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1 PLATELET DYSFUNCTION IN A MODEL OF COMPLEX MILITARY TRAUMA

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Background Haemorrhage is a principal contributory factor in trauma-related deaths in military (battlefield) and civilian settings. A significant proportion of severely injured casualties develop an early trauma- induced coagulopathy (TIC), which is an independent predictor of death. One aspect of TIC is an alteration in platelet function. In vivo trauma models are often essential to develop new treatments. The aim of this study was to evaluate whether an established model of trauma incorporates platelet dysfunction.

Method The study was conducted in accordance with the Animals (Scientific Procedures) Act, 1986. Blood was collected from terminally anaesthetised pigs before (Baseline) and 30 minutes after the induction of trauma/haemorrhagic shock (S30), and again after 90 minutes of hypotensive resuscitation (R90) with either 0.9% saline, 1:1 packed red cells and plasma (PRBC:FFP) or whole blood (WB). Platelet function was assessed by aggregometry in response to ADP and TRAP (Multiplate®). Platelet count was obtained using a haematology analyser, and shock was quantified by measuring Actual Base Excess (ABE) of arterial blood.

Results ABE fell significantly from Baseline to S30 and remained negative (-7 ± 1 mM) until R90 in all groups ($P<0.001$), without significant difference between groups ($P=0.4747$). Injury, shock and resuscitation were associated with a significant fall in platelet aggregation in response to ADP ($P<0.0001$) and TRAP ($P=0.0006$), but no difference between treatment groups ($P=0.9388$ ADP; $P=0.06385$ TRAP). The response to TRAP was markedly less than that to ADP. Platelet count fell significantly ($P<0.0001$), again without significant difference between treatment groups ($P=0.8574$). After 90 minutes of resuscitation, the response to ADP had fallen to 62% of the baseline response while platelet numbers had only fallen to 85% of baseline over the same period.

Conclusions Our model of trauma results in an attenuation of platelet function that, in the early phase, is independent of resuscitation strategy.

2 DEVELOPING A MILITARILY RELEVANT EX-VIVO MODEL OF TRAUMATIC INJURY AND HAEMORRHAGIC SHOCK

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Background Traumatic injury is a leading cause of death worldwide. There is a crucial need to develop therapies that improve critically injured patient outcomes. Current trauma research models are ethically and financially challenging, with poor translation. However, traumatic injury and haemorrhagic shock can be modelled using ex-vivo normothermic perfusion (EVNP), a methodology adapted from transplantation. The aim of this study was to develop a 24hr EVNP duel porcine limb and kidney model.

Method Eight porcine forelimbs, bilateral kidneys and blood were retrieved via standard protocols. Following <4hrs cold storage, the kidneys were connected to a bespoke Ex-Vivo Research Centre circuit via the renal artery, and a mean arterial pressure (MAP) of 80mmHg was maintained. The perfusate consisted of leukocyte-deplete blood and Ringer's solution. Once the kidney was haemodynamically stable, the limb was connected via the brachial and radial collateral arteries. Haemodynamic parameters were continuously monitored, biochemical perfusate assessment performed hourly and histopathology baseline and end timepoints samples taken.

Results Perfusion was maintained for 24hrs in all limbs, with blood flows of 345.03mls/min (±54.78 SD) and MAP of 77.57mmHg (±3.82 SD). Three kidneys achieved 24hr perfusion, with flows of 214.53mls/min (±41.6 SD) and MAP of 80.58mmHg (±0.51 SD). Biochemical analysis showed a statistically significant potassium elevation at 24hrs compared to baseline, $p=0.0078$. A further three kidneys were disconnected from the circuit at 7, 11 and 12hrs, and two kidneys showed decline in flow >15 hrs due to declining haemodynamics. Compared to baseline, evidence of cell death was observed in 24hr muscle samples. In the end-point kidney samples, tubular degeneration, protein loss and necrosis extended along the nephron.

Conclusions Limb EVNP can be successfully achieved for 24hrs, but further protocol improvements are required to sustain renal perfusion for 24hrs alongside adjustments to reduce the ischaemic insult and cell death.

3 CARDIOPULMONARY, FUNCTIONAL, COGNITIVE AND MENTAL HEALTH OUTCOMES POST COVID, ACROSS THE RANGE OF SEVERITY OF ACUTE ILLNESS, IN A PHYSICALLY ACTIVE WORKING AGE POPULATION: BASELINE FINDINGS FROM THE MCOVID STUDY

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Background The medium-long impact of coronavirus disease 2019 (COVID-19) on active populations is yet to be fully understood, with potential individual and operational impact on military service personnel (SP). The M-COVID study was established to investigate cardiopulmonary, functional, cognitive, and mental health post-COVID-19 SP outcomes, across the spectrum of acute COVID-19 severity.

Method Observational four-cohort study; hospitalised, community-based illness with on-going symptoms (community-symptomatic), community-based illness now recovered (community-recovered) and age, sex, job-role matched control. Participants underwent extensive clinical assessment involving

cardiopulmonary imaging, submaximal and maximal exercise testing, pulmonary function, cognitive assessment, blood tests, electrocardiogram and questionnaires on mental health and physical function.

Results 113 participants (aged 39±9, 86% male) were recruited; Hospitalised (n=35), community-symptomatic (n=34), community-recovered (n=18) and control (n=26), 159±72 days following acute illness. Hospitalised and community-symptomatic groups were older (p=0.003), with a higher body mass index (p<0.001), and worse mental health (anxiety,p=0.011;depression,p<0.001;post-traumatic stress, p<0.001), fatigue (p<0.001), and quality of life scores (p=0.001), with a mean of 2±2 and 2±1 symptoms, respectively. Hospitalised and community-symptomatic participants also performed less well on sub-maximal (p<0.001) and maximal exercise testing, with hospitalised individuals displaying impaired ventilatory efficiency (p<0.001), less work at the anaerobic threshold and at peak (both p<0.001), and significantly reduced forced vital capacity (p=0.004). Clinically significant abnormal cardiopulmonary imaging findings were present in 6% of hospitalised participants, lower than those seen in other studies. Those who recovered from community-based, mild-moderate COVID-19 had no significant differences from controls on any parameter.

Conclusions Recovered SP who suffered mild-moderate COVID-19 do not differ from an age, sex and job-role matched controls. This is reassuring for the vast majority of individuals who have had acute COVID-19 not requiring hospital management. Individuals who were hospitalised or continue to suffer symptoms may require a specific, comprehensive clinical and occupational assessment prior to a full return to duty.

4 IMPROVEMENTS IN ORTHOSTATIC TOLERANCE WITH PHYSICAL TRAINING ARE AUGMENTED WITH HEAT ACCLIMATION AND ASSOCIATED PLASMA VOLUME EXPANSION; A RANDOMISED CONTROLLED TRIAL

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Background Heat adaptation is protective against heat illness however its role in heat syncope, due to reflex mechanisms, has not been conclusively established. The aim of this study was to evaluate if heat acclimation (HA) was protective against heat syncope and to ascertain underlying physiological mechanisms.

Method 22 (17 males, 5 females) endurance trained cyclists were randomised to either 8 days of mixed active and passive HA (HEAT) or temperate exercise (CONTROL). Prior to, and following, the interventions participants underwent a HUT with graded lower body negative pressure (LBNP) continued until presyncope with measurement of cardiovascular parameters. Heat stress testing was performed to determine physiological and perceptual measures of HA.

Results There was a significant increase in orthostatic tolerance (OT), as measured by HUT/LBNP, in the HEAT group (pre-intervention; 28±9 mins, post-intervention; 40±7 mins) compared to CONTROL (pre-intervention; 30±8 mins, post-intervention; 33±5 mins) (p= 0.0116). Heat acclimation resulted in a significantly reduced peak and mean rectal and skin temperature (p<0.0141), peak heat rate (p<0.0033), thermal comfort (p<0.0411) and rating of perceived exertion (p<0.0251). There was a significantly increased plasma volume in the HEAT group in comparison to CONTROL (p=0.0293).

Conclusions Heat adaptation causes improvements in OT and is likely to be beneficial in patients with heat exacerbated reflex syncope. Heat acclimation mediated PV expansion is the likely predominant physiological mechanism underlying improved OT. These data offer opportunities to improve health and wellbeing of service personnel with economic, logistical and reputational benefits for the UK Armed Forces.

5 A NEW ANIMAL MODEL FOR INFECTED FRACTURE NON-UNION AFTER EXTERNAL FIXATION OF TIBIA WITH REAL-TIME IN-VIVO MONITORING OF INFECTION

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Background Non-union is a well-recognised complication after open fractures. Fifty percent of open extremity trauma returning from military operations developed non-union. Aetiology of non-union is multi- factorial, with infection reported as major contributory factor. The aim of this study was to develop an in-vivo model of infected fracture non-union managed with external fixation which allowed real time in-vivo monitoring of infection to evaluate potential therapeutic strategies.

Method Ten male Wistar rats underwent application of external fixator and midshaft tibia osteotomy. Osteotomy sites were inoculated with bioluminescent Staphylococcus aureus Xen36 (infected group; n=6); or phosphate buffer solution (control group; n=4). Animals were monitored for infection with in-vivo bioluminescent imaging and fracture healing with plain radiographs. Animals were sacrificed at eight weeks. Post-mortem micro-computed tomography (uCT) was used to assess fracture union; in-vivo bioluminescent imaging to assess persistence of Xen36 infection; tissue samples were processed for bacterial colony forming unit counts and histology to assess for fracture healing and infection.

Results Eight animals reached experiment endpoint (infected=5, control=3). All five infected animals demonstrated radiographic non-union on x-ray and uCT. Bioluminescence, at fracture site in infected cohort, peaked at week two and reduced to chronic baseline of 105 photons per second for duration of experiment. At experiment endpoint bioluminescence was confirmed at fracture site and bioluminescent bacteria was cultured from fracture site tissue samples in all of the infected cohort. Two of three control animals demonstrated radiographic non-union, none luminesced, one grew bacteria from tissue samples but was not bioluminescent.

Conclusions This study has developed an infected fracture non-union animal model. Use of bioluminescent bacteria allows for non-invasive and real-time monitoring of infection. This model is more representative of the military casualty than previously reported models and could be used to evaluate therapeutic strategies for prevention and management of infected fracture non-union.