DOES MILITARY TRAUMA ACCELERATE THE AGEING PROCESS?



CONTENTS

List of figures 3

Foreword 4

Summary infographic figures 5

Executive summary 8

Main report

Introduction 11

Background 12

Aim 14

Methods 15

Results 18

- **Physical injury and biological ageing** 19
- **Mental health and biological ageing** 19
- **Physical fitness, physical injury and biological ageing** 21
- **Physical fitness, mental health and biological age** 22
- **Other Potential Negative Influences on Biological Ageing** 23

Discussion 25

- **Physical and psychological injury** 25
- **Physical injury and biological ageing** 26
- **Mental health and biological ageing** 27
- **Benefits of Physical activity for biological ageing in veterans** 28
- **Other Negative Influences on Biological Ageing** 29

Limitations 29

Conclusions 29

Recommendations 30

References 31

List of Figures

Figure 1. Infographic summary of the impact of physical injury on the pace of ageing in the ADVANCE military cohort, with the beneficial effects of physical activity and fitness.

Figure 2. Infographic summary of the impact of mental health on the pace of ageing in the ADVANCE military cohort.

Figure 3. Infographic summary of the impact of Adverse Childhood Experience, Smoking and Sleep duration on the pace of ageing in the ADVANCE military cohort.

Figure 4. Life expectancy and healthy life expectancy for females in the UK.

Figure 5. Epigenetic clocks based upon the relationship between DNA methylation and chronological age, with deviations indicating a higher or lower biological age.

Figure 6. Study cohort demographic characteristics.

Figure 7. Dunedin Pace data for veterans who were uninjured, injured without amputations, and amputee veterans.

Figure 8. Dunedin Pace data for all ADVANCE veterans scored for Depression; Anxiety, or PTSD.

Figure 9. Pace of ageing in relation to physical fitness determined using pVO_2max and 6 minute walk test.

Figure 10. Impact of physical fitness, pVO2max, on Pace of ageing for veterans with depression, anxiety or PTSD.

Figure 11. Relationship between Adverse Childhood Experience, Smoking, BMI and sleep duration and Dunedin Pace of ageing.

Foreword

Whilst most ex-Service personnel enjoy good health outcomes, those deployed to conflict zones face an increased risk of both physical and psychological injury. Advances in medical care and technology have significantly improved survival rates following traumatic injury, transforming trauma care and improving outcomes for military and civilian populations alike. However, despite these advances, research indicates that individuals who survive major traumatic injury, including serving personnel, remain at increased risk of earlier mortality and the premature onset of certain long-term health conditions.

We were therefore pleased to be able to award a grant to the University of Birmingham to support this innovative study. Using data from the internationally recognised ADVANCE longitudinal military trauma study, the research enables us to better understand why ex-Service personnel who sustain major trauma experience poorer long-term health outcomes. The findings will inform more effective support for injured personnel, optimise recovery, and assist personnel to lead more fulling and healthier lives after they leave the Armed Forces.

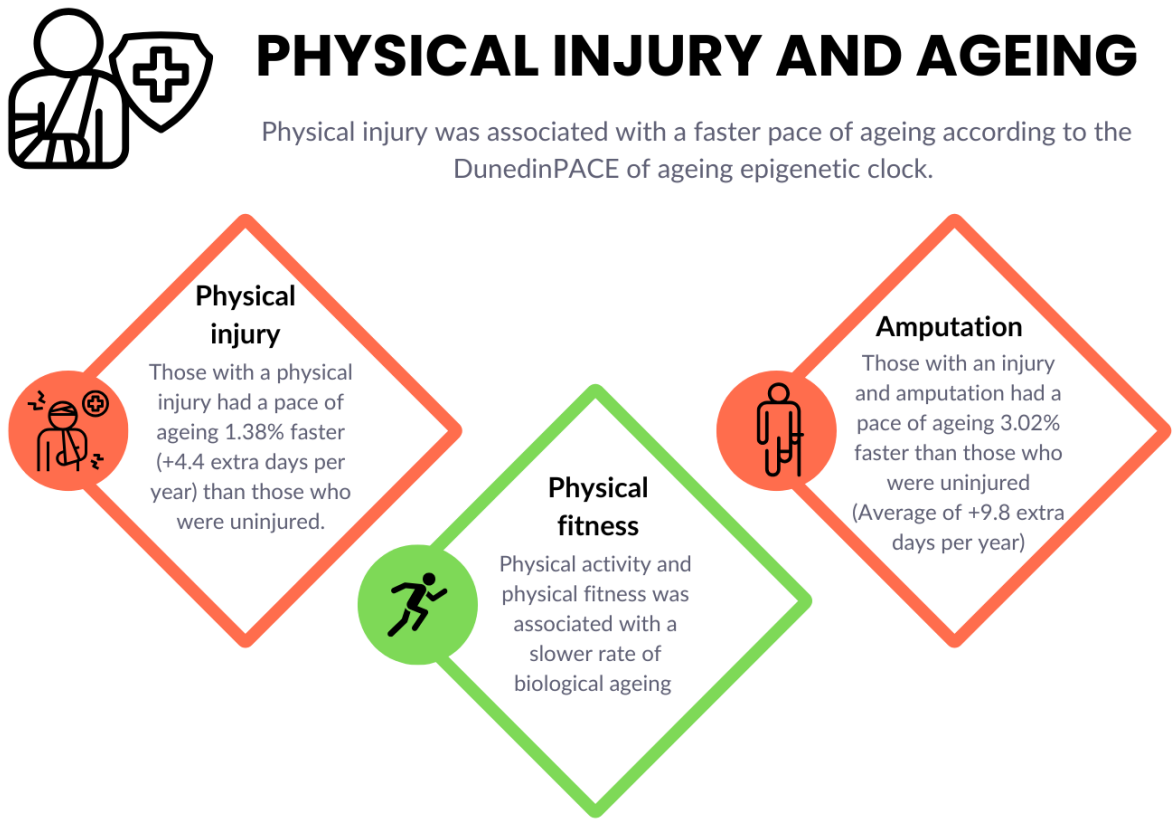
Recognising how the unique conditions of service can impact on the health and wellbeing of serving and ex-serving personnel and their families is key to improving their outcomes and the support they are offered. The study helps us to provide new evidence on the long-term effects of physical and psychological trauma on ageing, and the results are significant and compelling: finding that physical injury and mental ill health can accelerate the pace of ageing for ex-Service personnel who have experienced traumatic injury. But crucially, the research also shows that accelerated ageing is not inevitable. Physical activity, fitness, and maintaining a healthy lifestyle can help mitigate the impact of trauma on ageing.

These findings provide an important opportunity to improve the long-term outcomes for personnel who experience traumatic injury during service. I would therefore encourage all those involved in supporting personnel during and after service to read this report and consider the positive difference that lifestyle changes can make.

Michelle Alston, Chief Executive, Forces in Mind Trust.

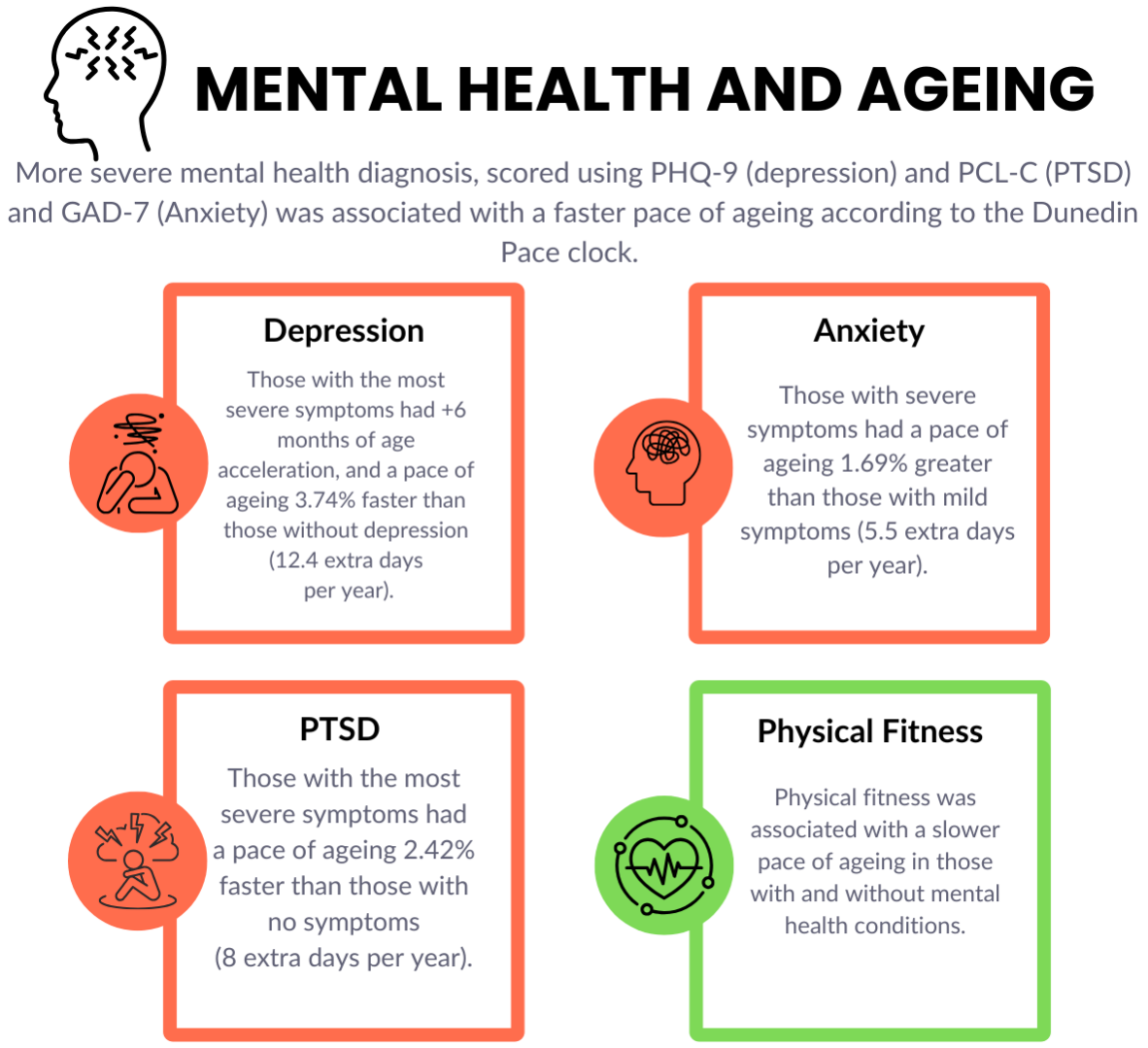
Physical injury and ageing in military veterans – Summary

Figure 1. Infographic summary of the impact of physical injury on the pace of ageing in the ADVANCE military cohort, and the beneficial effects of physical activity and fitness.



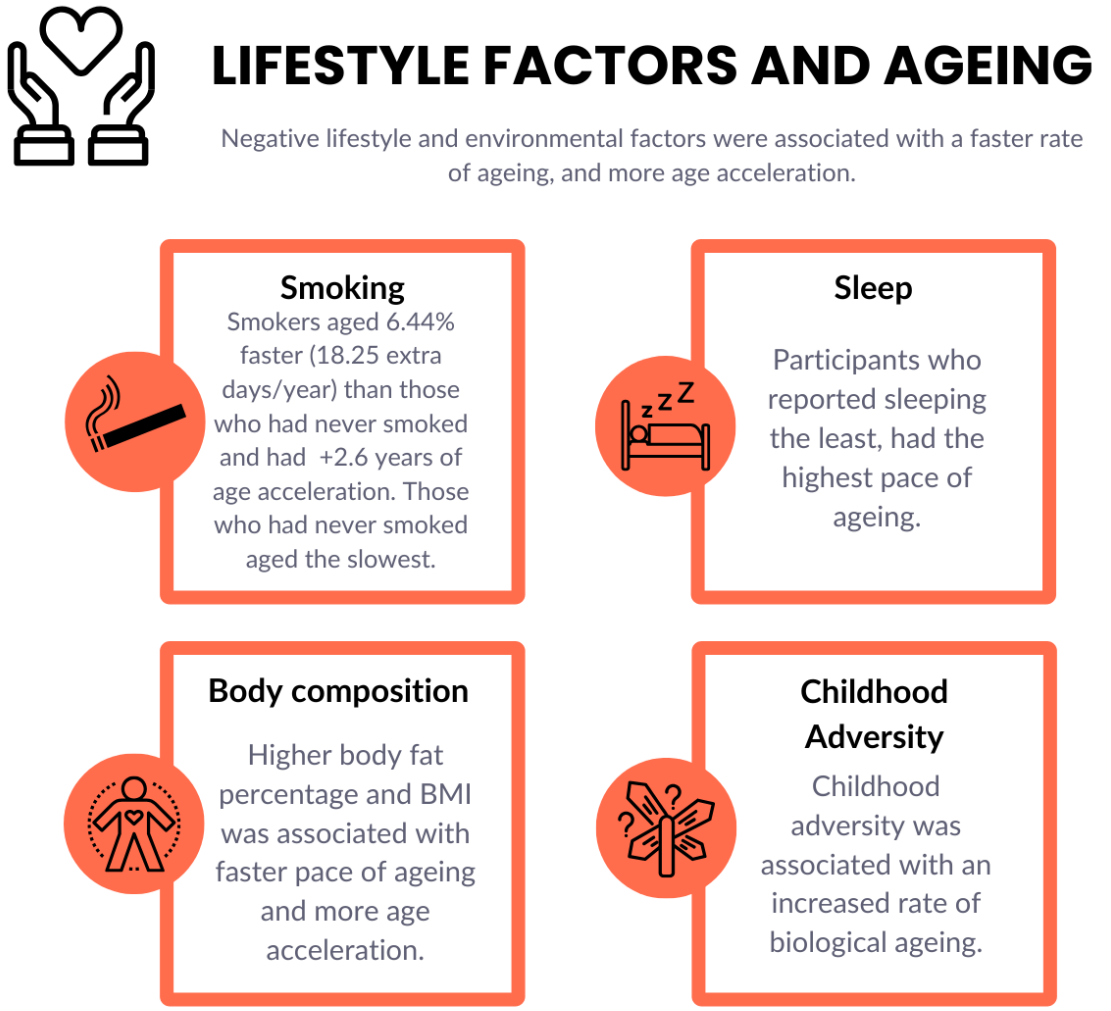
Mental health and ageing in military veterans - Summary

Figure 2. Infographic summary of the impact of mental health on the pace of ageing in the ADVANCE military cohort.



Lifestyle factors and ageing in military veterans - Summary

Figure 3. Infographic summary of the impact of Adverse Childhood Experience, Smoking and Sleep duration on the pace of ageing in the ADVANCE military cohort.



Executive Summary

Introduction

In the general population, traumatic injury is the major cause of death in young adults. Although most patients now survive their injuries, there is increased morbidity and mortality in these patients. Serving in the military, especially when deployed to a conflict zone, has increased risk of physical injury as well as psychological illness such as post-traumatic stress disorder (PTSD). Both major physical trauma and mental ill health are associated with increased prevalence of chronic diseases normally considered to be age-related, such as heart disease, cancer and dementia. One possible explanation is that major trauma is accelerating the biological ageing process. Until recently it was not possible to determine how quickly a person was ageing, but there are now blood tests that can accurately estimate a person's biological age as opposed to their chronological age. The aim of this study was to determine if ageing was accelerated in military veterans who had sustained an injury and those with mental health issues, including PTSD, and whether lifestyle factors such as maintaining physical fitness could ameliorate any accelerated ageing.

Methods

We worked with the ArmeD SerVices TrAuma and RehabilitatioN OutComE (ADVANCE) cohort, which consists of injured male UK veterans (N=579) and a comparison group of uninjured male UK servicemen and veterans (N=566) who had been deployed to the Afghanistan conflict. The groups were matched for age, rank, deployment period, service, regiment and role-in-theatre. The study has collected blood samples and extensive associated clinical, demographic and lifestyle data. We analysed DNA from 1125 blood samples in order to determine the degree of methylation[a], which was then used to estimate the participant's biological age, referred to as an epigenetic clock. We used four different epigenetic clock algorithms (Horvath, Hannum, Phenoage, GrimAge), to calculate the biological age at time of sampling, as well as the Dunedin Pace clock, which estimates the pace of ageing. We compared biological ageing in the injured and uninjured groups and in those with depression, anxiety or PTSD.[a] We assessed the impact of factors that might influence the acceleration of ageing, both

[a] DNA can have chemical groups added to it, in this case methyl groups. The degree of methylation is related to the biological ageing process, the greater the methylation the older the individual is biologically.

positive influences such as physical activity and fitness, and negative influences such as smoking, poor sleep, body mass index (BMI) and adverse childhood experiences.

Results

We found that biological ageing, specifically the pace of ageing, was increased by both physical injury and mental ill health. The increase in the pace of ageing was 4.4 extra days of ageing per year for those with a physical injury, rising to 9.8 days if amputation was involved. An extra 8 days of ageing per year was seen for those with PTSD, 5.5 days for those with anxiety and 12.4 days for those with depression. Physical fitness, evidenced by a higher predicted VO_2max and 6-minute walk test, was able to ameliorate the negative impact of ageing in those with a physical injury. The positive effect was also significant for those with mental health issues, including depression, anxiety and PTSD. Increases in the pace of ageing, as well as one of the epigenetic clocks determining biological age (GrimAge), was seen with smoking (both current and ex-smokers), a higher BMI and amount of body fat, adverse childhood experiences (especially school related) and shorter sleep duration.

Conclusions

Our main conclusion is that in a cohort of military veterans, several years after their deployment, both physical injury and mental ill health (depression, anxiety and PTSD) accelerated their biological age, specifically the pace at which they were ageing. Although the degree of this acceleration, ranging from 4.4 to 12.4 days per chronological year, may seem minimal, in such a young cohort this may have significant implications for their future health. Importantly, lifestyle factors such as physical activity and fitness, not smoking and having good sleep duration, were all modifiable factors that were able to ameliorate the negative impact of physical and psychological harm.

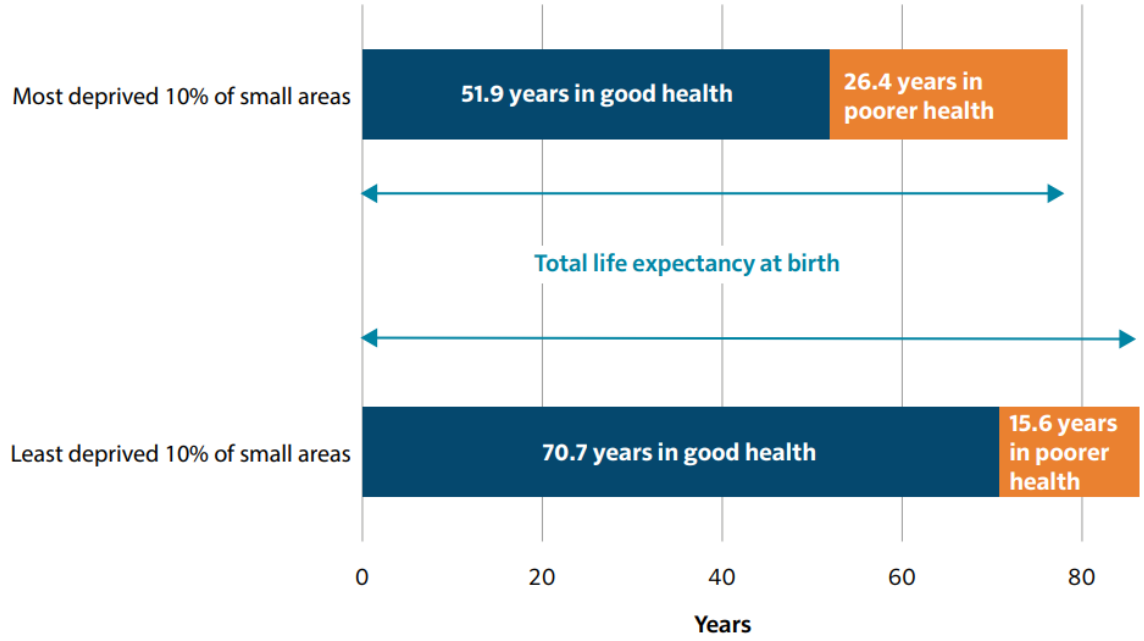
Key Findings, Recommendations and Limitations

- Physical injury and poor mental health (depression, anxiety and PTSD) both accelerated the pace of biological ageing in military veterans.
- Physical activity and fitness were able to reduce the impact of injury and poor mental health on biological age.
- Adverse childhood experiences, especially at school, increased the pace of ageing.
- Smoking, high BMI and high body fat, and short sleep duration all accelerated biological ageing.

- Advice on lifestyle changes, including increased physical activity, to reduce the impact of injury and mental health on biological ageing should be given to serving personnel and veterans.
- Support to maintain a healthy BMI, stop smoking and improve sleep should be given to veterans and serving personnel to optimise the pace of biological ageing.
- As there are also drugs and nutritional supplements that can reverse biological age, clinical trials to test these approaches in military populations with accelerated ageing would be beneficial.
- A limitation of the study is that the data were gained from a male cohort and whether the findings applied to women cannot be ascertained.
- The absence of data for participants before they were deployed is another study limitation.

Introduction

With falling birth rates and increased longevity, we are an ageing population. Life expectancy at birth in the UK between 2020 and 2022 was estimated at 82.6 years for women and 78.6 for men (Office for National Statistics, 2024). However, healthy life expectancy (HLE), the period of life we can expect to spend in good health, is considerably lower than overall life expectancy. Importantly both life expectancy and healthy life expectancy are influenced by a variety of factors, including non-modifiable variables such as socioeconomic status **(Figure 4**, Chief Medical Officer's Annual report 2023)[1] as well as modifiable influences such as diet and physical activity[2].



Source data: Office for National Statistics (ONS), Health state life expectancies by national deprivation deciles, England: 2018 to 2020[6]

Figure 4. Life expectancy and healthy life expectancy for females in the UK. Taken from the Chief Medical Officers annual report, 2023.

If we are to try and increase HLE, it is imperative to be able to assess how well someone is ageing; *Is their trajectory one that indicates a long and healthy old age, or a lifespan that is short and comprised of many years in poor health?* There are now several ways we can achieve this. For example, when considering the risk of poor heart health we can calculate the Q-risk score[3] and reduce this through lifestyle change or pharmacologically, e.g. with the use of statins. More recently, blood cell DNA-based measures that give an accurate estimate of the degree of overall ageing in an individual, termed epigenetic clocks[4] have emerged. Through

assessing the degree of methylation in a person's DNA, we can determine their biological age and compare this with their chronological age to determine if their ageing is accelerated or not[4].

These advances have broad implications for understanding the impact of a range of factors on how fast we are ageing. In the context of military veterans, this will be particularly useful in determining the impact of both physical injury and mental ill health on those deployed to conflict zones. Crucially, such information can also be used to determine lifestyle influences that may be able to moderate any negative impacts of injury on the rate of biological ageing in veterans and serving personnel.

Background

Injury, morbidity and mortality

Injury is the major cause of death amongst young adults[5] and each year over 5 million people die as a result of injury, accounting for 10% of annual deaths globally[6]. However, recent advances in emergency medicine have resulted in significant increases in survival post-injury, with a majority of major trauma victims now surviving their injuries[7]. Interestingly, there is growing evidence to suggest that sustaining a major injury is associated with the premature development of multiple, often "age-associated" diseases such as arthritis, heart disease and hypertension[8]. One study of survivors of burn and non-burn injuries has shown an increased incidence of heart disease[9] and a second study has reported increased mortality in burns survivors[10].

Biological ageing can be defined as "*the reduced resilience of a person over time that increases the risk of disease and death*" and we have suggested that one consequence of a major trauma that may explain these clinical outcomes is that injury is accelerating the biological ageing process[11]. To support this idea, studies have shown that injury increases some of the processes driving ageing, the so-called Hallmarks of Aging[12]. Examples of this are burns causing chronic low grade inflammation[13] and negative metabolic changes. To test our hypothesis, it is necessary to determine how rapidly an individual is ageing, comparing the injured and uninjured.

Physical Injury and Biological Ageing

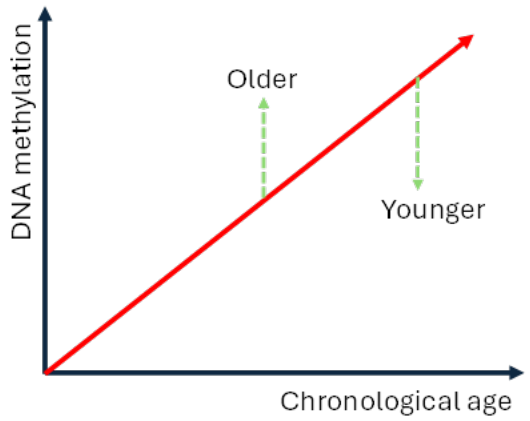


Figure 5. Epigenetic clocks based upon the relationship between DNA methylation and chronological age, with deviations indicating a higher or lower biological age.

It is now possible to assess how rapidly someone is ageing. For example, DNA can be extracted from blood cells and the degree of methylation of the DNA is correlated with the person's chronological age, termed an epigenetic clock **(Figure 5)**. Crucially, the more methylated the DNA, the higher the risk of disease and death[4], hence this is an indicator of biological age, or how quickly someone is ageing. We have shown previously that adults with a burn injury had an elevated rate of biological ageing compared to their uninjured peers at 6 months post-injury[14]. As injury is more likely in military personnel deployed to a war zone, the possibility that injured veterans may have poor health outcomes due to accelerated ageing is worthy of investigation. One small study in 2015 assessed DNA methylation and biological age in Dutch military veterans of the Afghanistan conflict 6 months after their injury. They showed an age acceleration of almost 2 years in those with a physical injury[15].

Psychological Ill Health and Biological Ageing

The literature concerning mental health and biological ageing is sparse and contradictory. The study of Dutch military veterans mentioned above reported no age acceleration in those diagnosed with PTSD[15]. However, in a more recent larger study of trauma in the general population, PTSD was associated with an increase in biological age in adults[16]. There is thus uncertainty in the literature as to whether trauma and injury, either physical or psychological, increases biological age and if this acceleration is long lasting. Studies of UK veterans have reported a high prevalence rate of PTSD (7.4% for ex-serving personnel vs 4% in the general population). Those veterans who served in a combat role on deployment to Iraq or Afghanistan had the highest prevalence rate at 17.1%[17]. Additionally, recent analysis has shown that 30.7% of UK veterans deployed in a combat role report common mental disorders such as depression and anxiety[17]. This issue therefore needs further research.

Factors influencing Biological Ageing

To the best of our knowledge none of the published studies have investigated factors that are able to modify the effect of trauma upon biological ageing, making it difficult to provide advice to veterans and civilian trauma survivors on how to optimise their recovery and long-term health outcomes. We know that in healthy adults, factors such as physical activity can influence the epigenetic clock positively[18], but whether this lifestyle factor could influence accelerated ageing after something as major as conflict acquired injuries is unknown. As veterans who have seen active service may have injuries not experienced by civilians, such as an injury caused by an Improvised Explosive Device (IED), as well as psychological trauma from deployment, it is important to know if lifestyle can overcome such trauma when seen individually or in combination with other interventions known to reverse biological age. For example, we have shown recently that a nutritional supplement intervention was able to reduce epigenetic age in older adults who had accelerated ageing[19].

Aim

Our aim was to test the hypothesis that biological ageing is accelerated in military veterans who have sustained a physical injury or have been diagnosed with chronic mental health conditions such as PTSD, as well as common mental disorders (depression, anxiety). We also aimed to ascertain the positive benefits of physical activity and fitness on any accelerated ageing. The aim was achieved by analysing DNA methylation in blood samples from veterans of the Afghanistan conflict, who are members of the ArmeD SerVices TrAuma and RehabilitatioN OutComE (ADVANCE) cohort, half of whom were injured and half were not. The following questions were posed to identify factors that may be driving any acceleration of ageing, or giving protection against it:

- Does physical injury accelerate biological ageing?
- Does the degree of physical injury, notably amputation, affect the amount of age acceleration?
- Does the presence of mental ill health (anxiety, depression, PTSD) influence biological ageing?
- Do adverse childhood events influence biological ageing in our cohort?
- Was biological ageing influenced by lifestyle factors such as sleep or smoking?

- Does body composition influence biological ageing in this cohort?
- Do physical activity and fitness moderate any acceleration of biological ageing?

Methods

Study population: To carry out the project, we worked with the ArmeD SerVices TrAuma and RehabilitatioN OutComE (ADVANCE) cohort of military veterans from the 2002-2014 Afghanistan conflict. This longitudinal study enrolled an injured group of male UK veterans (N=579) and a comparison group of uninjured male UK servicemen and veterans (N=566) between 2016 and 2020. The groups were matched for age, rank, deployment period, service, regiment and role-in-theatre. The study has collected blood samples and extensive associated clinical, demographic and lifestyle data[20]. We had previously performed a small pilot study of 150 veterans, 100 injured and 50 uninjured, assessing their biological age but focussing on the impact of physical injury only[21]. The data from this pilot were merged with the data generated in this study to give us coverage of the whole cohort, allowing us to consider the impact of both physical injury and psychological ill health on biological ageing.

Ethical approval: The ADVANCE study was approved by the Ministry of Defence Research Ethics Committee (MoDREC), with protocol number 357/PPE/12.

Estimation of biological age: To estimate biological age, we extracted DNA from stored blood samples and measured the amount of methylation at specific sites on the DNA. It has been established that there is a close association between the amount of DNA methylation and chronological age, termed an epigenetic clock[4]. There are many different epigenetic clocks, some of them were developed to relate to mortality and some to morbidity. We therefore calculated a range of clocks, namely: Horvath[4], Hannum[22], Phenoage[23], GrimAge[24]. In addition, a recent clock has been developed that gives an individual's pace of ageing, rather than their current biological age. This clock, the Dunedin Pace clock[25], is therefore useful for thinking about an individual's ageing trajectory, so we also included this clock in our analysis.

Estimation of physical fitness (VO_2max) and physical activity levels: One measure used to assess physical fitness in an individual is VO_2max. This involves a person exercising to their maximal ability (exhaustion) with oxygen consumption and carbon dioxide exhalation being measured. However, this measure was not available in the ADVANCE cohort, largely due to

the impact of injury on the ability to exercise to exhaustion in some participants. We therefore used a new method to estimate predicted VO_2max (pVO_2max) from proteins measured in the blood, using Somascan technology[26]. We determined if this correlated well with the self-reported data, the IPAQ questionnaire[27], from participants on their daily physical activity levels to give confidence in this as a measure of fitness and thus regular involvement in exercise. We also included analysis of the 6-minute walk test, which measures how far a person can walk in 6 minutes and is a measure of physical ability relating to ongoing physical activity levels.

Assessing mental health: For mental health we used data from three questionnaires covering the following conditions:

Depression: We used the Patient Health Questionnaire-9 (PHQ-9)[28], a nine-item self-report questionnaire that records depressive symptoms over the previous two weeks. Scores range from 0-27.

Clinical cut-offs: Score 0-4 – no depression; 5-9 – mild depression**;** 10-14 – moderate depression**;** 15-19 - moderately severe depression**;** 20+ - severe depression.

We combined the highest three categories for ascertaining probable depression, defined as a score of ≥10[29].

Anxiety: Generalised anxiety was measured using the Generalised Anxiety Disorder-7 (GAD-7), a seven-item self-report questionnaire that records anxiety symptoms over the previous two weeks[30]. Scores range from 0-21.

Clinical cut-offs: Score 0-4 – minimal anxiety**;** 5-9 – mild anxiety**;** 10-14 – moderate anxiety**;** 15-21 - generalised anxiety.

We combined the highest two categories with generalised anxiety defined as a score of ≥10.

PTSD: PTSD was measured using the PTSD Check List (PCL-C), a 17-item self-report questionnaire examining the symptoms of PTSD according to the DSM-IV over the past month[31]. Scores range from 17-85.

Clinical cut-offs: Probable PTSD was defined as a score of ≥50, a score of 40 to 49 was classified as sub-threshold PTSD indicating the individual was demonstrating several symptoms of PTSD but not meeting the full diagnostic criteria for PTSD.

Other Potential Negative Influences on Biological Ageing: We considered a range of other factors that have the potential to influence biological ageing, using appropriate self-report questionnaires, including:

- Adverse Childhood Experiences and Biological Ageing, this was assessed using the Adverse Childhood Experiences Questionnaire (ACE-Q), a 12-domain scale with a maximum score of 12, used to correlate childhood maltreatment and adverse rearing practices with adult health outcomes[32].
- Smoking: Smoking status was determined by self-report and was categorised as current smoker, previous smoker, and non-smoker.
- Sleep: Sleep duration was determined from the Pittsburgh Sleep Quality Index questionnaire[33].
- Body composition: BMI and waist to hip ratio were determined by physical examination. Muscle mass (lean mass) and fat mass were determined by Dual-energy X-ray Absorptiometry (DEXA).

Statistical analysis**:** Epigenetic age acceleration was defined as the residual of a linear regression of the epigenetic age on chronological age[34]**.** We excluded outliers that had epigenetic age measures (Pace or acceleration) exceeding 2 standard deviations. Military rank and ethnicity were controlled for in the analyses. Where appropriate, *P* values were corrected for multiple testing.

Results

From the 1145 participants in the ADVANCE study, we obtained 1125 blood samples and analysed them for DNA methylation status. The demographic details of the cohort are shown in **Figure 6**. All participants were male and the uninjured and injured groups were matched for age. The mean age of the whole cohort was 34.5 years (range 23.4 – 59.9), the mean age of the injured group was 34.4 years (range 23.4-55.9) and the uninjured was 34.7 years (range 23.8-59.9).

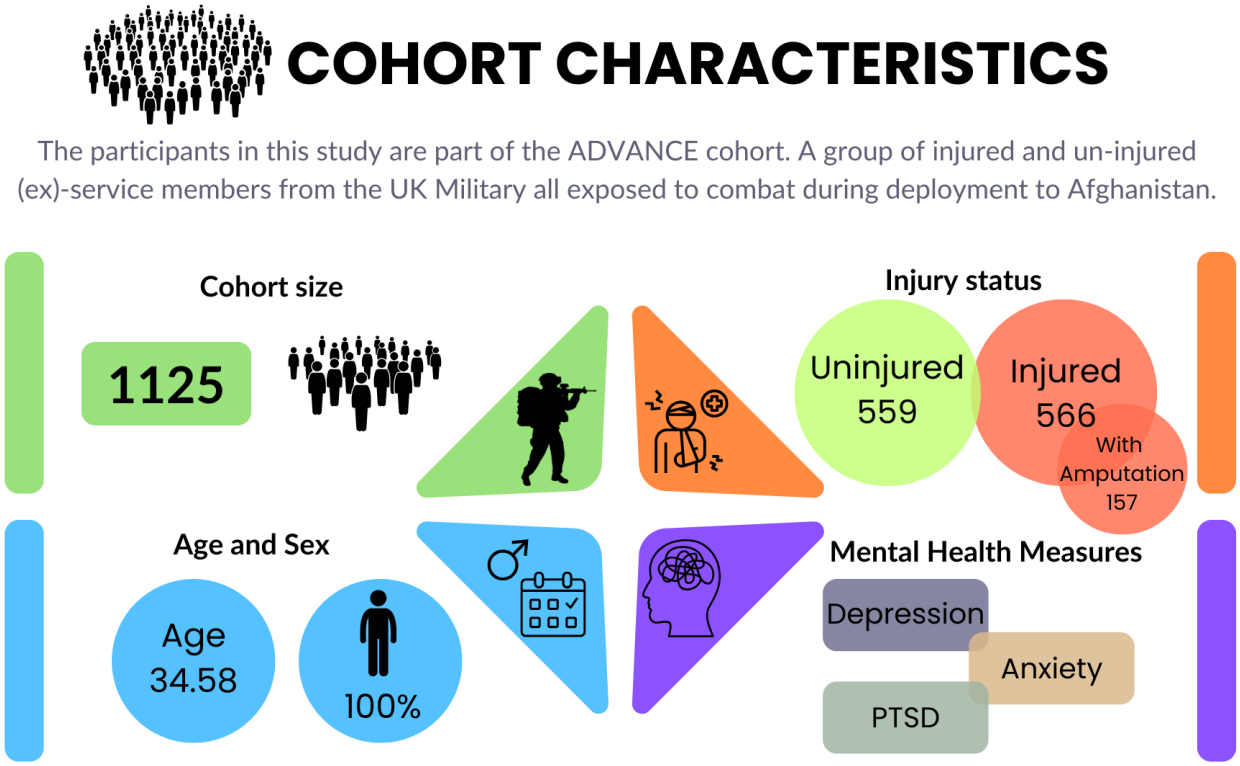


Figure 6. Study cohort demographic characteristics.

We confirmed that our DNA methylation was correlated with the chronological age of the veterans and so could be used to generate epigenetic clocks and biological ageing data. We then calculated biological age acceleration using four different clock algorithms: Horvath[4], Hannum[22], Phenoage[23] and GrimAge[24]. Finally, we also generated the Dunedin Pace clock[25], which assesses the pace of ageing of an individual rather than the actual biological age at time

of sampling. This gives useful information on their current ageing trajectory. All data were adjusted for military rank and ethnicity.

Physical Injury and Biological Ageing

The data from the 4 epigenetic clocks showed a broad spread of data, with no difference in biological age between the injured and uninjured groups. There was also no difference in biological age when we considered the severity of injury and split the injured group into those with an amputation and those without. However, the Dunedin Pace clock did show a significant difference between injured and uninjured participants, with the pace of ageing higher in the injured group. When the injured group was broken down into those with and without amputations, the higher rate of ageing was seen in those with an amputation (**Figure 7**).

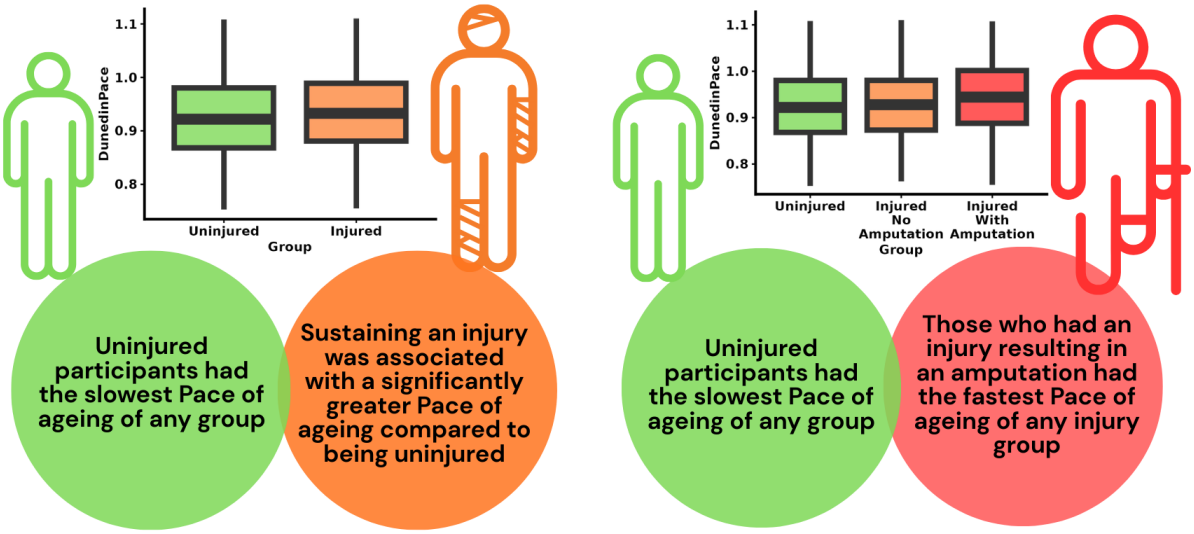


Figure 7. Pace of ageing in veterans who were uninjured, injured without amputation, or injured with amputation

Mental Health and Biological Ageing

Addressing the issue of mental health and biological ageing in veterans, we considered anxiety, depression, and PTSD. For this analysis, we initially used data for the whole cohort to ensure we had a sufficient sample size for the analysis, we later looked at the physically injured and uninjured separately. Data were not available for the whole of the cohort. For depression, 807 (71.7%) participants responded, 208 (25.7%) had symptoms of probable depression and 231 (28.6%) of mild depression. For anxiety, 1088 responses (96.7%) were gained, 175 (16%) were classified as having generalised anxiety and 204 (18.7%) with mild anxiety. PTSD data

were available for 1100 participants (97.7%), 135 (12.2%) had probable PTSD and 98 (8.9%) were deemed sub-threshold for PTSD, that is they displayed insufficient symptoms to meet the criteria for PTSD.

We found little impact of anxiety, depression or PTSD on the four epigenetic clocks, though GrimAge was mildly affected by depression ($p<0.05$). Again, it was the Dunedin Pace clock that was detecting major influences of mental health on the pace of ageing in the veterans. For depression, the group with probable depression had a significantly higher pace of ageing than those with no depression ($p<0.0001$) and also higher in comparison to those with mild depression ($p<0.01$) **(Figure 8)**. Those with generalised anxiety had a higher pace of ageing compared to those with either mild or minimal ($p<0.05$ for both) anxiety (**Figure 8**). For PTSD both those with subthreshold and probable PTSD had slightly higher Dunedin Pace scores than those without PTSD ($p<0.05$; **Figure 8**).

As there was only a modest impact of injury on one of the four epigenetic clocks when the whole cohort was analysed, unsurprisingly there was no effect when data were split for injured and uninjured groups. However, we found that in the uninjured group probable depression significantly impacted the Dunedin Pace score ($p<0.01$) and was significantly higher in those with sub-threshold PTSD ($p<0.05$), but there was no effect of anxiety. For the injured group, the impact of mental health was in general greater, with a significant impact on pace of ageing for probable depression ($p<0.0001$), generalised anxiety ($p<0.05$), or probable PTSD ($p<0.05$), the higher level seen for sub-threshold PTSD was not statistically significant.

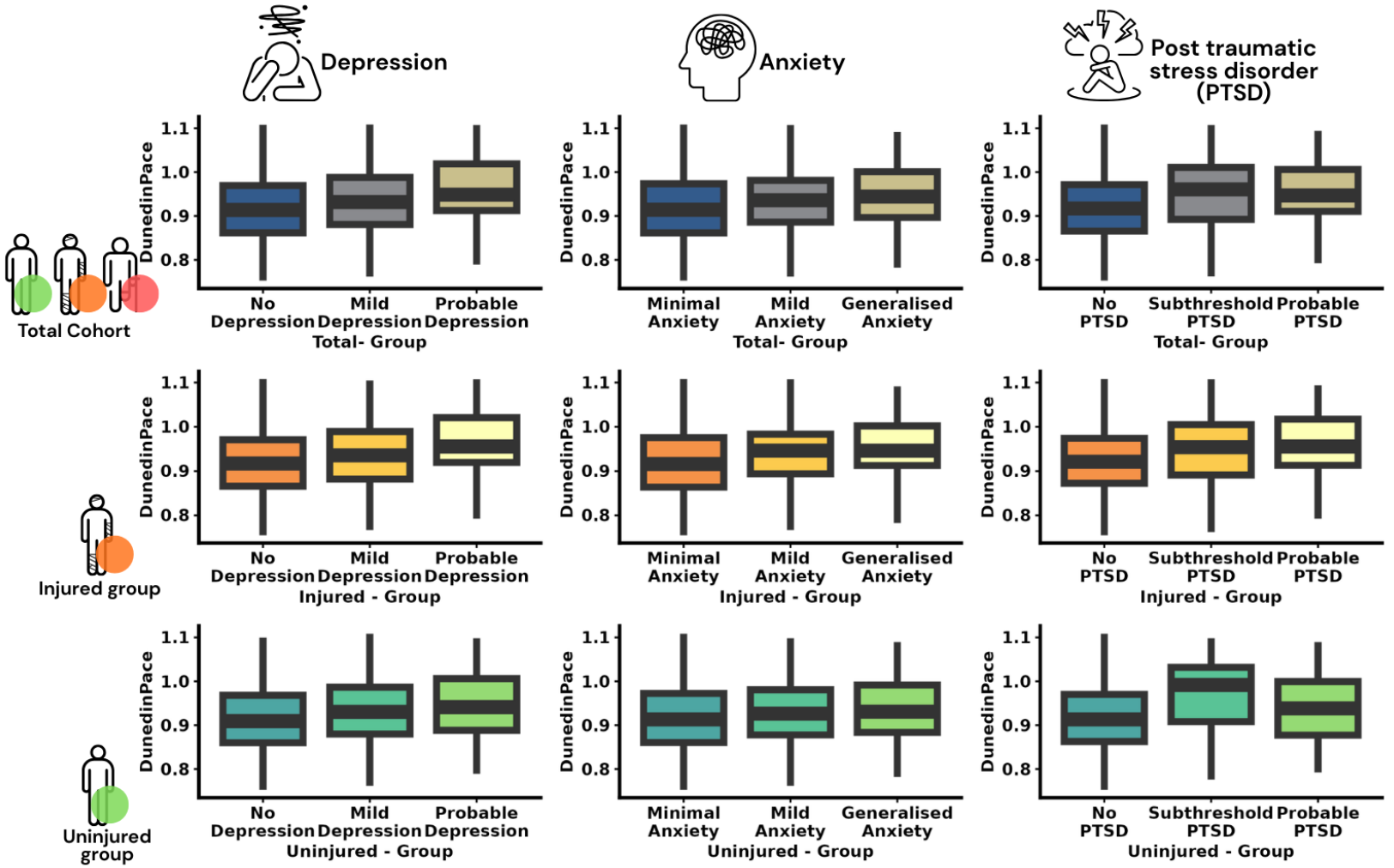


Figure 8. Pace of ageing in relation to veteran's mental health issues: Depression, Anxiety and PTSD. Results are shown for the whole cohort (top panel), the injured (middle panel) and the uninjured (bottom panel) separately.

Physical fitness, physical injury and biological ageing

We confirmed that the proteomic measure of physical fitness, pVO_2max, did relate to self-reported levels of vigorous physical activity in our cohort, we were therefore confident that this could be used as a proxy for their habitual physical activity levels and fitness. Considering the cohort as a whole, our analysis showed that the Dunedin Pace score was highly influenced by the physical fitness measures pVO_2max and also the 6-minute walk test **(Figure 9)**, with higher pVO_2max ($p<0.001$) and 6-minute walk test ($p<0.001$) values correlated with lower Dunedin Pace scores. Moreover, the data also showed a significant influence of these two measures on the Phenoage and GrimAge epigenetic clocks (all $p<0.001$), indicating a broad positive influence of physical activity and fitness on biological ageing.

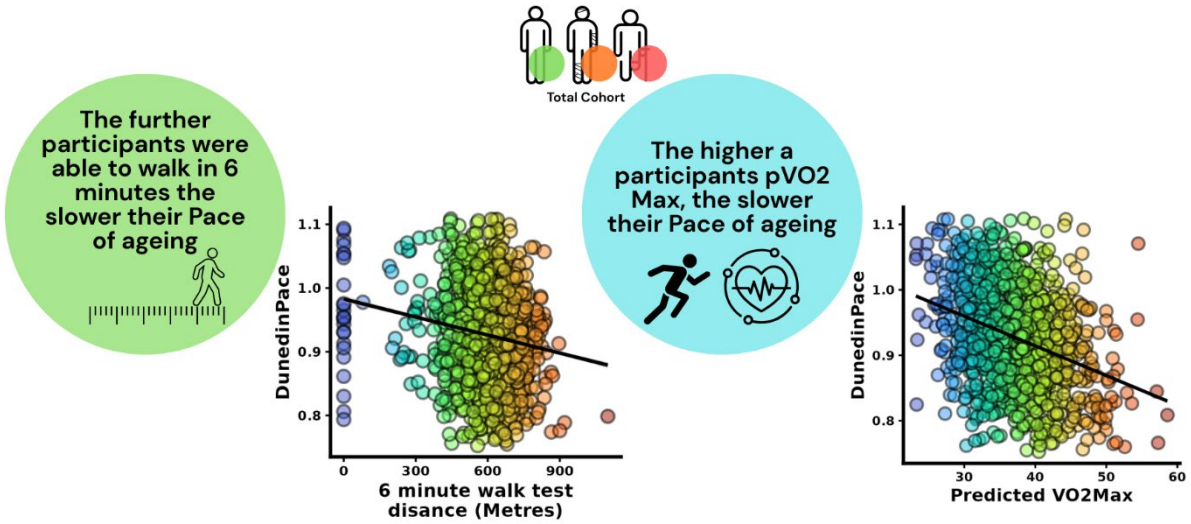


Figure 9. Pace of ageing for the whole cohort in relation to physical fitness determined using pVO_2max and 6 minute walk test.

Physical fitness, mental health and biological ageing

To assess the ability of physical activity and fitness to modulate the negative effects of mental health issues on biological ageing, we focussed on the Dunedin Pace clock as this was the marker of pace of ageing modulated by mental health. When comparing those in the top quartile for pVO_2max and those in the bottom quartile, there was a significant positive effect of pVO_2max on Dunedin Pace for those *without* mental health issues, (ie those with no depression, including mild depression, no anxiety, and no PTSD) (**Figure 10**; $p<0.0001$ for each). When we considered those with mental health issues (ie mild/probable depression; mild/generalised anxiety, sub-threshold/probable PTSD), we saw that those in the top quartile for pVO_2max had a significantly lower Dunedin Pace score than those in the bottom quartile for probable

depression ($p<0.01$), generalised anxiety ($p<0.01$) and probable PTSD ($p<0.05$) (**Figure 10**). A higher pVO_2max was therefore beneficial for those *with* a more severe common mental disorder or PTSD.

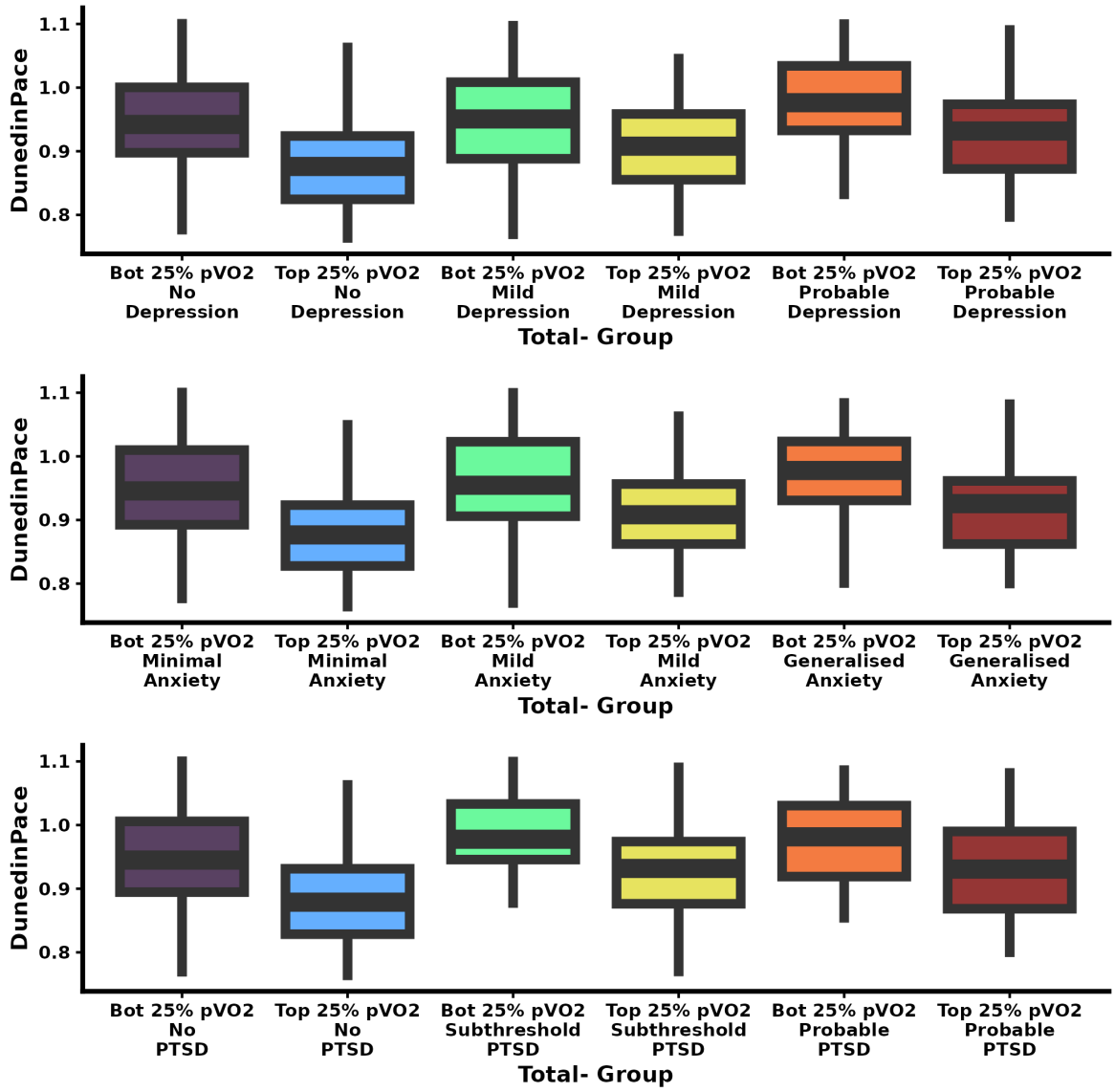


Figure 10. Impact of physical fitness, pVO2max, on Pace of ageing for veterans with depression, anxiety or PTSD.

Other Potential Negative Influences on Biological Ageing

Adverse Childhood Experiences and Biological Ageing: For Adverse Childhood Experiences (ACE), we found that the total score was correlated with increased pace of ageing as indicated by the Dunedin Pace score (**Figure 11**), and also increased biological age as indicated by the

GrimAge epigenetic clock, the latter is a clock which is associated with morbidity and mortality risk[24]. Interestingly, several of the individual items that make up the ACE score had major influences on biological ageing, notably issues at school, including truancy and expulsion being the major influences on both the DUNEDIN Pace and GrimAge scores.

Smoking: Smoking had a significant effect on the Dunedin Pace (**Figure 11**) and GrimAge scores, with both ex-smokers and current smokers having significantly higher scores than non-smokers ($p<0.001$ for all comparisons).

Body composition: We considered three measures of body composition namely: BMI and waist to hip ratio, which are both associated with health outcomes, as well as lean mass (muscle) and fat mass from DEXA data. A higher BMI ($p<0.001$), and to a lesser extent waist to hip ratio ($p<0.05$), both were correlated with a higher Dunedin Pace score, as was % fat mass ($p<0.001$. % fat mass also modestly correlated with a higher GrimAge score ($p<0.05$). None of the other epigenetic clocks were influenced by body composition.

Sleep: To determine an influence of sleep we used data from the Pittsburgh Sleep Quality Index (PSQI). Although the PSQI measures a range of aspects of sleep, including duration, time to fall asleep, sleep disruption, we focussed on sleep duration as the element that differed most among the cohort. The data show that sleep duration was negatively correlated with the Dunedin Pace score ($p<0.01$), so the less sleep a participant had the higher the Dunedin score. We also found that sleep duration influenced one of the epigenetic clocks, namely GrimAge ($p<0.05$), though to a lesser extent than the Pace score.

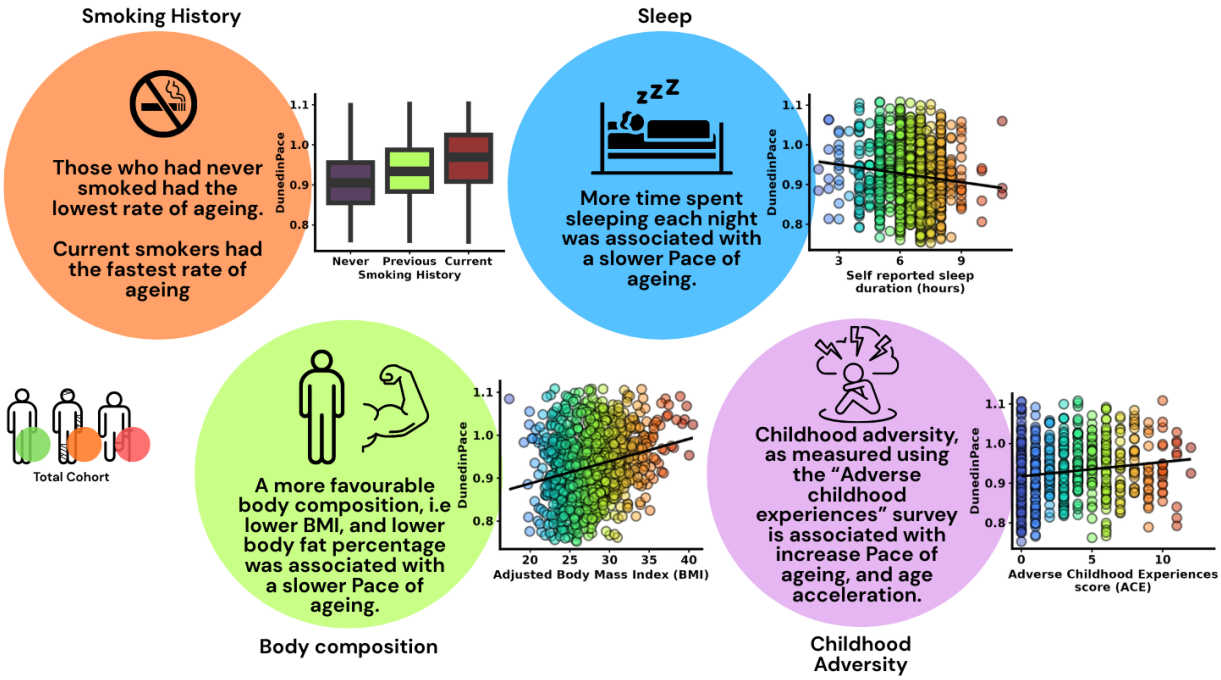


Figure 11. Dunedin Pace scores in relation to Adverse Childhood Experiences, Smoking, BMI and Sleep duration

Discussion

It is becoming clear that both mortality and morbidity are raised in major trauma survivors. One US study of major trauma outcomes found that 3-year cumulative mortality[b] was 16% in trauma patients compared to 5.9% for the expected population[c,35]. A longer-term study in Taiwan found that the 10-year cumulative survival for major trauma patients was 63.71% compared with 92.4% for the general population[36]. Psychological trauma can also impact upon health outcomes, for example PTSD is associated with an increase in all-cause morbidity[d] and premature mortality[37,38]. Interestingly, traumatic injury survivors often succumb to a range of age-related diseases at an earlier age than the general population. The aim of our study was to determine whether physical or psychological trauma in military veterans accelerates the ageing process and if lifestyle factors, such as physical activity and fitness, could modulate the impact of trauma on the acceleration of ageing.

Physical and psychological injury: The ADVANCE cohort, which recruited military veterans and serving personnel who had been deployed to the Afghanistan conflict, provided a suitable population for our study. With respect to mental health issues, data were not available for the whole cohort, with the response rates ranging from 71.7% for depression to 97.7% for PTSD.

It is possible that the relatively low number of responses to the depression questionnaire might be due to stigma associated with this condition amongst veterans (e.g. accessing mental health services for this condition has been reported to be low amongst this population[39)]. Looking at the prevalence of depression amongst those who responded, 25.7% is higher than that seen in a previous FiMT-funded study of mental health in veterans which reported a prevalence of 14.9% compared with 17.1% for the general population in the same health region[40]. However, our data are in closer agreement with a larger study of UK veterans by the King's Centre for Military Health Research (KCMHR) which assessed common mental disorders, which includes depression and anxiety, and reported a prevalence of 22% in personnel who were deployed[17]. If we take the number of respondents to the depression questionnaire (203) as a percentage of the whole cohort sampled, then the prevalence is approximately 20%. The closer agreement with the KCMHR study may reflect the fact that all the cohort had been deployed to a war zone and thus will have experienced higher levels of exposure to potentially stressful situations than veterans who had not been deployed. For anxiety, our prevalence of 16% was also slightly higher than that previously reported for UK military veterans at 12.8%[40] and US veterans at 7.9%. However, the US study did find that anxiety scores were higher in those that had served two deployments[41]. For PTSD, data was available for 1100 participants, 12.2% had probable PTSD, a level very close to the 12.4% [b]reported by a meta-analysis of 21 studies of military veterans who had been deployed[42]. Our population therefore appeared to have levels of PTSD similar to those reported by others but with higher levels of anxiety and depression.

Physical injury and biological ageing: We analysed DNA methylation to estimate the degree of biological ageing in the veterans, using four epigenetic clocks[4, 22-24] which give an estimate of biological age at the time of sampling. Two of the clocks are first generation clocks (Horvath[4] and Hannum[22]) and two are second generation (Phenoage[23] and GrimAge[24]). The benefit of the second-generation clocks is that they are more predictive of a range of adverse health outcomes, including those that are considered age-related diseases (cancer, frailty, Alzheimer's disease) and all-cause mortality, than the first-generation clocks[43]. We did not find an impact of injury

[b] Cumulative mortality means the total deaths occurring in a set time period. [c]Expected population means a population matched for features such as age and gender. [d]All cause morbidity means the total level of illnesses.

on biological age using any of the four epigenetic clocks studied, though an earlier much smaller study (n=96) of Dutch military veterans did report an age acceleration of approximately 2 years[15]. This may reflect the fact that that study was carried out on veterans just 6 months after their injury, whereas our participants were on average 7 years from injury, suggesting that the effect seen in the Dutch study was not long-lasting.

However, where we consistently found effects of injury was upon the Dunedin Pace clock, which was developed to predict longitudinal changes in multiple body systems and gives an estimate of the pace/rate of ageing of an individual. In this way, it predicts chronic disease risk, including time to cardiovascular disease and mortality[25]. In our relatively young cohort which had a mean age of 34 years, the pace of ageing was 1.38% faster in the injured group, ageing the individual by an extra 4.4 biological age days per year. For those with amputations, the situation was worse with the pace of ageing 3.02% higher than the uninjured group, adding an extra 9.8 days of biological ageing per year. These may seem like small and insignificant increases, but the pace of ageing is not linear and is normally less than one biological year per chronological year in young adults, only increasing to rates above one year per chronological year after middle age[25]. The seemingly small increases that we detected may therefore have significant impact in the longer term. As Dunedin Pace is a good predictor of long-term health outcomes and mortality, these values may well be suggestive of worse health and shortened lifespan in the injured veterans and interventions to reduce the pace of ageing may have significant benefits.

Mental health and biological ageing: Our data suggest that poor mental health is also impacting biological ageing, and the effects were greater than for physical injury, with effects again detected by the Dunedin Pace clock rather than the other epigenetic clocks. For those with depression, ageing was 3.74% faster than those without depression, which equated to 12.4 extra biological age days per chronological year. For those with anxiety there was a 1.69% increase, equating to an extra 5.5 days per year of ageing. With PTSD, the veterans were ageing 2.42% faster than those with no symptoms, which is equivalent to 8 extra biological age days per year. For depression, the literature is mixed with some reporting a negative effect of depression on epigenetic age[44] and others finding no effect[45]. However, a relevant study of twins from the Vietnam Era Twin Registry did find accelerated epigenetic age in the veterans and this age acceleration persisted for 11 years[46]. For anxiety, a recent small study (30 subjects with anxiety) actually found suppressed epigenetic ageing using a variety of clocks[47], whether this reflects a difference between a civilian and a military cohort warrants further investigation.

For PTSD, some researchers have found an acceleration of ageing with veterans diagnosed with PTSD. A study of 140 US military veterans who served in Iraq and/or Afghanistan found age acceleration with the GrimAge clock in veterans with PTSD compared to veterans without PTSD[48]. The study of Dutch military veterans mentioned earlier did not find an effect of PTSD on age acceleration, though they only used one of the first generation clocks[15]. Although there is disagreement in the literature and relatively few studies of mental health and age acceleration, an analysis of accelerated DNA methylation in 9 different studies found small but significant associations for childhood trauma exposure and lifetime PTSD severity[49]. However, PTSD diagnosis and lifetime trauma exposure in themselves were not associated with increased DNA methylation. Although our cohort was all male, a very recent study of trauma in women at mid-life found that those with two or more lifetime traumas had higher epigenetic age by both the GrimAge and Dunedin Pace clocks[50]. The effects of psychological trauma and mental health may therefore be age, severity, sex and context dependent and further research is required.

Benefits of physical activity and fitness for biological ageing in veterans: It is well established that physical activity and fitness have a broad range of health benefits and studies are now emerging to show that this includes an effect on biological age. A study of 3567 adults in Germany, mean age 55 years, found that time spent doing moderate to vigorous physical activity was negatively associated with epigenetic age assessed by the same four epigenetic clocks used in our study[18]. A second study assessed DNA methylation in 948 US adults, mean age 62 years, and found that higher physical activity was associated with lower epigenetic age assessed by eight different clocks. They found the greatest effects in non-Hispanic white adults and those with a BMI of 25-30[51]. In our study, we also found a positive influence of physical activity, using the proxy measures for physical fitness of pVO2max and 6-minute walk test, on two of the epigenetic clocks, Phenoage and GrimAge, as well as the Dunedin Pace clock, with all three showing higher physical fitness correlated with lower biological age. What is novel and encouraging about our finding is that this effect was seen in a group of young adults who had been deployed to a combat zone, half of whom were injured. Therefore, as long as the individual was able to maintain a good level of fitness, they were able to ameliorate the negative impact of injury upon their biological age. This is a positive health message for the veteran population.

When we considered physical fitness and biological age in veterans with different mental health issues the picture was also positive. When we compared pace of ageing in those in the highest

and lowest quartile for pVO_2max we found a significantly lower pace of ageing for those with mild and probable depression, generalised anxiety and probable PTSD. Thus good physical fitness was associated with a positive effect on biological age in those with mental health issues. This supports the literature which has shown a positive effect of physical activity in those with common mental disorders such as depression and anxiety, as well as PTSD. For example, a recent systematic review of 218 studies, covering over 14,000 participants, concluded that exercise is an effective treatment for depression, with walking or jogging, yoga, and strength training most effective, particularly when intense[52]. Our data complement the literature for PTSD as a systematic review of 11 studies revealed that exercise was an effective addition to PTSD treatment, with greater amounts of exercise providing more benefit[53]. What these studies did not consider was whether exercise could impact upon biological ageing in those with depression, anxiety or PTSD and our data now suggest that exercise can reduce mental health impacts on pace of ageing, at least in a military cohort.

Other negative influences on Biological Ageing: We considered a range of factors that might also influence biological ageing in our cohort. Adverse childhood experiences, notably schooling related, emerged as a negative influence on the pace of ageing but also the GrimAge clock. This is in agreement with other studies reporting an impact of childhood adversity (trauma exposure) on epigenetic age[49]. Our data was also in agreement with the literature on the negative effect of smoking on biological ageing, with both current and previous smokers affected. Other studies have reported increased epigenetic age associated with smoking in youth and cigarette pack years in adulthood [54]. Whether there is an effect of the length of time an individual had ceased smoking we could not ascertain from our data. However, the very significant impact of smoking supports the public health message for smoking cessation. Another health message that emerged from our study was the need to keep a healthy body weight and low adiposity, both of these factors influenced biological ageing, and similar results have been reported by others. One study of 1424 twins discordant for BMI found an association between BMI and GrimAge score[55]. As regular physical activity will help to maintain a healthy body weight, it is likely that this explains some of the positive effects of physical activity and fitness on the pace of ageing. Lastly, analysis of sleep duration data revealed poor sleep to be a negative impact on biological age. Short sleep duration is associated with poor mental and physical health[56], likely mediating its impact on epigenetic age. A study of 692 adults found that those with poor sleep had higher GrimAge and Dunedin Pace scores[57], in agreement with our data. Advice on improving sleep would therefore be beneficial to veterans.

Limitations: One of the main limitations of the study is that we do not have data for participants before their deployment to Afghanistan, therefore we do not know if they had higher biological age previously. It would also have been interesting to have data for military personnel who were not deployed, to ascertain if being in the military is associated with elevated ageing *per se*. The study cohort is also entirely male and thus we cannot make any conclusions with respect to sex and the impact of injury. Lastly, we are aware that substance misuse, including alcohol misuse, could influence epigenetic age[58]. However, although there are alcohol use (AUDIT) data from the cohort, we were advised by the ADVANCE data team that many of the participants had filled in the questionnaire incorrectly. We thus decided not to include this in our analysis. As this is a longitudinal study, it will be important to re-examine this issue at the next sampling as this could add to advice on lifestyle choices and health amongst veterans.

Conclusions: Our main conclusion is that in a cohort of military veterans, several years after their deployment, physical injury, depression, anxiety, or PTSD all accelerated their biological age, specifically the pace at which they were ageing. Although the degree of this acceleration, ranging from 4.4 to 12.4 days per chronological year, may seem minimal, in such a young cohort this may have significant implications for their future health. Importantly, lifestyle factors such as physical activity and fitness, not smoking and having good sleep duration, were all factors able to modify the negative impact of physical and psychological harm. As we have recently shown that a multivitamin supplement can reduce epigenetic age[19], this may represent another option for veterans with accelerated ageing.

Recommendations

- Improving physical fitness, or maintaining existing high levels, should be encouraged in military veterans who have sustained an injury, especially those with an amputation.
- We recommend that improving physical activity, should be encouraged in military veterans who have been diagnosed with common mental disorders (depression, anxiety) or PTSD.
- As our data are all associative, conclusive evidence for the benefits of physical activity for biological ageing would be gained by an intervention trial.
- Support for smokers to quit and those with poor sleep to improve sleep should be provided to veterans and those still in service to prevent acceleration of ageing.
- Advice on maintaining a healthy BMI should be given to veterans and serving personnel to prevent acceleration of ageing.

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