following visits – 3yr, 5 yr, 10 yr, 15 yr, 20 yr	0.002 mSv
DEXA scan for bone mineral density of total hip and spine and total body composition	1 at baseline and 1 at each of following visits – 3yr, 5 yr, 10 yr, 15 yr, 20 yr	<0.02 mSv

2. What is the total participant dose from all the exposures above, and what component of this is the additional dose over and above standard practice? What are the risks associated with these two doses (total and additional)?

The total effective dose for a participant receiving the full set of X-ray examinations at each of the 6 visits is estimated to be 2.5 mSv. Approximately 95% of the total dose results from the pelvis X-rays. Some participants will not receive X-ray examinations of one or both knees as a result of loss of the knee to amputation. The dose from the total body composition DEXA scan will also be lower for these patients.

The total effective dose of 2.5 mSv is equivalent to that from approximately 13 months of exposure to natural background radiation, based on the UK average.

The lifetime risk of fatal cancer for an effective dose of 2.5 mSv is approximately 1 in 8000 based upon the nominal risk coefficient for an adult published in the 2007 recommendations of the International Commission on Radiological Protection. This can be compared against the natural lifetime risk of fatal cancer of 1 in 3 to 1 in 4.

It is anticipated that the participants will receive some of these X-ray examinations as part of their normal clinical care. It is estimated that around 60% of the total dose from these examinations will on average be additional to that received in normal practice and solely received as a result of participating in the study.

3. Details of person acting as lead Medical Physics Expert

Name:	Mr David M Johnstone
Post:	Principal Physicist
Registration No.:	CS 09130
Organisation:	The Radiological Protection Centre, St. George's Healthcare NHS Trust

Address:	Unit 5 The Observatory 24 Deer Park Road London
Post Code:	SW19 3UA
Telephone:	020 8725 2552
Fax:	020 8417 1338
Email:	David.Johnstone@stgeorges.nhs.uk

15b. Does your study involve invasive procedures such as blood taking, muscle biopsy or the administration of a medicinal product?

Yes - Each participant will have up to 50mL of venous blood drawn at each visit.

If so, please provide details and complete appendix – details of medicines and/or healthcare products – as required:
N/A

15c. Please indicate the experience of the investigators in the use of these procedures:

Blood will be taken by staff fully trained in phlebotomy.

15d. If medical devices are to be used on any participant, do they comply with the requirements of the Medical Devices Directives?
Yes

15e. Please name the locations or sites where the work will be done:
DMRC Headley Court

15f. Will group or individual interviews / questionnaires discuss any topics or issues that might be sensitive, embarrassing or upsetting? If so, please list these topics and explain how you will prevent, or respond to, volunteer discomfort:
Participant's relationship status and sexual function will be investigated in the form of participant filled questionnaires. Participant can at any time decide not to complete any part of the study or withdraw completely without stating a reason. If participants are in anyway distressed they will be offered support by clinical staff and if necessary referred on for further clinical care.

15g. Is it possible that criminal or other disclosures requiring action (e.g. evidence of professional misconduct) could take place during the study? If yes, give details of what procedures will be put in place to deal with these issues:

This is not anticipated and regarded as being extremely unlikely. If however this does occur additional support and advice would be taken at the time.

15h. Please describe any expected benefits to the research participant:
All participants will be thoroughly assessed for cardiovascular risk and for the presence of musculoskeletal disease such as osteoarthritis and osteoporosis. These assessments are more detailed than in normal clinical practice and if any treatable risk factors or disease is identified then appropriate measures will be taken to do so. In most cases this would be to contact the participant's primary care physician.

15i. Under what circumstances might a participant not continue with the study, or the study be terminated in part or as a whole?
Any participant can decide to stop participating in the study at any time without needing to give an explanation and without prejudicing their future care and treatment.
If a participant dies, he will obviously no longer be participating in the study however data on that participant will be used up to that point.
There are no reasons anticipated why the study may be terminated early in part or whole.

16. FINANCIAL INCENTIVES, EXPENSES AND COMPENSATION

16a. Will travel expenses be given?

It is anticipated that participants will travel to DMRC Headley Court for the study visits. Travel cost will be covered to and from DMRC Headley Court.

16b. Is any financial or other reward, apart from travel expenses, to be given to participants? If yes, please give details and justification:
Yes- As suggested by MoDREC to help retention in the study all participant that attend each follow-up point will be entered into prize draw of 5 cash prizes.
As recognition for the participant's considerable time and effort in taking part in the study they will be given £100 per visit as a thank you.

16c. If this is a study in collaboration with a pharmaceutical company or an equipment or medical device manufacturer, please give the name of the company:
No

17. CONFIDENTIALITY, ANONYMITY AND DATA STORAGE

17a. What steps will be taken to ensure confidentiality (including the confidentiality and physical security of the research data)? Give details of the anonymisation procedures to be used, and at what stage they will be introduced:
See Section 9, subsection 7 above.

17b. Who will have access to the records and resulting data?
Study staff only

17c. Where, and for how long, do you intend to store the consent forms and other records?

See section 9, subsection 7 above.

18. PARTICIPANT INFORMATION SHEET AND CONSENT FORM

The participant information sheet and consent form should be composed according to the guidelines and submitted with this form.

The following, where applicable, are attached to this form (please indicate):

[x] Participant Information Sheet
[x] Consent Form
[ ] Appendix relating to medicines and/or healthcare products
[ ] Letter to general practitioners
[ ] Letter to parents/guardians
[ ] Letter of other research ethics committee approval or other approvals
[ ] Copy of e-mail recruitment circular/poster/press advertisement
[x] Questionnaire/ topic guide/ interview questions
[ ] Evidence of permission from organisation (e.g. hospital) where research is to take place
[x] List of acronyms
[x] CVs of named investigators
[ ] CV of supervisor
[x] CV of Independent Medical Officer

Please list any other supporting documents:

Appendices A-Z- study outcome measures.

Comments about form

If you have any suggestions for improving this form please e-mail them to ***ethics@mod.uk***

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ADVANCE Study- List of Acronyms

AI	-	Augmentation Index
AMPQ	-	Amputee Mobility Predictor Questionnaire
AUDIT		Alcohol Use Disorder Identification Test
BMD	-	Bone Mineral Density
BMI	-	Body Mass Index
CAD	-	Coronary Artery Disease
CRP	-	C-reactive protein
CVD	-	Cardiovascular Disease
DASH	-	Disability Arm Shoulder Hand
DEXA	-	Dual energy X-ray absorptiometry
DM	-	Diabetes Mellitus
DMRC	-	Defence Medical Rehabilitation Centre
DMS	-	Defence Medical Services
ECG	-	Electrocardiogram
EQ5D	-	European Quality of Life 5 Domains
FEV1	-	Force expiratory velocity in 1 second
FVC	-	Forced Vital Capacity
HbA1C		Haemoglobin A1c
HRV		Heart Rate Variability
HsCRP		High Sensitivity C-reactive protein
HDL	-	High Density Lipoprotein
IBP	-	Inflammatory Back Pain
IPAQ	-	International Physical Activity Questionnaire
ISI		Insomnia Severity Index
HLA-B27		Human Leucocyte Antigen B27
IHD	-	Ischaemic Heart Disease
KCMHR		Kings College Mental Health Research
KOOS	-	Knee Osteoarthritis Outcome Score

MACE -	Major Adverse Cardiovascular Endpoint
MSPSS	Multidimensional Scale of Perceived Social Support
NAHS -	Non Arthritis Hip Score
NHS -	National Health Service
NRS -	Numerical Rating Scale
QoL -	Quality of Life
PCL -	PTSD Check List
PHQ-9	Patient Health Questionnaire-9
PSQI	Pittsburgh Sleep Quality Index
PTG	Post Traumatic Growth
PTG-I	Post Traumatic Growth Inventory
PTSD -	Post Traumatic Stress Disorder
PWV -	Pulse Wave Velocity
SIGAM-	Specialist Interest Group in amputee Medicine
SpA -	Spondyloarthropathy
TIA -	Transient Ischaemic Attack
6MWT-	6 Minute Walk Test

Appendix B:
ANNEX G- REQUEST TO AMEND A MODREC APPROVED PROTOCOL

ANNEX G- RREQUEST TO AMEND A MODREC APPROVED PROTOCOL

Amendment Request

General Information.

Protocol No: /MODREC/357/PPE/12

Protocol Title: The ADVANCE Study

Chief Investigator: Gp Capt Alex Bennett

Organisation: Academic Department of `Military Rehabilitation, DMRC, Headley Court

Date of Approval: 15th Jan 2013

Date of Approval of 1st Set of Amendments: 12th March 2015

Date of Approval of 2nd Set of Amendments: 23th Sep 2015

Date of Approval: **3rd Set of Amendments**: 7th Feb 2016

Date of Approval: **3rd Set of Amendments**: 2nd May 2018

Specific Information.

Date research started: 31/08/2015

No. of volunteers involved: 1200

No. of under 18s involved: 0

Amendments

Description of amendment required and rationale for Amendment:

The ADVANCE Study, the 20yr cohort study investigating long term medical (primary outcomes: cardiovascular disease and bone health/osteoarthritis) and psychosocial outcomes of military combat casualties was given initial MoDREC approval in January 2013 and amendments were approved in March 2015, September 2015, Feb 2016 and May 2018.

It is a large and ambitious study that has been logistically complex to organized however it is progressing well with 750 participants recruited. The target for participant recruitment is 1200 which we aim to complete by the end of 2019. The first round of follow up participants (3yr from initial visit) are due in the early spring 2019 and the investigators have reviewed the data collection and have decided it appropriate/necessary to make some minor amendments. The investigators would therefore be extremely grateful if MODREC would consider approving this additional set of amendments. These proposed amendments are outlined below.

All additions to the protocol (Version 14. 21.01.19) or appendices are highlighted in track changes/comments or in yellow and any deletions are detailed in the track-changed boxes in the right-hand margin.

Brief Summary of Proposed Amendments:

***Additional/Changes to questionnaires*:**

a. Manchester-Oxford Foot Questionnaire (MOXFQ). New questionnaire.

b. Drug Use Disorders Identification Test (DUDIT). New questionnaire to replace existing Drug/Medication history questionnaire.

c. Reason for leaving Armed Forces questionnaire. New questions

d. Highest level of education attained question. New question

e. Veteran specific outcomes questions. New questions

f. Who lives with you? New question.

g. Friends and family network. New questions

h. Mild traumatic brain injury questionnaire. New questionnaire

i. Rivermead Post Concussion Questionnaire. New Questionnaire

j. Replacing existing PCL (PTSD questionnaire) with updates PCL-5 questionnaire

k. Brief Pain Inventory (BPI)

l. Neuropathic Pain Symptom Inventory (NPSI)

m. Pain Catastophising Scale (PCS)

Additional assessments:

n. Collection/storage/testing of Blood and Urine at all follow up visits (3yrs, 5yrs, 10yrs, 15yrs and 20years post baseline visit) as per currently approved MoDREC protocol for initial/baseline visit.

o. Additional testing of male hormone profile on blood samples. No extra blood required.

Additional wording of consent:

p. Additional sentence added to consent form to confirm that participants are content with their study data being shared with existing and future research collaborators

q. Change existing consent from "…in accordance with the provisions of the Data protection Act 1998" to "... in accordance with the provisions of the data protection Act 2018."

Detailed Proposed Amendments:

***Additional/Changes to questionnaires*:**

a. **Manchester-Oxford Foot Questionnaire (MOXFQ). (Appendix Z9)**

It has become apparent during the baseline data collection from the participants in the ADVANCE study that we are currently not capturing problems associated with foot and ankle injury and that this is a common and significant problem. The investigators therefore propose including the internationally used and validated MOXFQ (Morley et al., 2013)

1. Morley, D., Jenkinson, C., Doll, H., Lavis, G., Sharp, R., Cooke, P., & Dawson, J. (2013). The Manchester-Oxford Foot Questionnaire (MOXFQ): Development and validation of a summary index score. *Bone & Joint Research*, *2*(4), 66–69. http://doi.org/10.1302/2046-3758.24.2000147

b. **Drug Use Disorders Identification Test (DUDIT). (replacement appendix Z1)**

The current drug brief drug history (appendix Z1) has been found by many participants to be complicated and long winded and we have had a number of negative comments about the questionnaire. We therefore propose exchanging it for the DUDIT (Berman, Palmstierna, Källmén, & Bergman, 2007) an internationally recognised and validated and concise questionnaire.

1. Berman, A. H., Palmstierna, T., Källmén, H., & Bergman, H. (2007). The self-report Drug Use Disorders Identification Test: Extended (DUDIT-E): reliability, validity, and motivational index. *Journal of Substance Abuse Treatment*, *32*(4), 357–369. http://doi.org/10.1016/j.jsat.2006.10.001

c. Personal reason for leaving the armed forces. (Appendix Z10)

Although the method of discharge (end of service, medical, redundancy etc) from the Armed forces is recorded the participant's personal reasons was not and a simple question has been added to explore this.

d. Highest level of education attained question (Appendix Z11)

Highest level of education attained is often linked with many psychosocial outcomes. This was not previously recorded so the investigators propose a simple question to ascertain this.

e. Veteran specific outcome questions. (Appendix Z12)

It is well recognised that some veterans run in to trouble with criminal convictions and/or with financial problems. This is not currently being recorded in this cohort we therefore proposed 2 simple questions to cover this.

f. Who lives with you? (Appendix Z13) and *g.* Family and friends' network (Appendix Z14)

The investigators propose 2 simple questionnaires to investigate who the participants are currently living with the who are important in their family and social network to achieve a better understanding of their social outcomes.

h. **OSU TBI Identification (Appendix Z15) and** *i.* **Rivermead Post Concussion Questionnaire (Appendix Z16)**

Mild Traumatic Brain injury has been recognised as a common and significant injury from the Afghanistan war. Currently the ADVANCE Study is not capturing the prevalence of mTBI in the study cohort or the potential consequences of it. This additional investigator administrated questionnaire is comprehensive but concise and is reliable and valid (Corrigan et al 2007)

Corrigan JD, Bogner JA. 2007. Initial reliability and validity of the OSU TBI Identification Method. J Head Rehabil; 22(6):318-329.

The outcome of TBI also needs to be assessed. The most commonly used and validated questionnaire for this is a quick and simple to use patient reported outcome measure, The Rivermead Post concussion Questionnaire (King et al 1995)

King, N. S., Crawford, S., Wenden, F.J., Moss, N.E.G. Wade, D.T. (1995). The Rivermead Post Concussion Symptoms Questionnaire: a measure of symptoms commonly experienced after head injury and its reliability *Journal of Neurology*, 242, 587-592.

j. **PTSD Checklist – 5 (PCL-5) (Appendix V)**

We are currently investigating symptoms of PTSD with the PTSD check list (PCL). This questionnaire has been superseded by the PCL-5 (Blevins, Weathers, Davis, Witte, & Domino, 2015) and therefore we now propose to use the PCL-5 for the ADVANCE Study.

Blevins, C. A., Weathers, F. W., Davis, M. T., Witte, T. K., & Domino, J. L. (2015). The
Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5): Development and Initial Psychometric
Evaluation. *Journal of Traumatic Stress*, *28*(6), 489–498. http://doi.org/10.1002/jts.22059

k. Pain assessment, characterisation and impact by the Brief Pain Inventory (Appendix Z17), *l.* the Neuropathic Pain Symptom Inventory (Appendix Z18) and the *m.* Pain Catastrophising scale (Appendix Z19)

Chronic pain is a predominant symptom of major trauma patients. Detailed information on the degree, character and impact of pain is essential to gather. Therefore, it is proposed to use the Brief Pain Inventory (Tan G et al 2004) (Appendix Z17), the Neuropathic Pain Symptom Inventory (Bouhassira et al 2004) (Appendix Z18) and the Pain Catastrophising scale (Sullivan et al 1995) (Appendix Z19)

Validation of the Brief Pain Inventory for Chromic non-malignant Pain. Tan G, Jensen MP, Thornby JI, Shanti BF. J Pain. 2004 Mar;5(2):133-7.

Development and Validation of the Neuropathic Pain Symptom Inventory. Bouhassira D, Attal N, Fermanian J, et al. Pain. 2004 Apr;108(3):248-57.

Sullivan MJ, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. Psychol Assess. 1995;7:524–532.

Additional assessments:

n. **Collection/storage/testing of Blood and Urine at all follow up visits**

We are currently taking blood and urine for testing/storage on all participants at the baseline visit. This is extremely well tolerated by the participants. It was an oversight of the investigators not to include blood and urine storage at all follow up visits. To fully track the medical outcomes of this cohort the investigators have decided it necessary to follow the same procedures of blood and urine testing/storing from baseline at all follow up visits and request this be approved by MoDREC.

o. **Additional testing of male hormone profile on blood samples.**

The investigators request that MoDREC approve a standard profile of male hormones to include testosterone, follicular stimulating hormone (FSH), luteinising hormone (LH) and sex hormone binding globulin (SHBG) to be tested on each follow up visit. This was not included in the original blood test battery however on consultation with a patient group (Afghan injured) they have requested that male hormones be measures due to concerns over long term fertility with major trauma. This has been discussed and confirmed as an appropriate investigation with Major Martin Moody Defence Consultant Advisor for Urology.

Additional wording of consent: (Appendix C)

p. An additional sentence has been added to the consent form to confirm that participants are content with their study data being shared with existing and future research collaborators

q. Change existing consent form wording from "…in accordance with the provisions of the Data protection Act 1998" to "... in accordance with the provisions of the data protection Act 2018."

Proposed method of data collection:

The DUDIT and the PCL-5 are replacement questionnaires. The PCL-5 may take a minute or 2 extra however the DUDIT is likely to save several minutes over the existing questionnaire that it is replacing. The additional 11 questionnaires/questions are short and will be able to be completed in 15 minutes maximum (tested on volunteers) however as mentioned time will bw saved (approximately 5 minutes) with the exchange of the DUDIT for the existing drug history questionnaire. All questionnaires will be collected prospectively in an anonymised format during the 6 hour study visit. The additional questions/questionnaires are not expected to impact significantly on participant's time or well-being. Staff will continue to be available to answer questions on any specifics in the questionnaire pack.

The additional hormones tests will not require further blood to be drawn but will be tested on the existing volume of 50ml of blood. If an abnormal hormones results are identified then MO/GP will be informed and Maj Martin Moody DCA Urology will be available to give specialist advice if needed.

Blood and rine collection and storage at follow up visits will follow the exact same protocol as the baseline visit with the exception that the genetic test for HLA-B27 will not be repeated,

Impact of Amendment not being approved:

This is a unique study in a unique population. The additional requested amendments have been carefully thought though by the project board of collaborating investigators from MoD, Imperial College London and Kings College London. We now have the benefit of working on the ADVANCE Study for 4 years and have the experience of having over 750 participants go through the data collection process. The additional data that will be collected from these suggested amendments will add valuable information to the long term medical and psychosocial outcomes of this severely and uniquely injured cohort.

Date Amendments approved/rejected (with reasons):

Date of Approval: 15th Jan 2013

Date of Approval of 1st Set of Amendments: 12th March 2015

Date of Approval of 2nd Set of Amendments: 23th Sep 2015

Date of Approval: **3rd Set of Amendments**: 7th Feb 2016

Date of Approval: **3rd Set of Amendments**: 2nd May 2018

Summary of changes to questionnaire (Ref:MODREC/357/PPE/12)

Table 1: Changes to questionnaire

Version 2	Action	Details
Questionnaire	Addition	MOXFQ
	Replacement	DUDIT to replace brief drug history
	Addition	Personal reason for leaving Armed Forces Question
	Addition	Highest level of educational attainment
	Addition	Veteran specific outcome questions
	Addition	Who Lives with you question
	Addition	Family and friend network question
	Addition	Ohio State University TBI Identification method
	Addition	Rivermead Post Concussion Questionnaire
	Replacement	Replace PCL with new version PCL-5
	Addition	Brief Pain Inventory
	Addition	Neuropathic Pain Symptom Inventory
	Addition	Pain Catastrophising Scale
Assessment	Addition	50mls or blood and 50ml of urine (as per baseline protocol) to be taken/tested/stored (as per baseline protocol) at all follow up visits (3yr, 5yr, 10yr, 15yr and 20yr)
	Addition	Male hormone profile of testosterone, FSH, LH and SHBG to be tested on existing volume of blood at all follow up visits

Reference

Berman, A. H., Palmstierna, T., Källmén, H., & Bergman, H. (2007). The self-report Drug Use Disorders Identification Test: Extended (DUDIT-E): reliability, validity, and motivational index. *Journal of Substance Abuse Treatment*, *32*(4), 357–369. http://doi.org/10.1016/j.jsat.2006.10.001

Blevins, C. A., Weathers, F. W., Davis, M. T., Witte, T. K., & Domino, J. L. (2015). The Posttraumatic Stress Disorder Checklist for DSM-5 (PCL-5): Development and Initial Psychometric Evaluation. *Journal of Traumatic Stress*, *28*(6), 489–498. http://doi.org/10.1002/jts.22059

Bouhassira D, Attal N, Fermanian J, et al. Development and Validation of the Neuropathic Pain Symptom Inventory.. Pain. 2004 Apr;108(3):248-57.

Corrigan JD, Bogner JA. 2007. Initial reliability and validity of the OSU TBI Identification Method. J Head Rehabil; 22(6):318-329.

King, N. S., Crawford, S., Wenden, F.J., Moss, N.E.G. Wade, D.T. (1995). The Rivermead Post Concussion Symptoms Questionnaire: a measure of symptoms commonly experienced after head injury and its reliability *Journal of Neurology*, 242, 587-592.

Morley, D., Jenkinson, C., Doll, H., Lavis, G., Sharp, R., Cooke, P., & Dawson, J. (2013). The Manchester-Oxford Foot Questionnaire (MOXFQ): Development and validation of a summary index score. *Bone & Joint Research*, *2*(4), 66–69. http://doi.org/10.1302/2046-3758.24.2000147

Sullivan MJ, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. Psychol Assess. 1995;7:524–532.

Validation of the Brief Pain Inventory for Chromic non-malignant Pain. Tan G, Jensen MP, Thornby JI, Shanti BF. J Pain. 2004 Mar;5(2):133-7.

Appendix C:
MODREC APPROVAL
Protocol 357

MOD Research Ethics Committee
(Personnel, Protection and Effectiveness)

From the Chairman
Professor Allister Vale
National Poisons Information Service (Birmingham Unit),
City Hospital, Birmingham B18 7QH

Telephone: 0121 507 4123
e-mail:allistervale@npis.org

Wg Cdr Alex Bennett
PhD FRCP
Head of Research DMRC
Headley Court,
Epsom,
Surrey

Our Reference: 357/PPE/12

Date: 15 January 2013

Dear Wg Cdr Bennett,

First of all I would like to congratulate you for not only submitting a revised protocol with tracked changes but also a detailed letter indicating your responses. This was most helpful to the Committee and as I said at the time is an excellent example of how investigators should resubmit their protocols.

The revised protocol has been approved by MODREC but you may like to correct a small number of typographical errors in the PIS.

I wish you and your colleagues a successful study and we look forward to receiving in due course a brief summary of the results so that these can be filed in accordance with the arrangements under which MODREC(PPE) operates.

With kindest regards,

Allister Vale

Allister Vale MD FRCP FRCPE FRCPG FFOM FAACT FBTS FBPharmacolSocHon FRCPSG

Cc Dr John Scadding, Professor David Jones, Dr Paul Rice OBE, Marie Jones