The Endocrine and Metabolic Response in Male Survivors of Major Trauma

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Abstract (250 words)

Background: Survival rates after severe injury are improving, but complication rates and outcomes are variable. This study addressed the lack of data on the endocrine response, particularly adrenal steroid secretion, during recovery from major trauma, exploring potential targets for intervention.

Methods: We undertook a prospective, observational cohort study from time of injury to six months post-injury at a major UK trauma centre and a military rehabilitation unit, studying patients within 24 hours of major trauma (estimated New Injury Severity Score (NISS) >15) and at regular intervals for six months. We recorded clinical outcomes (ventilator days, length of hospital stay, organ dysfunction, sepsis), measured serum steroids by tandem mass spectrometry, and assessed muscle loss by ultrasound and nitrogen excretion.

Findings: We screened 996 multiply injured adults, approached 106, and recruited 95 eligible patients; 87 survived. We analysed all male survivors <50 years not treated with steroids (N=60; median age 27 [interquartile range 24-31] years; median NISS 34 [29-44]). Urinary nitrogen excretion and muscle loss peaked after one and six weeks, respectively. Glucocorticoid secretion remained within the reference range, though the serum cortisol over cortisone ratio increased, normalising by eight weeks. Serum testosterone, DHEA and DHEAS decreased immediately after injury and took two, four and more than six months, respectively, to recover; all three correlated with SOFA score and probability of sepsis.

Interpretation: The acute catabolic response to severe injury is accompanied by sustained androgen suppression. The impact of androgen supplementation on health outcomes after major trauma should be systematically explored.

Introduction

Over 5 million people worldwide die each year from serious injury[1] , with almost 25% caused by road traffic collisions (RTC)[2]. In England alone, there are 5400 trauma deaths and 20,000 severe injuries treated by the National Health Service annually[3]. Since 2012, the establishment of 22 Trauma Centres in England has been accompanied by a 19% improvement in survival odds following injury[4]. During this time, the UK also received severely injured military trauma patients from the conflict in Afghanistan[5,6].

Improvements in short-term outcomes have been achieved through early resuscitation and acute care[7], often informed by approaches pioneered on the battlefield. However, the impact of improvements on long-term outcomes are often offset during the weeks following acute major trauma, mainly by a dysregulated Systemic Inflammatory Response Syndrome (SIRS), associated with compromised immunity increasing the risk of infection, multi-organ dysfunction/failure (MOD/MOF) and late deaths[8,9]. Simultaneously, the hypothalamic-pituitary-adrenal axis (HPA) is thought to drive a hypermetabolic and overtly catabolic response. Pro-inflammatory cytokines activate the enzyme 11β-hydroxysteroid dehydrogenase type (11β-HSD1) that is responsible for tissue-specific activation of glucocorticoids through conversion of inactive cortisone to active cortisol[10], providing a link between SIRS and catabolism. Importantly, in this profound catabolic state, patients lose valuable lean muscle and suffer from increased rates of infection and poor wound healing. Nutritional strategies have proven disappointing [11,12]. Moreover, the dynamic nature of this response, in particular beyond the first few days after injury and during recovery remains poorly described and understood, limiting the evidence base for novel therapeutic interventions.

To address this issue, we have undertaken a detailed prospective study of the endocrine and metabolic response to severe injury in military and civilian populations, recruiting patients within 24 hours of major trauma and following up on them for the six subsequent months. This was undertaken to identify predictive biomarkers and therapeutic targets as well as to explore the optimal timing for therapeutic interventions targeting the endocrine response that could promote better recovery after severe traumatic injury.

Methods

Study Design and Protocol

This prospective cohort study was conducted in the Royal Centre for Defence Medicine and the Critical Care Unit at the Queen Elizabeth Hospital Birmingham UK (QEHB), one of 22 UK Major Adult Trauma Centres (MTC) and the primary receiving facility for UK military personnel injured abroad. Military and civilian major trauma patients with an estimated New Injury Severity Score (NISS) greater than 15 [13] were recruited on arrival at QEHB. NISS was used to ensure those with significant extremity trauma but lower ISS were included[14]. Patients were not eligible if they had a significant head injury or if any pre-injury neoplastic conditions were identified.

Informed consent was obtained from a personal consultee and confirmed subsequently by surviving patients following recovery of capacity. The protocol was approved by the NRES Committee South West – Frenchay 11/SW/0177 and MOD REC 116/Gen/10.

A daily patient review allowed the injured to be clinically phenotyped. Bespoke study management software (Clinical RESearch Tool – CREST) tracked the patient through the study and their clinical data was entered prospectively. Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), and Simplified Acute Physiology Score (SAPS) II were calculated from the ICU charts [15,16]. Sepsis was defined using Bone's criteria of an infection associated with SIRS[17], current at the time of the study.

Blood and urine samples were collected within 24 hours of acute injury and at 3, 5, 10, 14, 21, 28 days and 2, 3, 4 and 6 months after injury. This challenging sampling schedule was supported by junior clinical staff to ensure blood sampling was conducted simultaneously between 0730 and 0900hrs on the day of sampling on multiple patients; their serum was then separated and frozen at -80^{o}C for batched analysis.

We separately collected morning blood samples from 37 healthy control subjects, who were matched by age and sex to the major trauma patients included in the final analysis group, to provide a comparator cohort for steroid analysis.

Assessment of protein catabolism

Muscle thickness was measured by portable ultrasound to assess lean body mass as described by Campbell *et al*[18,19]. Ultrasound measurements were taken from 4 different muscle sites; biceps brachii, radial forearm,

rectus femoris and rectus abdominis at weekly intervals while in hospital and at 3, 4, 5 and 6 months following discharge.

Urinary urea excretion was measured by the routine laboratory at QEHB and used to estimate Total Urinary Nitrogen (TUN) excretion as described by Milner et al[20] [Estimated Nitrogen Excretion: Urinary Urea Excretion (mmol/l) x 0.028 x 1.25 = Total Urinary Nitrogen excretion (g/l)].

Serum steroid analysis

Serum concentrations of adrenal and gonadal steroids were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis[21]. In brief, serum steroids were extracted via liquid/liquid extraction with tert-butyl-methyl-ether (MTBE), evaporated, reconstituted and analysed by LC-MS/MS for the quantification of cortisol and cortisone. Serum androgens and androgen precursors (DHEA, androstenedione, testosterone) were measured following oxime derivatisation[22,23]. DHEA sulphate (DHEAS) was measured following protein precipitation[24,25].

Serum concentrations of sex hormone binding globulin (SHBG) and luteinizing hormone (LH) were analysed on the Roche Modular System (Roche Diagnostics, Lewes, UK) by two-site sandwich immunoassay using electrochemiluminesence technology.

Statistical Analysis

The raw data were evaluated by analysis of variance (ANOVA); in addition, paired and unpaired Student's t-test, Chi-Square Analysis and Mann-Whitney tests were used where appropriate. Data modelling techniques were employed to allow further interrogation of the data. Generalised linear mixed-effects models[26] were used to examine the change in variables over time. Patients were included in the models as random effects to account for repeat measures over time on the same individuals. Time was modelled using restricted cubic splines[27] to allow for flexible relationships[28]. Severity scores were modelled as Poisson distributions due to their skewness and non-negative ranges. Plots of predicted average fixed effects with 95% confidence intervals were produced for the first 4 weeks and first 6 months after injury as required. Analyses were conducted in R using libraries lme4, effects, rms and ggplot2.

RESULTS

Patient recruitment and final cohort

996 multiply injured adults were assessed for eligibility soon after injury. The majority of the 889 excluded patients had a NISS≤15; others had a significant head injury as their major injury component, and two were excluded due to a pre-injury diagnosis of cancer. Of the 106 severely injured patients approached, 102 patients were recruited into the study, with two withdrawn and re-assessment in five revealing an actual NISS≤15, leaving a final study cohort of 95 patients (**Fig. 1A**).

After exclusion of fatalities (n=8) and patients given steroids as part of their treatment (n=7), the cohort comprised 80 survivors with completed sample collection over 6 months. To minimise confounders from age-related comorbid disease and sex, we excluded women (n=9) and age-advanced men (n=11), arriving at our final study cohort of 60 men <50 years (**Fig. 1B**).

A summary of the cohort characteristics is shown in **Fig. 2**. Median age was 27 (interquartile range (IQR) 24-31) years, median NISS was 34 (IQR 29-44), and patient day 1 (=day of major trauma) APACHE II score was 21 (IQR 14-25). Patients remained ventilated and stayed on the intensive care unit (ICU) for a median of 9 (IQR 5-16) days. Median length of stay at QEHB was 36 (IQR 19-56) days. Improvised Explosive Device (IED) (n=33; 55%) and Road Traffic Collision (RTC) (n=11; 18%) were the most common causes of injury. Twenty-five (42%) patients had at least one septic episode and most occurred in the second week following injury (**Fig 2C**).

Glucocorticoid synthesis and metabolism after major trauma

Serum concentrations of the major catabolic glucocorticoid cortisol increased after injury, peaking at 408 (IQR 249-511) nmol/L at two weeks (**Fig. 3A; Suppl. Fig. 2**). However, concentrations remained within the wide range seen in healthy controls. Serum concentrations of the inactive metabolite cortisone were lower than normal after injury, and increased slowly over time, but this trend was not significant (p=0·08) (**Fig. 3B**). The serum cortisol to cortisone ratio, a marker of glucocorticoid activation by 11β-HSD1 activity (**Fig. 3C**), peaked at two weeks post-injury and returned to normal at around eight weeks.

Androgen synthesis and activation after major trauma

The adrenal-derived steroid dehydroepiandrosterone (DHEA) serves as an androgen precursor through downstream conversion to androstenedione and subsequent activation to the active androgen testosterone. Serum concentrations of DHEA were very low after injury ($p<0{\cdot}0001$), as compared to controls, but recovered by 3 months post-injury (**Fig. 3D, Suppl. Fig. 2**). In contrast, its sulphate ester, DHEAS, failed to recover to values within the healthy reference range even at the end of the 6-month study period (**Fig. 3E**). Consequently, the serum DHEA to DHEAS ratio (**Fig. 3F**) increased by week 2 compared to controls and failed to return to normal during the entire study period. The serum cortisol to DHEAS ratio (**Fig. 3G**) increased after injury, peaking at 2 weeks, before slowly declining over the study period, but without returning to normal by 6 months.

Serum concentrations of the androgen precursor androstenedione (**Fig. 3H**) were below the reference range immediately after injury, recovering to the mid reference range at 2 weeks after injury, therefore, recovering much faster than serum DHEA, suggestive of rapid downstream activation of DHEA to androstenedione.

Serum concentrations of the active, androgen receptor-activating androgen testosterone (**Fig. 3I**) were low following injury, but started to increase after two weeks, recovering to the healthy sex- and age-matched reference range approximately eight weeks after injury.

Serum luteinising hormone (LH), the pituitary gonadotrophin responsible for gonadal stimulation of testosterone synthesis, was acutely suppressed immediately after injury, and recovered to the normal range approximately 2 weeks after injury (**Suppl. Fig. 2A**). Serum sex hormone-binding globulin (SHBG) (**Suppl. Fig. 2B**) concentrations were subnormal immediately post-injury but returned to the healthy reference range between injury and day 7.

Protein catabolism after major trauma

The 24-hour total urinary nitrogen (TUN) excretion increased immediately after trauma, to reach a peak at $25{\cdot}0\pm16{\cdot}1$ g/day at the end of the first week, returning to below 15·0 g/day by week 4. The measured mean maximum rate of nitrogen excretion was $33{\cdot}0\pm21{\cdot}3$ g/day **(Fig. 4A)**. The normalisation of TUN excretion coincided with the gradual recovery of adrenal and gonadal androgen production (**Figs. 4B+C**).

The biceps brachii muscle was the most reliable site for ultrasound measured muscle thickness; dressings, amputations and other wounds hampered the measurements in other areas. Changes in biceps brachii muscle thickness followed a U-shaped curve after injury that reached a nadir at week 6 (day 1 after trauma compared with week 6, p=0·001). The mean maximum loss was 22·7% (±12·5) **(Fig. 4D)**. Similar to TUN, the recovery of muscle thickness took place alongside gradually increasing adrenal and gonadal androgen production (**Figs. 4E+F**).

Clinical course of post-traumatic recovery and serum androgen dynamics

The relationship between adrenal and gonadal androgens and the Sequential Organ Failure Score (SOFA) and probability of sepsis are illustrated in **Fig. 5**. During the first four weeks after trauma, serum concentrations of DHEA, DHEAS, and testosterone all correlated with the clinical SOFA score (autocorrelation factor (ACF) = 0·85, 0·90 and -0·79, respectively). The serum concentrations of all three steroids also showed strong associations with the probability of sepsis (R=-0·85, 0·85 and -0·97 for serum DHEA, DHEAS and testosterone, respectively). SOFA score and probability of sepsis also correlated strongly with the DHEA:DHEAS ratio (autocorrelation factor (ACF) = -0·94 and -0·96 respectively) and with the Cortisol:DHEAS ratio, negatively for the SOFA score but positively for probability of sepsis (autocorrelation factor (ACF) = -0·81 and 0·89 respectively) (**Suppl. Fig 3**).

Discussion

In our study, we have characterised the response of adrenal and gonadal steroids and catabolic metabolism to severe injury, describing the related dynamic changes from the day of acute major trauma through to six months post-injury in a prospectively recruited cohort. Modelling the resulting data has allowed us to provide a detailed description of the post-injury transition from catabolism to anabolism, a point following injury where anabolism becomes the dominant process as the severely injured individual recovers. This analysis revealed an initial phase of glucocorticoid activation and immediate, sustained suppression of adrenal and gonadal androgen synthesis, which took two to more than six months to recover and revealed associations between specific endocrine parameters and patient outcomes including risk of sepsis. These novel data, comprising in-depth phenotyping and kinetics of the endocrine response to severe trauma, provide a rationale for targeting androgen balance with interventions aimed at improving health outcomes for critically injured patients.

Our data are the first to provide detailed adrenal and gonadal steroid data beyond the first days after trauma in a large cohort of patients, with all patients recruited prospectively and steroid analysis carried out by tandem mass spectrometry. As summarised in a recent meta-analysis, previous data on serum cortisol after injury is usually limited to small cohorts derived from patients undergoing elective surgery, rarely followed up for more than two days[29]. In our study, serum cortisol quickly returned to normal following initial increases after acute trauma. In contrast, serum cortisone remained low for three months post-injury, resulting in a significant increase in the cortisol to cortisone ratio, an *in vivo* measure of the activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 11β-HSD1 is the major enzyme converting inactive cortisone to cortisol and has been shown to be upregulated systemically and locally in response to inflammation in sepsis and trauma, possibly in an attempt to dampen the inflammatory response[30,31]. As our data reveal, the downside of this increased activity is increased local catabolic potential, affecting tissues such as skeletal muscle which express 11β-HSD1[32]. Previous research has shown increased 11β-HSD1 activity in an animal model of trauma haemorrhage[33] and a recent study has shown improved wound healing in mice treated with 11β-HSD1 inhibitors[34]; however, human data after trauma were previously lacking. Employing 11β-HSD1 inhibitors in the recovery phase after injury may help to prevent muscle loss by

reducing the preponderance of cortisol and may also stimulate adrenal androgen synthesis, due to activation of the hypothalamic-pituitary-adrenal feedback response to reduced tissue-specific glucocorticoid activation. Our finding of an increase in active cortisol over inactive cortisone in circulation during the early post-injury phase could also reflect a decreased metabolism of cortisol by 11β-HSD2, converting active cortisol to cortisone, or by the enzyme 5α-reductase, responsible for downstream metabolism of both cortisol and cortisone. A study investigating the *in vivo* metabolism of deuterated cortisol and cortisone in 11 patients with critical illness concluded that increased glucocorticoid activation in the context of ICU treatment might be due to decreased cortisol clearance rather than increased glucocorticoid activation [35].

In this study, we observed a pronounced and sustained loss of adrenal and gonadal androgen synthesis immediately following acute major trauma. The recovery of circulating DHEA and testosterone concentrations to the mid-reference range took two and four months post-injury, respectively, and DHEAS remained pathologically decreased at the end of the six-month follow-up period. Previous reports have described a suppression of the enzyme SULT2A1, the major DHEA sulphotranspherase, and its sulphate donor enzyme, PAPSS2, in a mouse model of acute inflammation[36]. We reviewed 23 previous studies that measured serum DHEA and DHEAS in critically ill patients (**Suppl. Table 1**), but most of them followed patients only for a few days and relatively few patients suffered from acute trauma. One previous study[37] described decreased circulating concentrations of DHEAS in 181 patients with septic shock and 31 patients with acute hip fracture; serum DHEA was increased in the sepsis patients but decreased in the trauma patients. However, as the hip fracture patients in that study were mostly elderly (mean age 82 years) and female, it is not clear if this finding was trauma-related or due to confounding factors.

A number of previous studies in trauma patients described an association of infection rate and mortality with low circulating DHEAS concentrations and also a raised cortisol to DHEAS ratio[38-41]. In vitro studies have demonstrated that cortisol decreases neutrophil superoxide production, which is counteracted by coincubation with DHEAS[38]. Furthermore, we have previously shown that DHEAS, but not DHEA, directly enhances neutrophil superoxide generation, a key mechanism of human bactericidal function, via activation of protein kinase C-β, independent of androgen receptor signalling[42]. In this study, analysing a large cohort

of men younger than 50 years who suffered major trauma, we observed suppression of both serum DHEA and DHEAS post-injury, indicating that the loss of adrenal androgen synthesis represents a trauma-related event. Importantly, we show for the first time that this decrease in circulating adrenal androgen precursors is sustained for several months and that DHEAS remains low 6 months post-injury. We speculate that there may be a potential therapeutic benefit from restoring the levels of these steroids during recovery.

Alongside the decrease in adrenal androgen synthesis, we observed a near complete loss of gonadal testosterone production and pituitary LH secretion immediately after trauma. Both the gradual recovery of adrenal and gonadal androgen production paralleled the decrease in catabolism, as assessed by urinary nitrogen excretion and biceps muscle thickness. The suppression of the hypothalamus-pituitary-gonadal (HPG) axis after severe injury that is shown in our study is supported by the literature[43-46]. We demonstrate that suppression of the HPG is shorter than for the HPA axis with LH returned to normal ranges after 1-2 weeks and testosterone levels were returning to normal levels by 4 weeks. In traumatic brain injury studies, a significant proportion of patients go on to develop anterior pituitary dysfunction[47,48], which is even more pronounced in patients exposed to a blast injury mechanism[49]. However, in this study, significant head injury represented an exclusion criterion for recruitment. More recently, Gang *et al* reported low testosterone immediately after injury in a study with 95 patients who had suffered severe injury[50], but they did not provide follow-up data. While limited data from patients with burns and critical illness have suggested a central, hypothalamic-pituitary cause of trauma-related hypogonadism[51,52], the evidence base prior to our study has been limited. The current study suggests a central cause of suppression to the gonadotrophic axis, with a decrease in both pituitary LH and gonadal testosterone secretion. However, it cannot be excluded that the administration of opiates, as typically required after major trauma, could causally contribute to the induction of central hypogonadism[53].

Importantly, our study shows for the first time that young men suffering from major trauma experience a sustained loss of androgen production. Androgens are important in wound healing, erythrocytosis, bone density and muscle mass[54]. The catabolic state that occurs following trauma thus presents a significant challenge to these patients. The use of androgens to ameliorate catabolism has some precedent, as evidenced by the use of the synthetic androgen, oxandrolone, that has some proven benefit in treating burn injury[55]. A

meta-analysis of 15 RCTs including 806 burns patients by Li et al, showed significant benefits ($P < 0.05$) for using oxandrolone including less net weight loss, lean body mass loss, nitrogen loss, donor-site healing time, and length of stay in the catabolic and rehabilitative phases[56]. The use of oxandrolone in major trauma was investigated in two studies but no benefit was demonstrated. However, one of the two trials was underpowered[58] and the other was stopped early due to re-intubation rates being higher in the treatment group but failed to acknowledge the increased death rate in the placebo arm[57,58].

The strengths of our study include its prospective nature, relatively narrow age range of the patients, single gender, single site for recruitment and analysis and detailed follow-up over six months post injury as well as the measurement of circulating steroid hormones by state-of-the-art tandem mass spectrometry. Analysing a young cohort has also reduced the confounding effects of age-related c-morbidities. Our study is limited by the diverse nature of major trauma patients in relation to injury pattern and the involvement of military casualties. Using NISS>15 as an inclusion criterion, we attempted to use a measure of both the injury burden and include injuries to multiple extremities. The timing and number of observations during our study was pragmatic and some statistical comparisons were made using modelled data.

In conclusion, in this to date most detailed and first prospective study of the endocrine response after trauma, we were able to follow the acute response to severe injury for 6 months, revealing severe DHEAS deficiency and decreased adrenal and gonadal androgen production that is sustained for many months. Recovery of androgen production in the severely injured patients was mirrored by a switch from catabolism to anabolism. We were also able to show associations between the adrenal and gonadal androgens and risk of sepsis. Therefore, the effects of an anabolic intervention addressing the pronounced decrease in androgen we identified warrants detailed study in men with major trauma, to assess its impact on morbidity including infectious episodes and recovery of normal muscle strength and health status.

Implications

This observational study identifies adrenal and gonadal androgens and androgen precursors such as DHEA as potential agents to counteract catabolism but also enhance immune function during the early recovery after major trauma. There are no studies to date to assess DHEA supplementation in a trauma population. A randomised controlled trial is now needed to prove efficacy in a population still vulnerable to prolonged morbidity and assess the potential of such treatment to reduce hospital stay and their long-term burden of care.

Contributors

MF, CB, JFB, MM, JL and WA designed the study. JB and MF performed the statistical analysis. MF, CB, NH, JF, DW collected samples, ultrasound measurements and clinical data from patient and controls. AT and WA carried out steroid analysis. MF, AT, NH, CB, JB, JFB, JF, DW, MM, JL and WA undertook data analysis. IB and MF reviewed the literature. MF drafted the manuscript and AT, NH, CB, JB, JFB, JF, DW, IB, MM, JL and WA co-wrote the final report. All authors contributed to writing the final report and approved the version to be published.

Declaration of interests

There is no conflict of interests for any of the authors. JML and WA are supported by the National Institute for Health Research (NIHR) through the NIHR Birmingham Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

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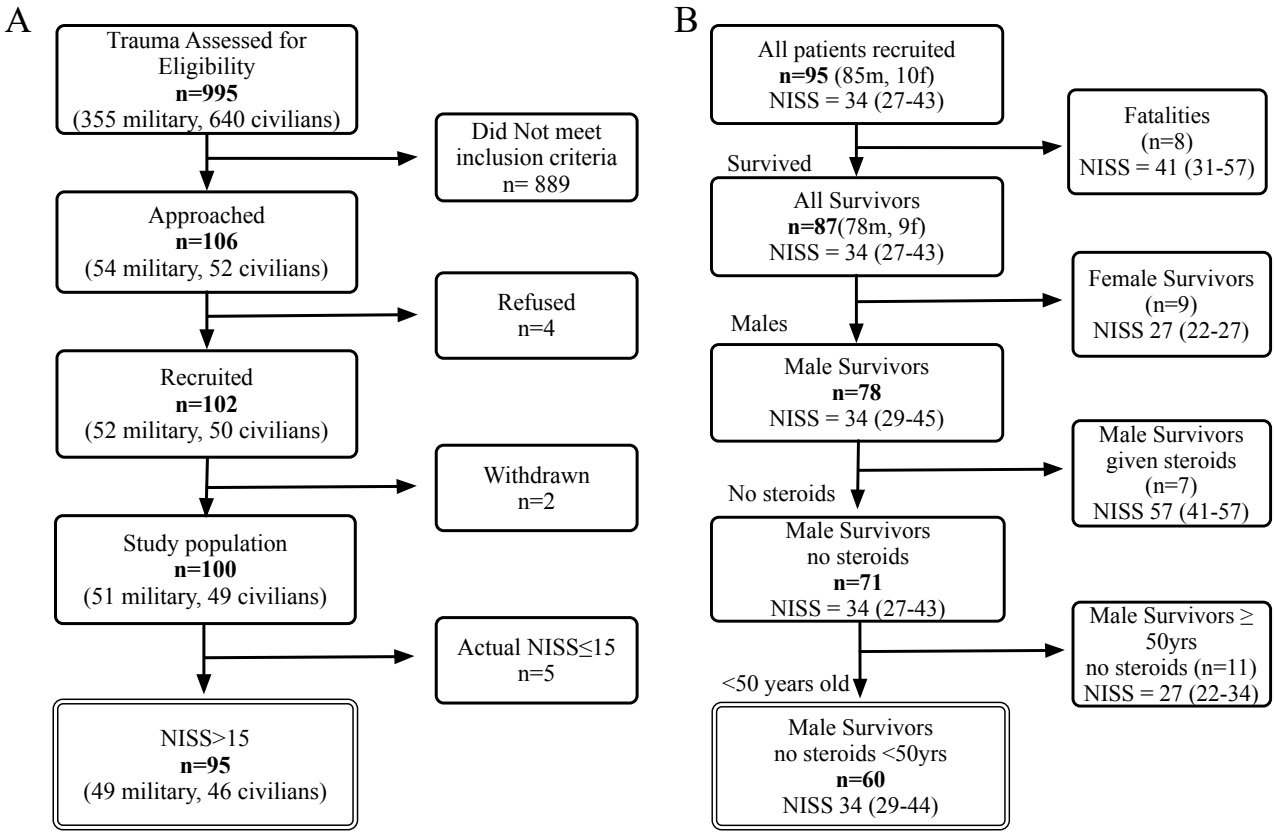

Figure 1. Consort diagram. (A) recruitment process and (B) subgroup selection for analysis for sixty male survivors of severe injury (NISS>15) under 50 years of age who had not been given exogenous steroids were analysed**.**

A

Demographics	
Patients n=60	Median (IQR)
Age	27 (24-31)
GCS	14 (3-15)
ISS	25 (17-32)
NISS	34 (29-44)
TRISS	94 (56-98)
APACHE 2 (Dayl)	21 (14-25)
SAPS 2 (Dayl)	48 (26-54)
SOFA (Dayl)	9 (6-10)
Septic Episodes	1 (0-2)
RBCs (Day1), Units	10 (2-17)
FFP (Day 1), Units	9 (1-15)
Crystalloid/colloid (Day1), L	0 (0-1)
Ventilator Days	9 (5-12)
ICU Length of Stay, Days	9 (5-16)
Operative procedures	5 (3-7.5)
Hospital Length of Stay, Days	36 (19-56)

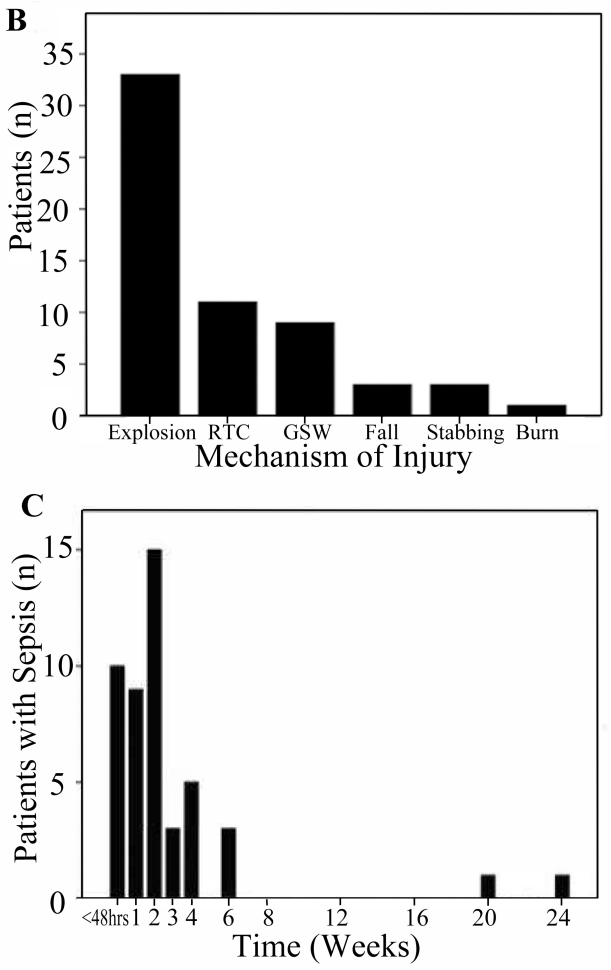

Figure 2. Patient Characteristics of the Analysis Cohort. (A) Demographics, (B) Mechanism of Injury and (C) the distribution of septic episodes for 60 male survivors from severe injury (NISS>15) under 50 years of age.

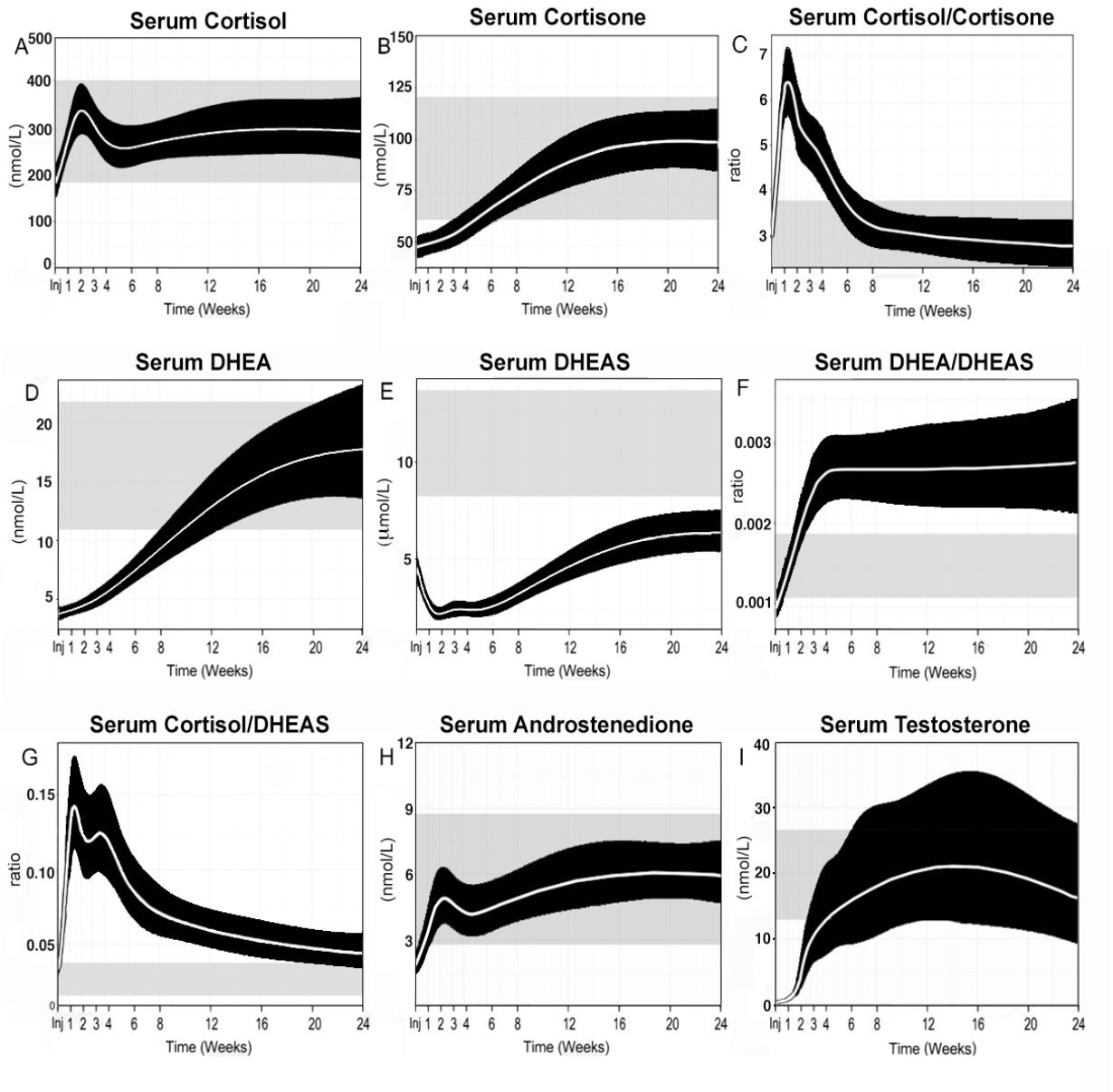

Figure 3. Serum steroids in 60 male survivors of severe injury (NISS>15) under 50 years of age. Serum concentrations shown include cortisol (A), cortisone (B), the cortisol/cortisone ratio (C), DHEA (D), DHEAS (E), the DHEA/ DHEAS ratio (F), the cortisol/DHEAS ratio (G), androstenedione (H), and testosterone (I). Data are represented after modelling of the raw data (Suppl. Fig. 1) using a non-linear mixed effects model that accounts for unbalanced repeated measures using a 4-knot cubic spline. Modelled data are shown as means and 95% confidence intervals.

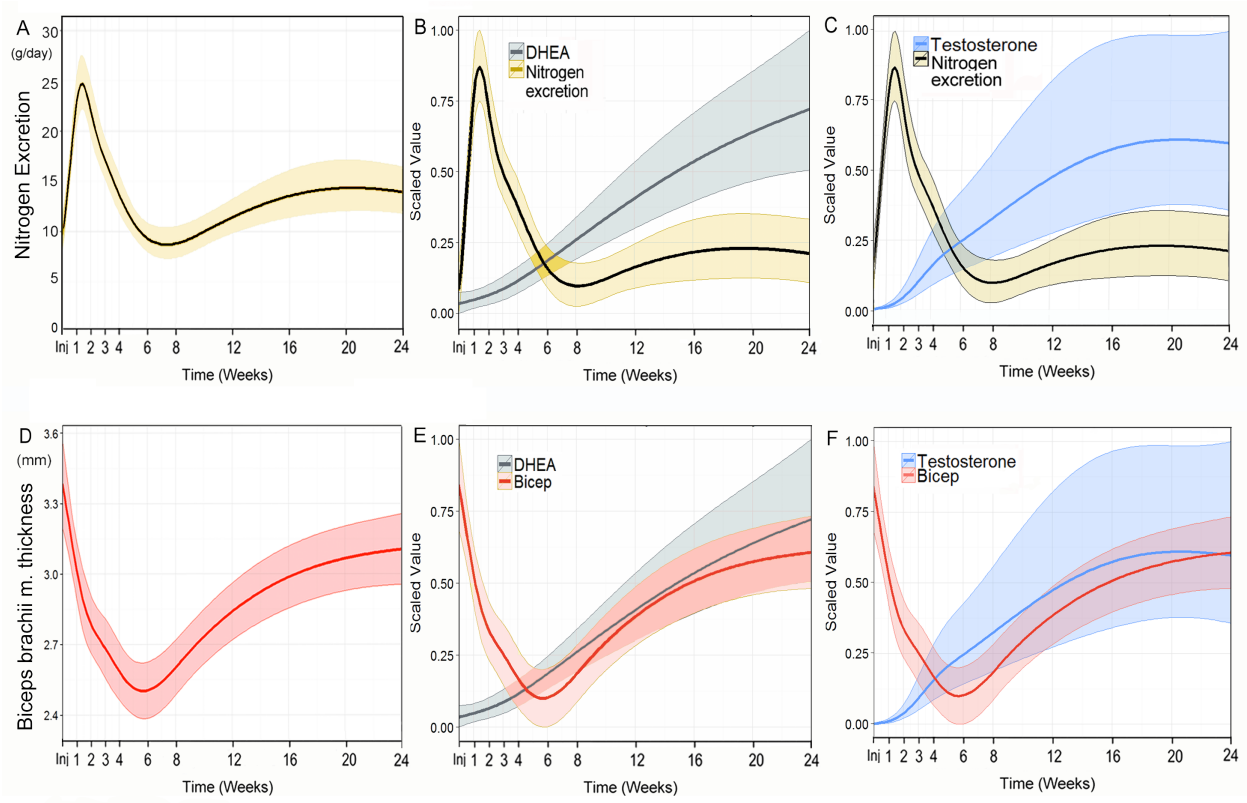

Figure 4. The relationship between (A) Urinary Nitrogen Excretion or (B) biceps muscle thickness with (B and D) DHEA and (C and F) testosterone, over time for young (<50), severely injured (NISS>15) males who had survived and not been given anabolic steroids. Muscle thickness data was modelled using a mixed effects technique; modelling time as a 6 and 7-knot restricted cubic spline respectively provided the best fit. Data are means and 95% confidence intervals for model-based predicted fixed effects of time are shown.

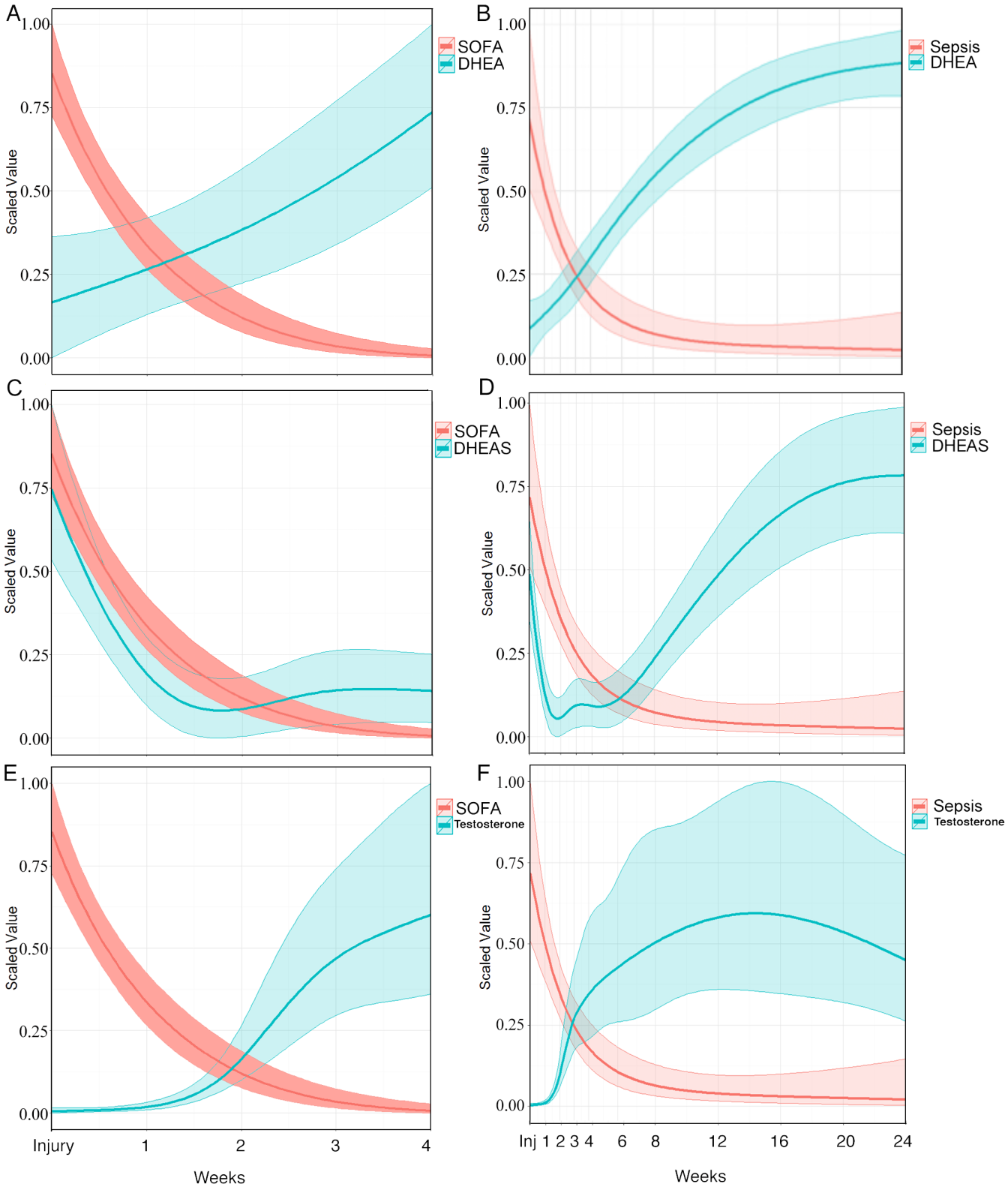

Figure 5. Sequential Organ Failure Assessment (SOFA) score and probability of sepsis in relation to endocrine response. SOFA and sepsis are related serum concentrations of DHEA (Panels A+B), DHEAS (Panels C+D), and testosterone (E+F). Data were modelled using a non-linear mixed effects model that accounts for unbalanced repeated measures using a 4-knot cubic spline. Modelled data are reported as means and 95% confidence intervals.