Mapping the steroid response to major trauma from injury to recovery: a prospective cohort study

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Abstract

Context: Survival rates after severe injury are improving, but complication rates and outcomes are variable. **Objective:** This cohort study addressed the lack of longitudinal data on the steroid response to major trauma and during recovery.

Design: We undertook a prospective, observational cohort study from time of injury to six months post-injury at a major UK trauma center and a military rehabilitation unit, studying patients within 24 hours of major trauma (estimated New Injury Severity Score (NISS) >15).

Main outcome measures: We measured adrenal and gonadal steroids by tandem mass spectrometry, assessed muscle loss by ultrasound and nitrogen excretion, and recorded clinical outcomes (ventilator days, length of hospital stay, incidence of organ dysfunction and sepsis); results were analyzed by generalized mixed-effect linear models.

Findings: We screened 996 multiply injured adults, approached 106, and recruited 95 eligible patients; 87 survived. We analyzed 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; the serum cortisol over cortisone ratio increased, normalizing eight weeks post-injury. Serum testosterone, DHEA and DHEAS decreased immediately after trauma and took two, four and more than six months, respectively, to recover; all three correlated with SOFA score and probability of sepsis.

Conclusion: The catabolic response to severe injury is accompanied by acute and sustained androgen suppression. Whether androgen supplementation improves health outcomes after major trauma should be investigated.

Précis

A cohort study in male survivors of major trauma revealed acute and sustained androgen suppression and protein catabolism including muscle loss. Serum androgens correlated with probability of sepsis.

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 improvement in survival is often offset during the weeks following acute major trauma, by the systemic inflammatory response syndrome (SIRS), associated with increased risks of infection, multi-organ dysfunction or failure (MOD/MOF), and death(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) 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 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 Defense Medicine and the Queen Elizabeth Hospital Birmingham, a major UK trauma center and the primary receiving facility for UK military personnel injured abroad. Military and civilian trauma patients with an estimated New Injury Severity Score (NISS) >15 were recruited(13). NISS was used to ensure those with significant extremity trauma but lower ISS were included(14). Patients with significant head injury or pre-injury neoplastic conditions were not eligible. Informed consent was obtained from personal consultees until recovering 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 and their clinical data were entered prospectively and used to calculate Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), and Simplified Acute Physiology Score (SAPS)(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 during recovery from major trauma. This challenging sampling schedule was supported by junior clinical staff to ensure blood sampling between 0730 and 0900hrs; serum was then separated and frozen at -80°C for batched analysis. We separately collected morning blood samples from 37 healthy age- and sex-matched controls, to provide a comparator for the steroid data.

Assessment of protein catabolism

As a surrogate marker for muscle mass, we undertook longitudinal measurements of muscle thickness by a well validated method using portable ultrasound as described by Campbell *et al*(18,19). Ultrasound measurements were taken from four 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. All ultrasound assessments were performed by two designated operators. Measurements were performed three times at each muscle site and the mean of the three measurements was recorded. The dominant arm was favored for

ultrasound assessment of muscle mass unless it was missing or unable to be measured where wounds were extensive. This non-invasive method was chosen over others such as creatine (methyl-d3) dilution (D3-creatine) (20) due to the strict dietary requirements for methy-d3 estimation, which was not practical in the context of major trauma; similarly, we did not undertake MRI measurement of muscle mass due to the risk associated with repeated transport of critically ill patients to scanning facilities (21).

Urinary urea excretion was measured and used to estimate Total Urinary Nitrogen (TUN) excretion as described by Milner et al(22) [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, employing a validated multi-steroid profiling method(23). In brief, serum steroids were extracted via liquid/liquid tert-butyl-methyl-ether (MTBE), evaporated, reconstituted, and analyzed by LC-MS/MS for cortisol and cortisone. Serum androgens and androgen precursors (DHEA, androstenedione, testosterone) were measured following oxime derivatisation(24,25). DHEA sulfate (DHEAS) was measured following protein precipitation(26,27).

Serum concentrations of sex hormone-binding globulin (SHBG) and luteinizing hormone (LH) were analyzed 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.

Generalized linear mixed-effects models(28) were used to examine the change in variables over time. Patients were included in models as random effects to account for repeat measures over time on the same individuals. Time was modelled using restricted cubic splines(29) to allow for flexible relationships(30). 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 four weeks and first six months after injury as required. Analyses were conducted in R using libraries lme4, effects, rms and ggplot2. Power calculation based on previous work[36] indicated a minimum sample size of 25 patients accommodate variability in steroid analysis.

RESULTS

Patient recruitment and clinical characteristics of the final study cohort

We screened 996 multiply injured adults. 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 102 patients recruited into the study, two withdrew and re-assessment in five revealed an actual NISS ≤15, leaving a study cohort of 95 patients (**Fig. 1A**).

Excluding eight fatalities and seven patients that had received steroid therapy, 80 survivors completed sample collection over 6 months. To minimize confounders, we excluded the small groups of women (n=9) and age-advanced men (n=11), leaving our final study cohort of 60 men <50 years of age (**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 on the intensive care unit (ICU) for a median of 9 (IQR 5-16) days. Median length of hospital stay 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 (**Fig 2C**).

Glucocorticoid biosynthesis and metabolism after major trauma

Serum cortisol concentrations increased slightly 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 observed in healthy controls. Serum concentrations of the inactive glucocorticoid 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 11β-HSD activity (**Fig. 3C**), peaked at two weeks post-injury and returned to normal at around eight weeks.

Androgen biosynthesis and activation after major trauma

Serum concentrations of adrenal androgen precursor dehydroepiandrosterone (DHEA) were very low after injury ($p<0{\cdot}0001$, as compared to healthy controls), but recovered to the normal range by three months post-injury (**Fig. 3D, Suppl. Fig. 1**). In contrast, its sulfate ester, DHEAS, demonstrated sustained suppression and median concentrations did not 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, followed by a gradual decrease, but without returning to normal by the end of the 6-month study period.

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 testosterone (**Fig. 3I**) was very low following injury, starting to increase after two weeks, and recovering to the healthy sex- and age-matched reference range approximately eight weeks after injury. This was mirrored by acute suppression of serum LH immediately after injury, followed by recovery to the normal range approximately 2 weeks after injury (**Suppl. Fig. 2C+D**). Serum sex hormone-binding globulin (SHBG) (**Suppl. Fig. 2E+F**) concentrations were subnormal immediately post-injury but quickly 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, peaking at $25{\cdot}0 \pm 16{\cdot}1$ g/day at the end of the first week, returning to below $15{\cdot}0$ g/day by week 4. The mean maximum rate of nitrogen excretion was $33{\cdot}0 \pm 21{\cdot}3$ g/day **(Fig. 4A)**. The normalization 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 measurement of muscle thickness; dressings, amputations and other wounds hampered the measurements in the other muscle areas. Changes in biceps brachii muscle thickness followed a U-shaped curve after injury, reaching a nadir at 6 weeks (day 1 after trauma compared with week 6, p=0·001). The mean muscle loss was 22·7±12·5% **(Fig. 4D)**. Similar to TUN, muscle thickness recovered 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, serum 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 serum cortisol-to-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 this study, we have characterized the response of adrenal and gonadal steroids and catabolic metabolism to severe injury, describing the related dynamic changes for six months post-injury. Modelling the resulting data has allowed us to provide a detailed description of the transition from catabolism to anabolism during recovery from severe injury. 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.

Our study revealed an initial phase of minor glucocorticoid activation with a transient increase in the serum cortisol-to-cortisone ratio. As summarized in a recent meta-analysis, previous data on serum cortisol after injury is limited to small cohorts derived from elective surgery, rarely followed up for more than two days(31).

In our study, serum cortisol quickly returned to normal following slight 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 11β-hydroxysteroid dehydrogenase (11β-HSD) activity. The cortisol-activating enzyme 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, thereby dampening the inflammatory response(32,33). Skeletal muscle expresses 11β-HSD1(34) and previous studies reported increased 11β-HSD1 activity in an animal model of trauma haemorrhage(35) and recently improved wound healing in mice treated with 11β-HSD1 inhibitors(36); human data after trauma are lacking. Mirroring this, there is substantial evidence indicating a reduced cortisol clearance in critical illness, due to decreased cortisol inactivation in liver and kidney (37); this mechanism could also be responsible for the slight changes in cortisol and cortisone we observed.

In our 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 took two and four months post-injury, respectively, and DHEAS remained pathologically decreased at the end of the six-month follow-up period. In a mouse model of acute inflammation, sustained suppression of the expression of the DHEA sulfotransferase SULT2A1 and its sulfate donor enzyme, PAPSS2, have been described(60). We reviewed 23 previous studies that measured serum DHEA and DHEA(**Suppl. Table 1**) patients (**Suppl. Table 1**), but most followed patients only for a few days and relatively few patients suffered from acute trauma. One previous study measured both serum DHEA and DHEAS in 181 patients with septic shock and 31 patients with acute hip fracture(48); serum DHEAS was decreased in both groups, while DHEA was increased in the sepsis patients but decreased in the trauma patients, suggesting an inflammation-mediated downregulation of DHEA sulfation in the latter group, resulting in a dissociation between serum DHEA and DHEAS concentrations.

A number of previous studies have described an association of infection and mortality with low circulating DHEAS concentrations and a raised serum cortisol-to-DHEAS ratio in patients with trauma(42,43,61,62). In vitro studies have demonstrated that cortisol decreases neutrophil superoxide

production, which is counteracted by coincubation with DHEAS(42). 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(63). In this study in severely injured men younger than 50 years of age, we observed suppression of both serum DHEA and DHEAS post-injury, indicating that the loss of adrenal androgen synthesis is 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 even six months post-injury.

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 shown in our study is supported by the literature(56,57,59,64). Our longitudinal data demonstrate that suppression of the HPG axis is of shorter duration than that of the HPA axis. In traumatic brain injury studies, a significant proportion of patients go on to develop anterior pituitary dysfunction including secondary hypogonadiasm(65)' (50,51), however, in our study traumatic brain injury was an exclusion criterion. While limited data from patients with burns and critical illness have suggested a central, hypothalamic-pituitary cause of trauma-related hypogonadism(66,67), the evidence prior to our study has been limited. Out data indicate a central cause of suppression to the gonadotrophic axis, with a decrease in both pituitary LH and gonadal testosterone. However, it cannot be excluded that the administration of opiates could causally contribute to the induction of central hypogonadism(68).

Our cohort study reveals a loss of both adrenal and gonadal androgen production in young and middle-aged men after major trauma. Androgens are important in wound healing, erythrocytosis, bone density and muscle mass(69). The catabolic state that occurs following trauma thus presents a significant challenge. 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(70). 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(71). The use of oxandrolone in major trauma was investigated in two intensive care studies but no benefit was demonstrated(72,73).

The strengths of our study include its prospective nature, narrow age range of the patients, single gender, single site for recruitment and analysis and detailed follow-up over six months as well as the measurement of circulating steroid hormones by state-of-the-art tandem mass spectrometry. Analyzing a young cohort has also reduced the confounding effects of age-related co-morbidities.

Our study is limited by the diverse nature of major trauma patients in relation to injury pattern and the involvement of military casualties. The timing and number of observations during our study was pragmatic and some statistical comparisons were made using modelled data. While we measured total cortisol and cortisone by tandem mass spectrometry, we did not measure free cortisol or cortisol-binding globulin. The estimation of nitrogen excretion was pragmatic due to the diverse nature of the patients and we were not able to record nitrogen intake. Ultrasound estimation of muscle thickness was performed at four different body sites but many individuals had limbs missing or extensive wounds that prevented measurements. While imperfect, the longitudinal nature of these measurements allowed us to model these changes over time.

In conclusion, in this most detailed and first prospective study of the steroid response to major trauma, we followed the patients from severe injury to six months of recovery, revealing pronounced and sustained decreases in adrenal and gonadal androgen biosynthesis. Recovery of androgen production in the severely injured patients was mirrored by a switch from catabolism to anabolism as reflected by recovery of muscle mass and a decrease in nitrogen loss. Adrenal and gonadal androgens correlated with risk of sepsis. It is tempting to suggest that an anabolic intervention with androgens or androgen precursors could have a beneficial effect on health outcomes during recovery from major trauma. However, this will need to be investigated by future intervention studies.

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Legends

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**.**

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.

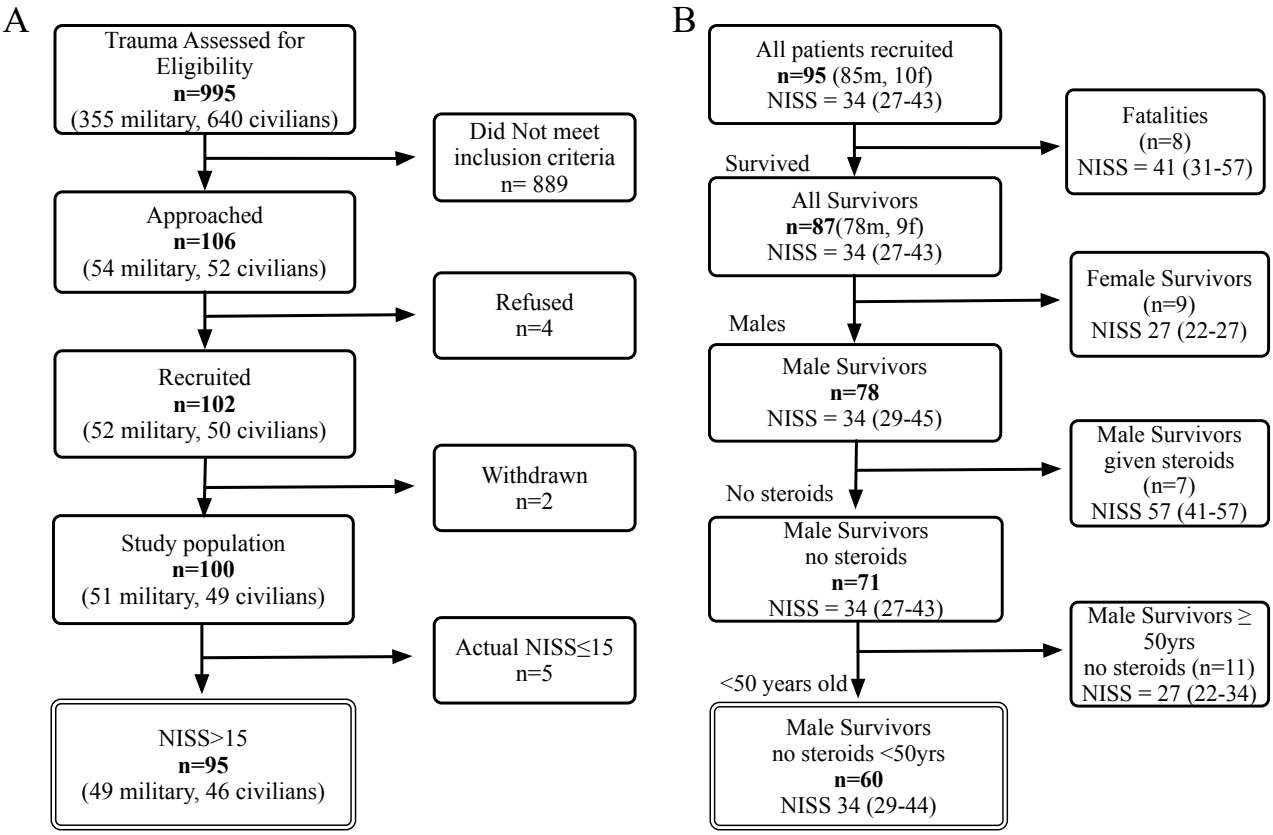
Figure 5. Sequential Organ Failure Assessment (SOFA) score and probability of sepsis in relation to endocrine response. SOFA and sepsis are related serum concentrations of DHEA (Panels A+B), DHEAS (Panels C+D), and testosterone (E+F). Data were modelled using a non-linear mixed effects model that accounts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

Suppl. Table 1. Longitudinal Studies in critically ill patients analysing DHEA and DHEAS.

Suppl. Fig. 1. Serum steroid concentrations in 60 male survivors from severe injury (NISS>15) under 50 years of age. Serum concentrations of cortisol (A), cortisone (B), DHEA (C), DHEAS (D), androstenedione (E), and testosterone (F) are shown as medians and interquartile ranges; the normal reference range is indicated by the grey shaded box.

Suppl. Fig. 2: Male gonadal axis parameters during the first 4 weeks following injury. Luteinising hormone (LH), sex hormone binding globulin (SHBG) and serum testosterone concentrations in male survivors <50 years after severe injury (NISS>15). LH and SHBG were only measured in the military cohort, therefore represent results from 43 individuals. Data are represented after modelling based on the raw data, using a non-linear mixed effects model that accounts for unbalanced repeated measures using a 4-knot cubic spline.

Suppl. Fig 3. Sequential Organ Failure Assessment (SOFA) score and probability sepsis in relation to endocrine response. SOFA and sepsis are related to the ratios of serum DHEA over serum DHEAS (Panels A+B), and the ratio serum cortisol over serum DHEAS (Panels C+D) compared to serum (A), DHEA/DHEAS ratio (C) Cortisol/DHEAS. 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.



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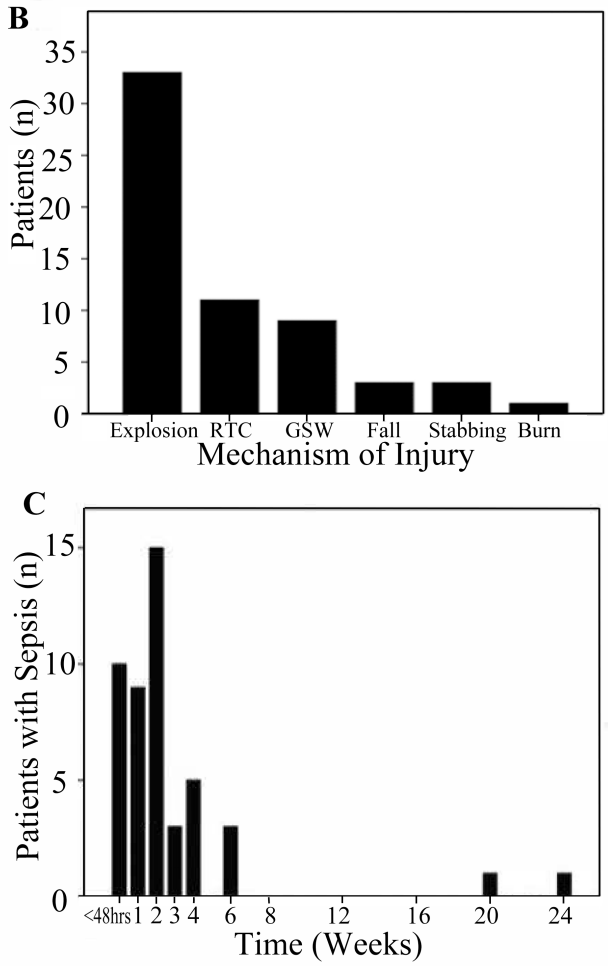
A

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)



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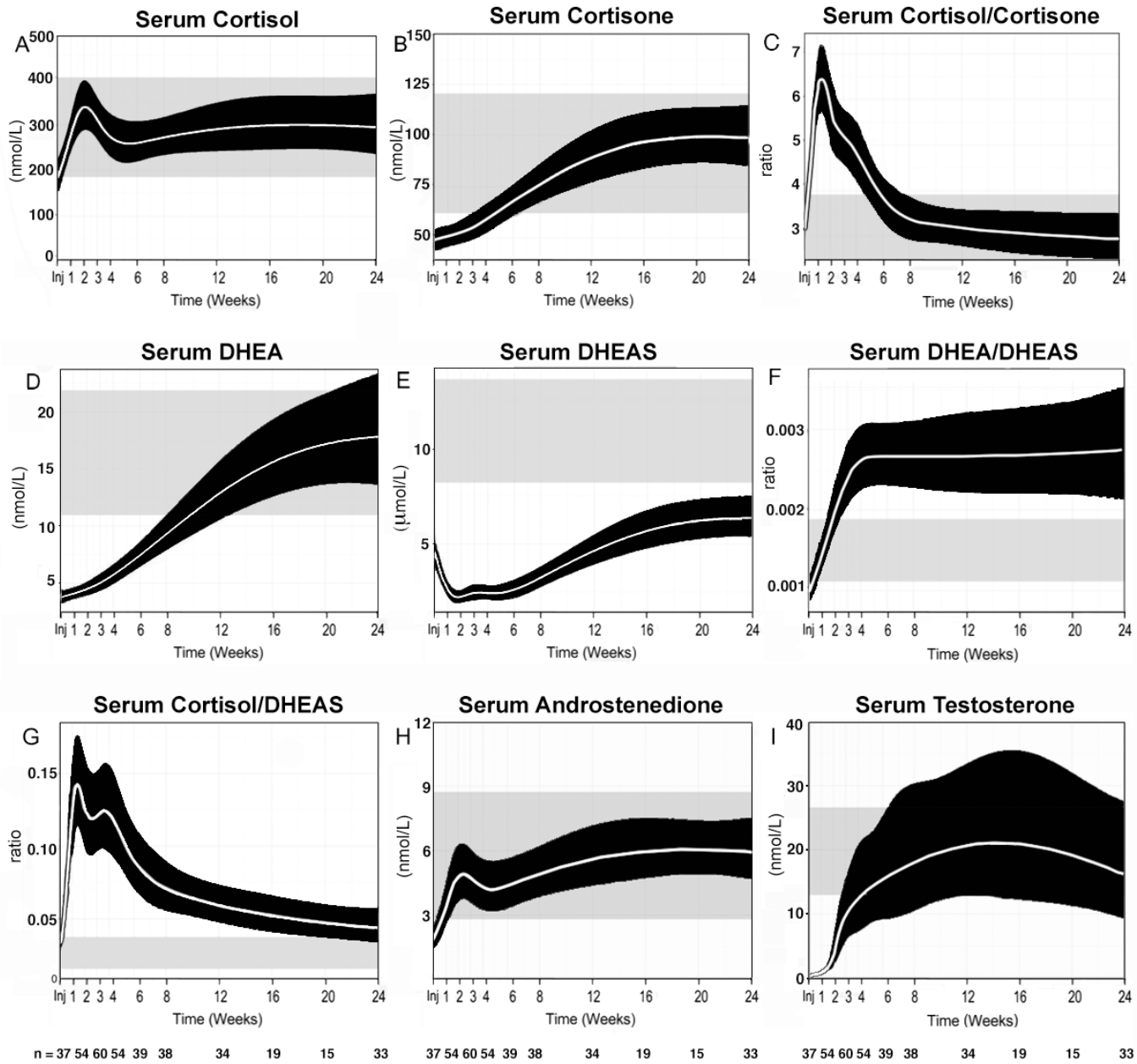

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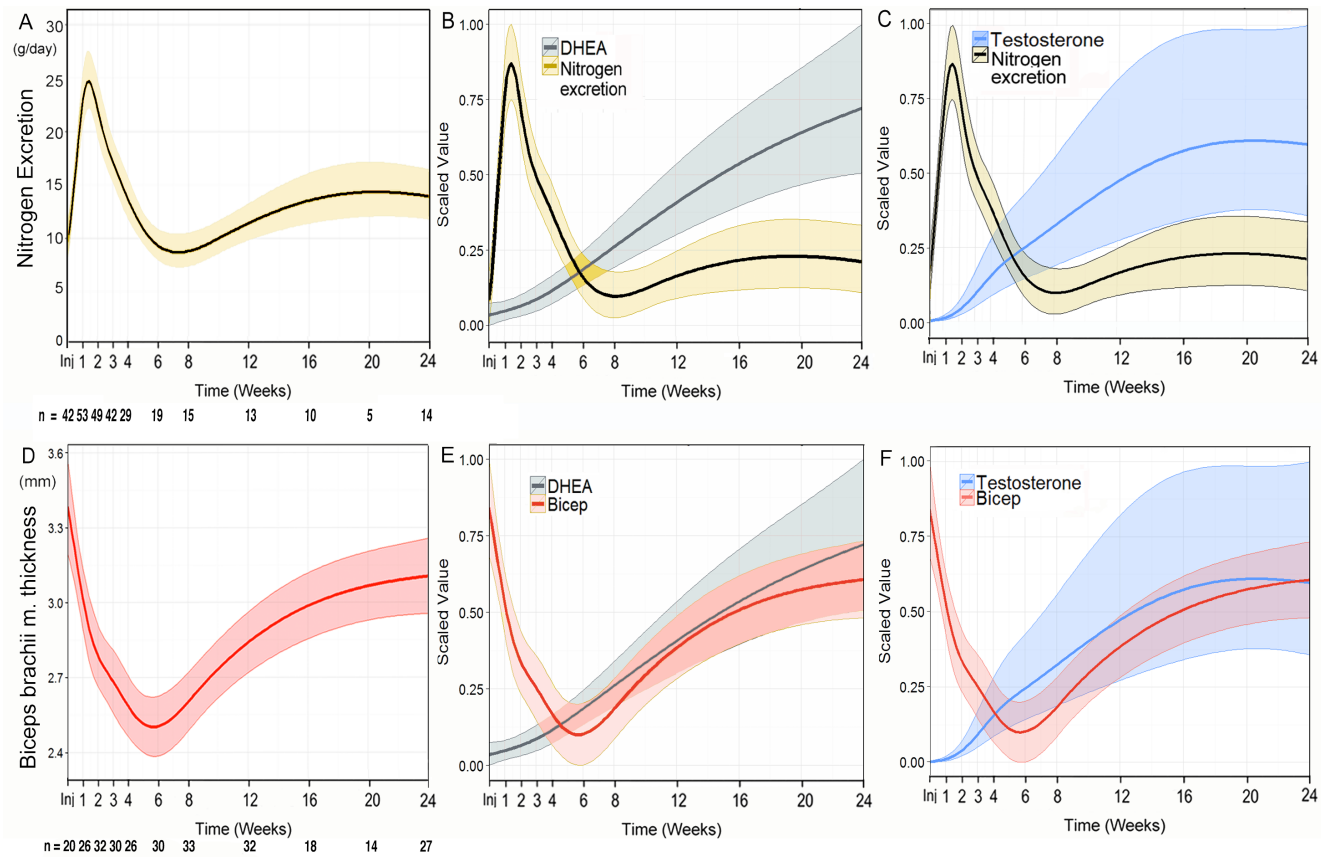

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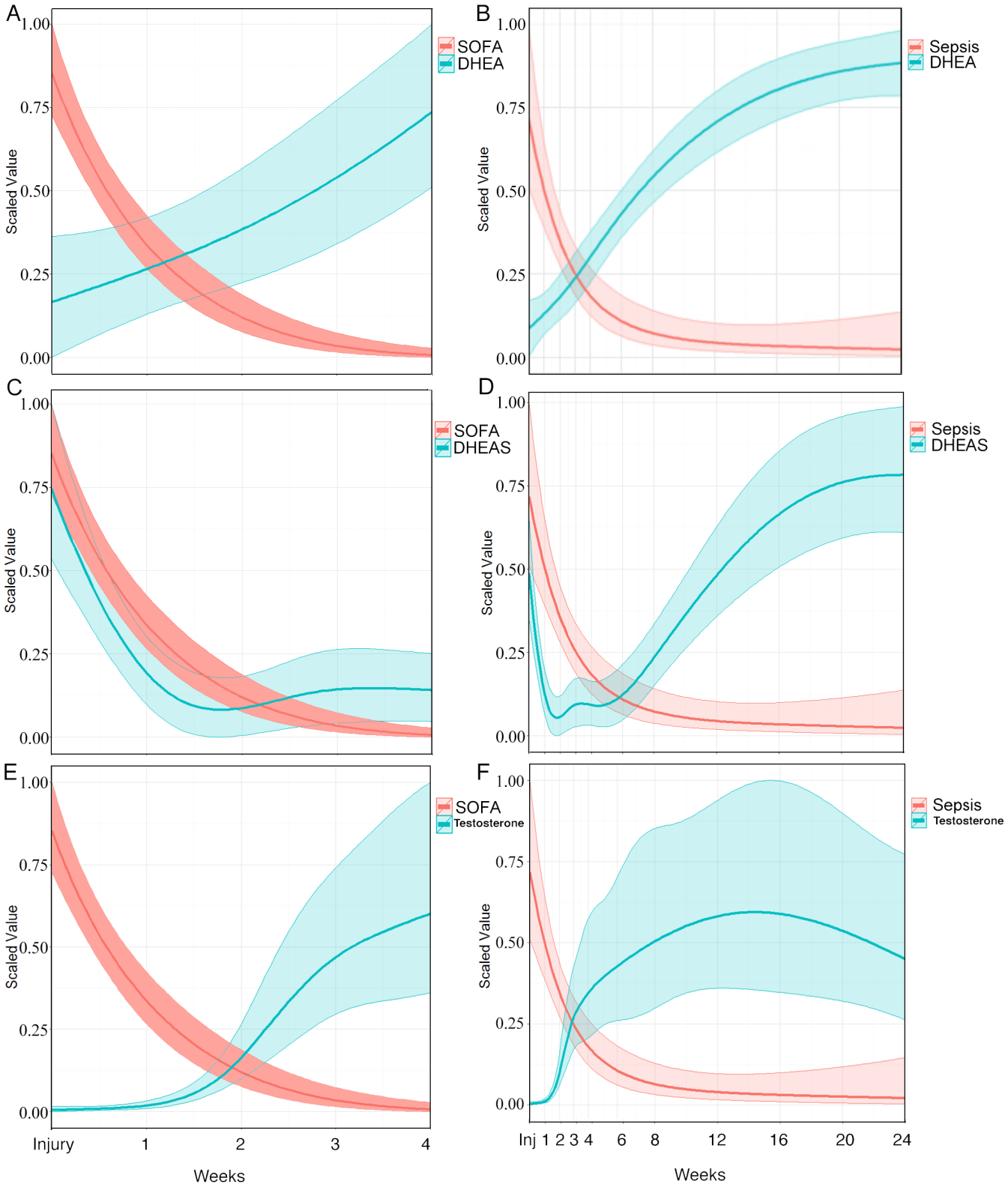

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