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Mapping the Steroid Response to Major Trauma From Injury to Recovery: A Prospective Cohort Study

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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 6 months postinjury 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).

Main outcome measures: We measured adrenal and gonadal steroids in serum and 24-hour urine by mass spectrometry, assessed muscle loss by ultrasound and nitrogen excretion, and recorded clinical outcomes (ventilator days, length of hospital stay, opioid use, incidence of organ dysfunction, and sepsis); results were analyzed by generalized mixed-effect linear models.

Findings: We screened 996 multiple 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 1 and 6 weeks, respectively. Serum testosterone, dehydroepiandrosterone, and dehydroepiandrosterone sulfate decreased immediately after trauma and took 2, 4, and more than 6 months, respectively, to recover; opioid treatment

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delayed dehydroepiandrosterone recovery in a dose-dependent fashion. Androgens and precursors correlated with SOFA score and probability of sepsis.

Conclusion: The catabolic response to severe injury was accompanied by acute and sustained androgen suppression. Whether androgen supplementation improves health outcomes after major trauma requires further investigation. (*J Clin Endocrinol Metab* 105: 1–13, 2020)

Key Words: major trauma, systemic inflammatory response syndrome, stress response, steroids, DHEA, testosterone

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 centers in England has been accompanied by a 19% improvement in survival odds following injury (4). During this time, the United Kingdom also received severely injured military trauma patients from the conflict in Afghanistan (5).

Improvements in short-term outcomes have been achieved through early resuscitation and acute care (6), often informed by approaches pioneered on the battlefield. However, improvement in survival is often offset during the weeks following acute major trauma by the systemic inflammatory response syndrome (SIRS), which is associated with increased risks of infection, multiorgan dysfunction or failure (MOD/MOF), and death (7, 8). Simultaneously, the hypothalamic-pituitary-adrenal axis (HPA) is thought to drive a hypermetabolic and overtly catabolic response. Importantly, in this profound catabolic state, patients lose valuable lean muscle and suffer from increased rates of infection and poor wound healing. Moreover, the dynamic nature of this response, especially beyond the first few days following injury and during recovery remains poorly described and understood, limiting the evidence base for novel therapeutic interventions. Burn injury also produces an extreme inflammatory and catabolic response after injury, previously targeted by anabolic steroid analogues (9) and beta-blockade (10). The dynamic changes in endogenous glucocorticoids and their influence on adrenal steroid metabolism after severe injury are not well characterized. We know that proinflammatory cytokines activate the enzyme 11 β -hydroxysteroid dehydrogenase type (11 β -HSD1) responsible for tissue-specific activation of glucocorticoids through the conversion of inactive cortisone to active cortisol (11). However, only scarce data exist on what happens to early sex steroids and their precursors during this catabolic state.

To address these gaps in knowledge, 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 for the 6 months post-trauma.

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.

Materials and 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 (12). The NISS was used to ensure those with significant extremity trauma but lower injury severity score were included (13). Patients with significant head injury or preinjury neoplastic conditions were not eligible. None of the patients received etomidate during their treatment. 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 an Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), and Simplified Acute Physiology Score (SAPS) (14, 15). Sepsis was defined using Bone's criteria of an infection associated with SIRS (16), current at the time of the study.

Details of opioid administration were collected from the electronic health record and prescribing system (PICS) (17) on each of the study patients. The total amount of opioid given during their hospital stay was appropriately weighted and totaled with equivalence to an oral dose of 10 mg morphine, adjusting for potency, delivery method, and opioid preparation (18).

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 post-trauma. Blood sampling occurred between 7:30 AM and 9:00 AM; 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 (19, 20). 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. All ultrasound assessments were performed by 2 trained operators. Measurements were performed 3 times at each muscle site and the mean of the 3 measurements was recorded. The dominant arm was favoured for ultrasound assessment of muscle mass unless it was missing or unable to be measured where wounds were extensive. This noninvasive method was chosen over others such as creatine (methyl-d3) dilution (D3-creatine) (21) due to the strict dietary requirements for methyl-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 (22).

Urinary urea excretion was measured and used to estimate total urinary nitrogen (TUN) excretion as described by Milner et al (23) (estimated nitrogen excretion: urinary urea excretion (mmol/l) \times 0.028 \times 1.25 = total urinary nitrogen excretion (g/l)).

Steroid analysis

Serum concentrations of adrenal and gonadal steroids were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, employing a validated multisteroid profiling method (24). 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 derivatization (25, 26). Serum DHEA sulfate (DHEAS) was measured following protein precipitation (27, 28). Steroid metabolite excretion analysis in 24-hour urine samples was carried out by gas chromatography-mass spectrometry (GC-MS) in selected-ion-monitoring (SIM) mode, as previously described (29).

Serum concentrations of sex hormone-binding globulin (SHBG) and luteinizing hormone (LH) were analyzed on the Roche Modular System (Roche Diagnostics, Lewes, UK) by 2-site sandwich immunoassay using electrochemiluminescence 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 (30) 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 (31) to allow for flexible relationships (32). Severity scores were modeled 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 postinjury as required. Analyses were conducted in R using libraries lme4, effects, rms, and ggplot2.

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 \leq 15,

others had a significant head injury as their major injury component, and 2 were excluded due to a preinjury diagnosis of cancer. Of the 102 patients recruited into the study, 2 withdrew, and reassessment in 5 revealed an actual NISS \leq 15, leaving a study cohort of 95 patients (Fig. 1A).

Excluding 8 fatalities and 7 patients who 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 RTC ($n = 11$; 18%) were the most common causes of injury. Twenty-five (42%) patients had at least 1 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 2 weeks (Fig. 3A) (33). 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) (33). The serum cortisol-to-cortisone ratio, a marker of systemic 11 β -HSD activities (Fig. 3C), peaked at 2 weeks postinjury and returned to normal at around 8 weeks. Consistent with these findings, urinary steroid metabolite excretion analysis revealed an increase in glucocorticoid metabolite excretion in weeks 2, 4, and 8 after major trauma, alongside changes in steroid metabolite ratios indicative of increased systemic 11 β -HSD1 and decreased 11 β -HSD2 activities, as assessed by (5 α -tetrahydrocortisol + tetrahydrocortisol)/tetrahydrocortisone and cortisol-to-cortisone ratio, respectively (33).

Androgen biosynthesis and activation after major trauma

Serum concentrations of the adrenal androgen precursor dehydroepiandrosterone (DHEA) were very low after injury ($P < 0.0001$, compared with healthy controls) but recovered to the normal range by 3 months

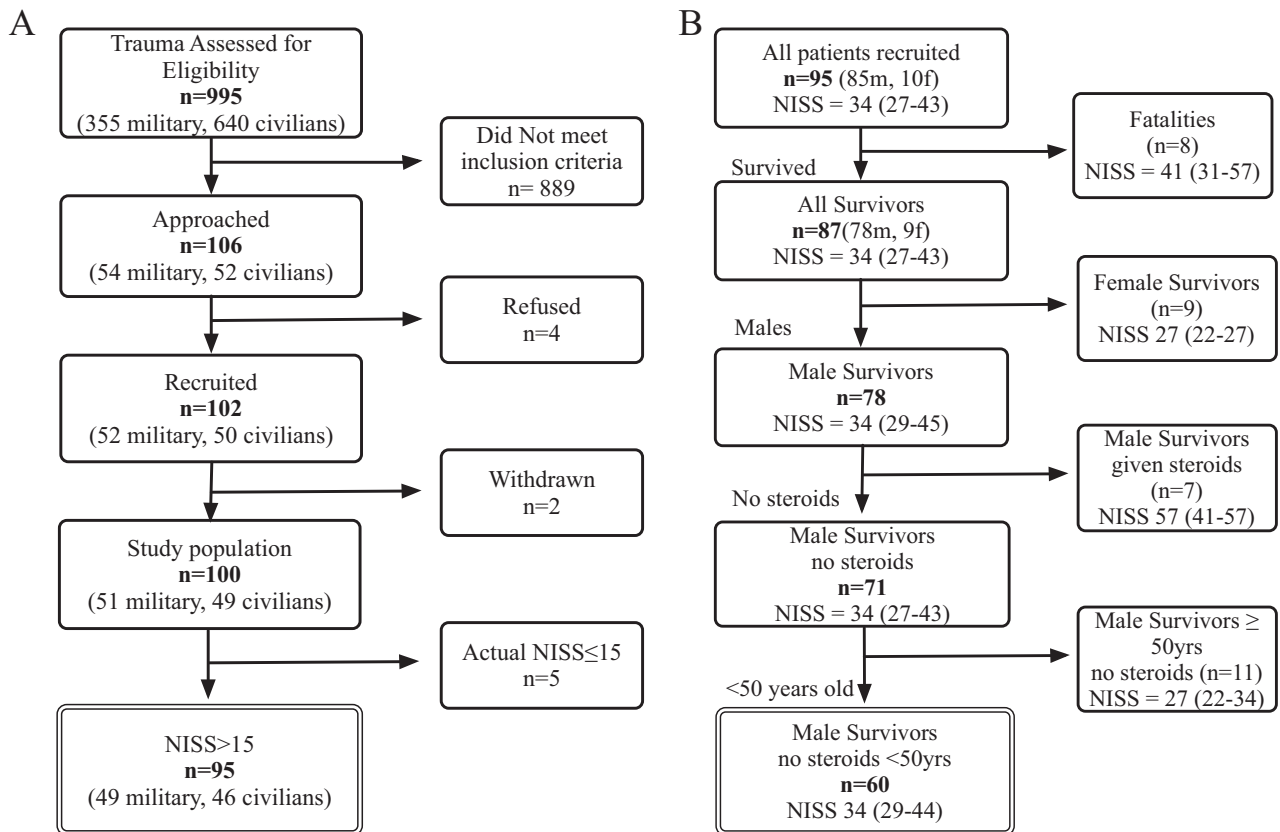


Figure 1. Consort diagram. A, recruitment process. B, subgroup selection for analysis for 60 male survivors of severe injury (NISS > 15) under 50 years of age who had not been given exogenous steroids were analyzed.

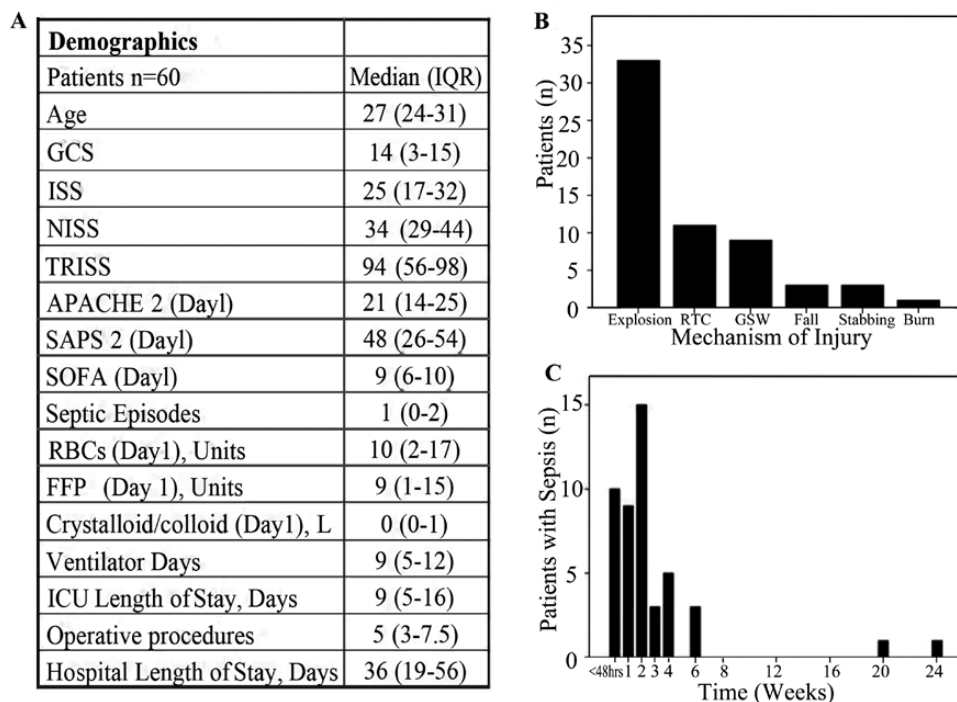


Figure 2. Patient characteristics of the analysis cohort. A, Demographics. B, Mechanism of injury. C, The distribution of septic episodes for 60 male survivors from severe injury (NISS > 15) under 50 years of age.

postinjury (Fig. 3D) (33). In contrast, its sulfate ester, DHEAS, demonstrated sustained suppression; median serum DHEAS 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

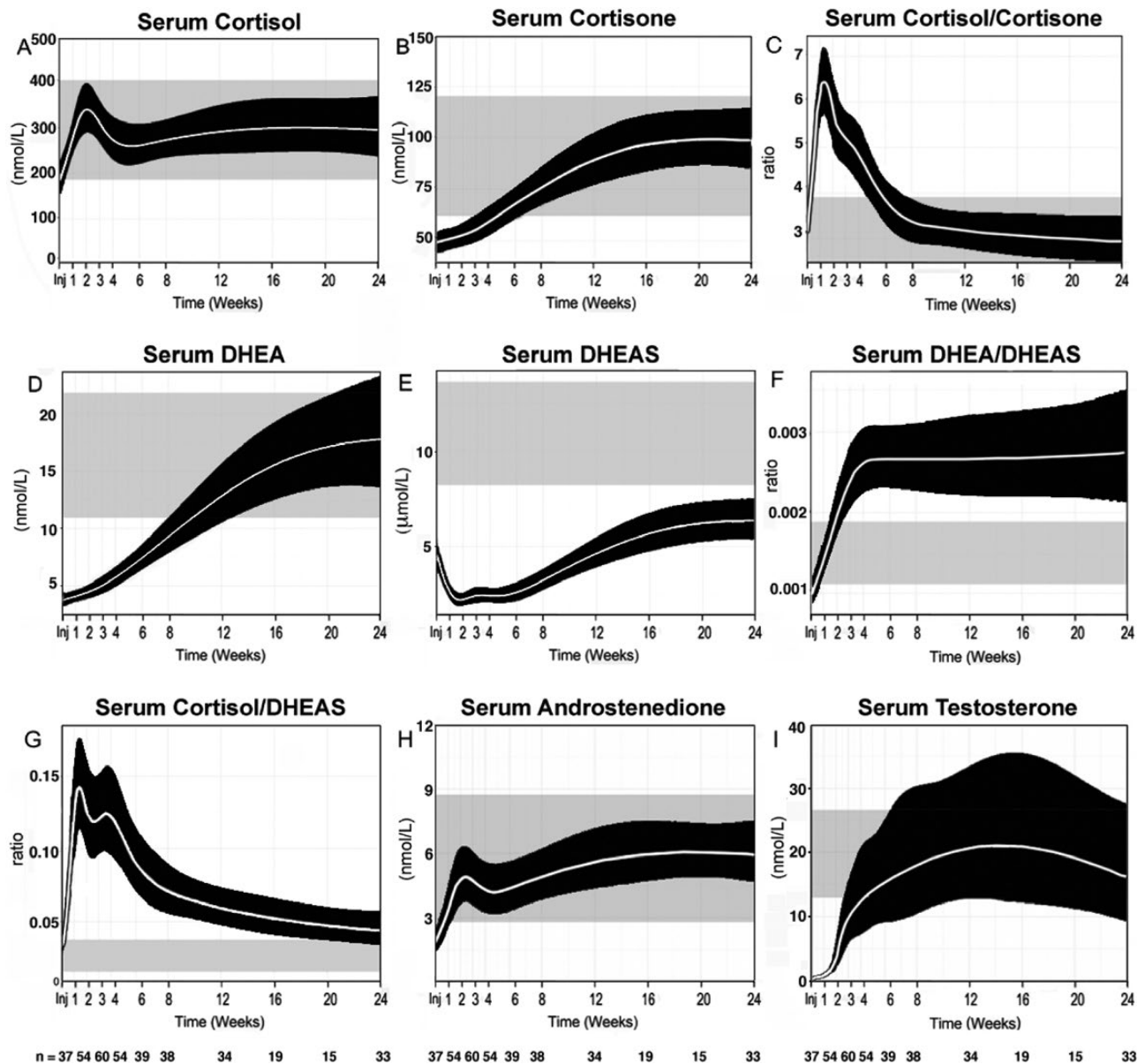


Figure 3. Serum steroids in 60 male survivors of severe injury (NISS > 15) under 50 years of age. Serum concentrations shown include A, cortisol; B, cortisone; C, the cortisol-to-cortisone ratio; D, DHEA; E, DHEAS; F, the DHEA-to-DHEAS ratio; G, the cortisol-to-DHEAS ratio; H, androstenedione; and I, testosterone. Data are represented after modeling of the raw data (33) using a nonlinear mixed effects model that accounts for unbalanced repeated measures using a 4-knot cubic spline. Modeled data are shown as means and 95% confidence intervals.

week 2 compared with controls and failed to return to normal during the 6-month study period. The serum cortisol-to-DHEAS ratio (Fig. 3G) increased postinjury, 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 midreference range at 2 weeks postinjury. Thus, serum androstenedione concentrations recovered much faster than DHEA, suggestive of rapid downstream activation of DHEA to androstenedione.

Serum testosterone (Fig. 3I) (33) was very low following injury, starting to increase after 2 weeks, and recovering to the healthy sex- and age-matched reference range approximately 8 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 (33). Serum sex hormone-binding globulin (SHBG) (33) concentrations were subnormal immediately postinjury, but quickly returned to the healthy reference range between injury and day 7.

Consistent with the observed decrease in circulating androgens, 24-hour urinary steroid metabolite excretion

analysis revealed a steep decrease in the major androgen metabolites androsterone and etiocholanolone at 2, 4, and 8 weeks (33). Similarly, urinary DHEA excretion, representing the sum of unconjugated DHEA and DHEA sulfate, sharply decreased to very low concentrations at 2, 4, and 8 weeks, with a transient increase in 16α -hydroxylation of DHEA at 2 weeks (33), possibly linked to the systemic decrease in DHEA sulfation (Fig. 3D–F). The overall decrease in androgen production was paralleled by a profound decrease in systemic 5α -reductase activity (33), and hence in androgen activation, as 5α -reductase is responsible for converting testosterone to the most potent androgen 5α -dihydrotestosterone.

Protein catabolism after major trauma

The 24-hour TUN excretion increased immediately after trauma, peaking at 25.0 ± 16.1 g/day at the end of the first week, returning to below 15.0 g/day by week 4. The mean maximum rate of nitrogen excretion was 33.0 ± 21.3 g/day (Fig. 4A). The normalization of TUN excretion coincided with the gradual recovery of adrenal and gonadal androgen production (Fig. 4B and 4C).

The biceps brachii muscle was the most reliable site for ultrasound measurement of muscle thickness; dressings, amputations, and other wounds hampered the measurements of 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 $22.7 \pm 12.5\%$ (Fig. 4D). Similar to TUN, muscle thickness recovered alongside gradually increasing adrenal and gonadal androgen production (Fig. 4E and 4F).

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 4 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 3 steroids also showed strong associations with the probability of sepsis ($R = -0.85, 0.85, \text{ and } -0.97$ for serum DHEA, DHEAS, and

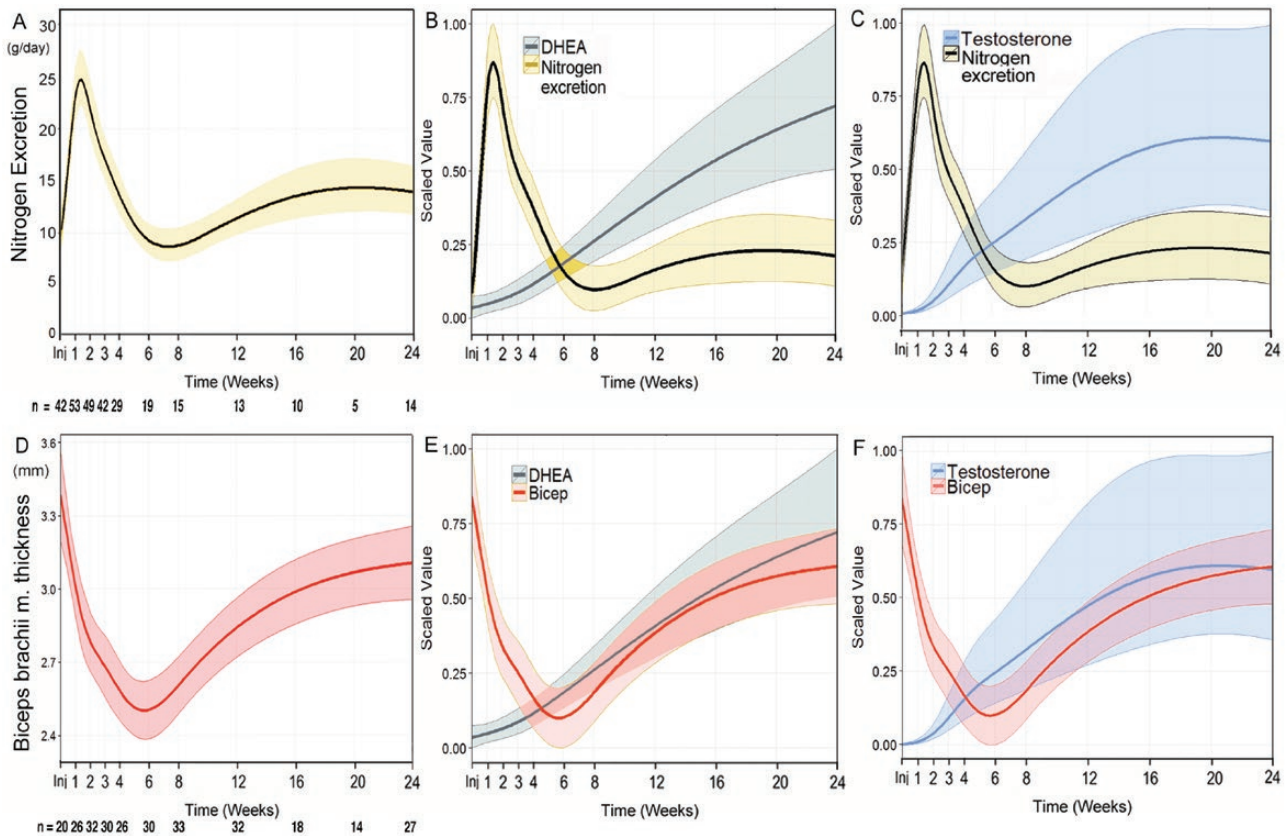


Figure 4. The relationship between A, urinary nitrogen excretion or B, biceps muscle thickness with (B and D) DHEA and (C and F) testosterone, over time for young (<50), severely injured (NISS > 15) males who had survived and not been given anabolic steroids. Muscle thickness data was modeled using a mixed effects technique; modeling 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.

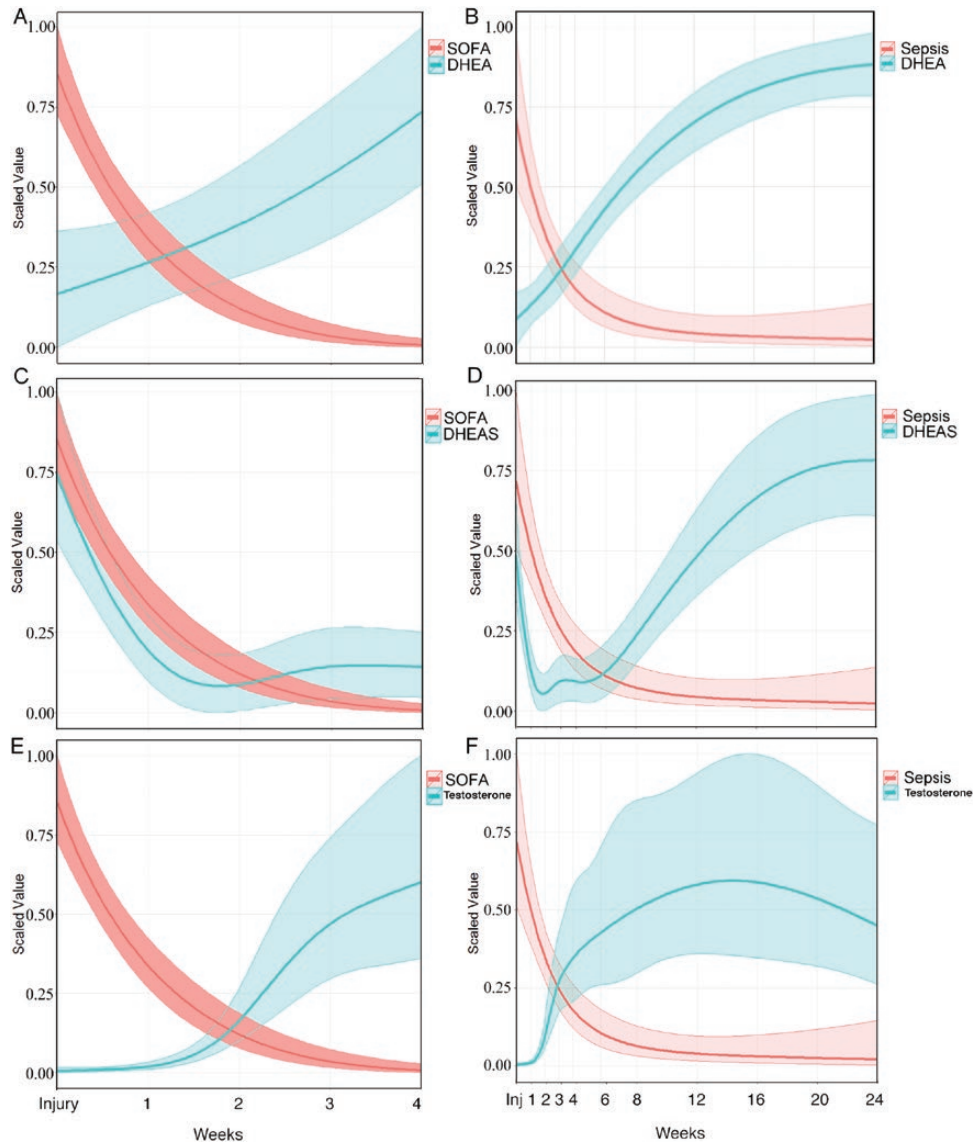


Figure 5. Sequential Organ Failure Assessment (SOFA) score and probability of sepsis in relation to endocrine response. The SOFA and sepsis are related serum concentrations of DHEA (A and B), DHEAS (C and D), and testosterone (E and F). Data were modeled using a nonlinear mixed effects model that accounts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

testosterone, respectively). The SOFA score and probability of sepsis also correlated strongly with the DHEA:DHEAS ratio (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 (ACF = -0.81 and 0.89, respectively) (33).

Opioid administration and endocrine recovery

To examine whether opioid administration affected endocrine recovery, we modeled the impact of the total cumulative in-patient opioid dose on circulating steroid concentrations during recovery from major trauma. For this purpose, we categorised patients according to cumulative opioid dose. Modeling took into account the

differences in injury severity score, length-of-stay (LOS), ICU LOS, and SOFA score.

The adjusted modeling revealed a dose-dependent impact of opioid treatment, with a higher initial peak of serum cortisol and the cortisol/cortisone ratio in those on higher doses (≥ 3000 mg), while those on lower doses had initially lower serum cortisol concentrations but showed better recovery of cortisol and cortisol/cortisone 2 months into the recovery period, with broad interindividual variability in those with high cumulative opioid doses (Fig. 6).

Opioid administration showed a pronounced, dose-dependent effect on adrenal and gonadal androgen production, with significantly delayed recovery of serum DHEA and DHEAS in patients on higher opioid doses

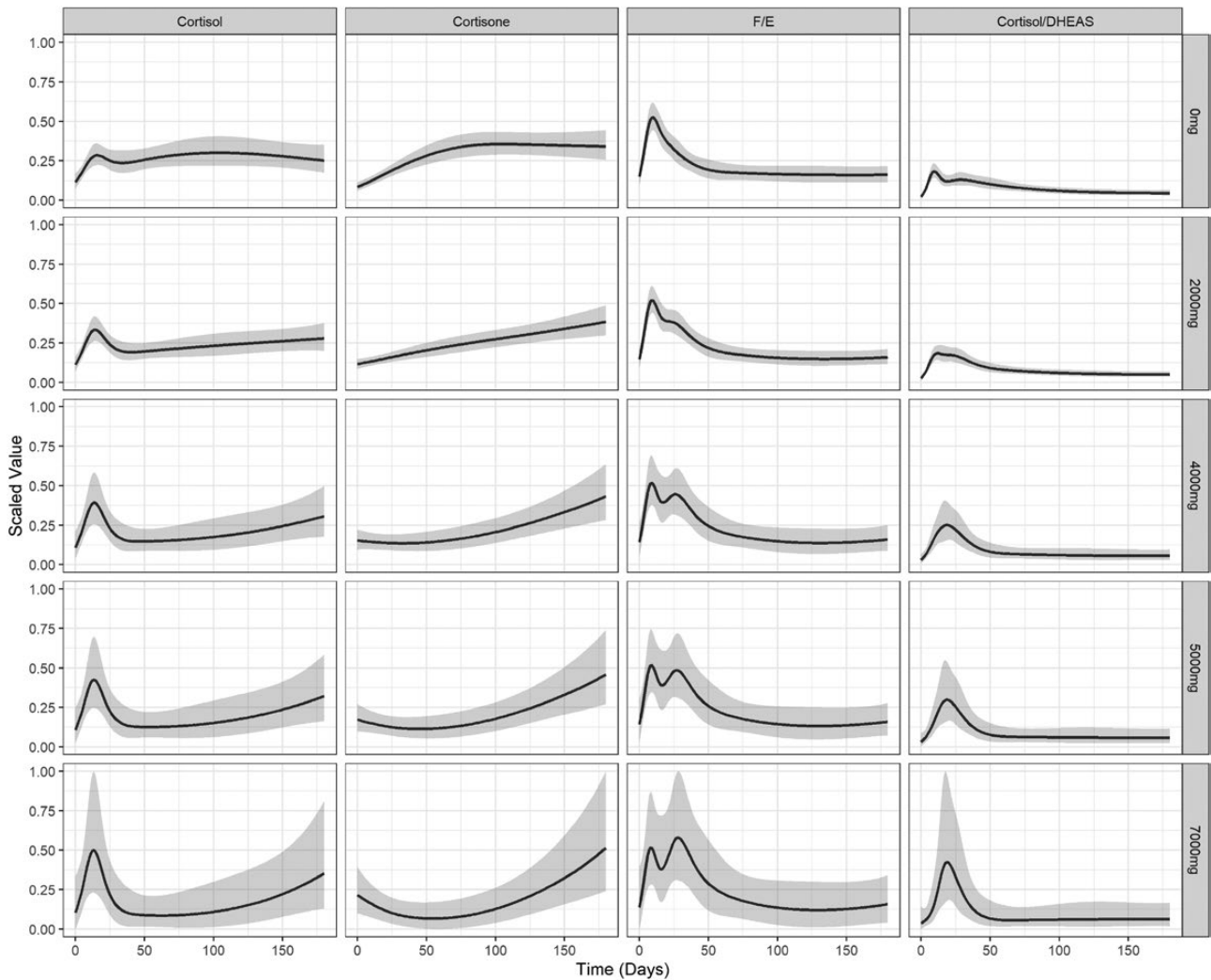


Figure 6. Impact of total inpatient opioid dose on circulating glucocorticoids after major trauma. Serum concentrations are scaled for cortisol, cortisone, the cortisol-to-cortisone ratio, and the cortisol-to-DHEAS ratio. Data are represented after modeling of the raw data using a nonlinear mixed effects model that accounts for unbalanced repeated measures using a 4-knot cubic spline. Modeled data are shown as means and 95% confidence intervals.

($P = 0.029$, $P = <0.001$, respectively; Fig. 7). By contrast, serum testosterone concentrations, which were initially equally suppressed in all cumulative dose groups, showed a much faster recovery in individuals who received higher (≥ 3000 mg) total cumulative opioid doses. However, these confidence intervals were large for these model estimates (Fig. 7).

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 6 months postinjury. Modeling the data has allowed us to provide a detailed description of the transition from catabolism to anabolism during recovery from severe injury, including investigating the impact of cumulative in-patient opioid dose. Our data are the first to provide

detailed adrenal and gonadal steroids beyond the first days after trauma in a large cohort of young patients, with all patients recruited prospectively and steroid analysis carried out by tandem mass spectrometry.

As summarized in a recent meta-analysis (34), previous data on serum cortisol after injury are limited to small cohorts derived from elective surgery, rarely followed-up for more than 2 days. In our study, serum cortisol quickly returned to normal following slight initial increases after acute trauma. In contrast, serum cortisone remained low for 3 months postinjury. Our study revealed an initial phase of minor glucocorticoid activation with a transient increase in the serum cortisol-to-cortisone ratio, with changes in urinary glucocorticoid metabolites indicative of increased 11β -HSD1 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

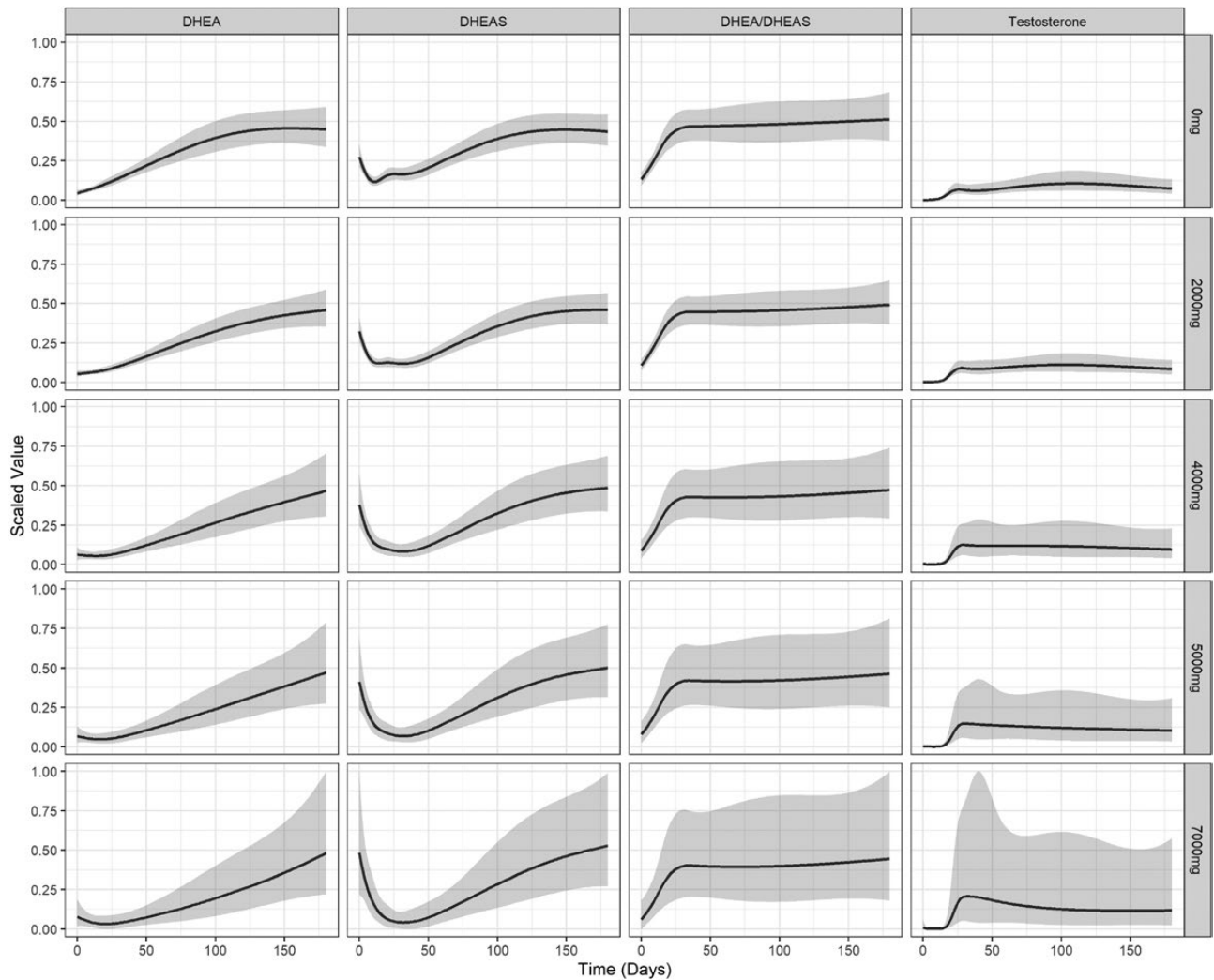


Figure 7. Impact of total inpatient opioid dose on serum androgen and androgen precursors after major trauma. Serum concentrations are scaled for DHEA, DHEAS, the DHEA/DHEAS ratio, and testosterone. Data are represented after modelling of the raw data using a non-linear mixed effects model that accounts for unbalanced repeated measures using a 4-knot cubic spline. Modelled data are shown as means and 95% confidence intervals.

in response to inflammation, thereby dampening the inflammatory response (35, 36). Skeletal muscle expresses 11β -HSD1 (37). Previous studies reported increased 11β -HSD1 activity in an animal model of trauma haemorrhage (38) and improved wound healing in mice treated with 11β -HSD1 inhibitors (39). However, human data after trauma are lacking. There is substantial evidence indicating a reduced cortisol clearance in critical illness, due to decreased cortisol inactivation in liver and kidney (40); this mechanism could also be responsible for the slight changes in cortisol and cortisone we observed. This was corroborated by the observed reduction in the urinary cortisol-to-cortisone ratio, which is reflective of 11β -HSD2 activity.

Interestingly, patients on higher opioid doses showed a higher early peak in cortisol production after trauma, followed by persistently lower circulating cortisol during the recovery period, as compared with patients on lower

opioid doses. Previous reports have described suppressive effects of opioids on the HPA axis, though studies in smaller mammals have indicated an acute stimulatory effect of opioid administration on serum cortisol concentrations (41, 42).

We observed a pronounced and sustained loss of adrenal and gonadal androgen synthesis within the first 24 hours following acute major trauma. The recovery of circulating DHEA and testosterone concentrations took 2 and 4 months postinjury, respectively, and DHEAS remained pathologically suppressed at the end of the 6-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 (43). We reviewed 23 previous studies that measured serum DHEA and DHEAS in critically ill patients (33, 44), but most studies followed patients

for only 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 (45). Serum DHEAS was decreased in both groups, while DHEA was increased in sepsis but decreased after trauma. This suggested an inflammation-mediated downregulation of DHEA sulfation after trauma, resulting in a dissociation of serum DHEA and DHEAS. In our study, this was also observed, as indicated by a sustained increase in the serum DHEA/DHEAS ratio and persistently low serum 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 (46–49). In vitro studies have demonstrated that cortisol decreases neutrophil superoxide production, which is counteracted by coincubation with DHEAS (46). 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 (50). In the present study, carried out in severely injured men younger than 50 years of age, we observed suppression of both serum DHEA and DHEAS postinjury, indicating that the loss of adrenal androgen synthesis is a trauma-related event. Importantly, we showed for the first time that this decrease in circulating adrenal androgen precursors is sustained for several months, and that DHEAS remains low even 6 months postinjury.

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 (51–54). Our prospective, 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 hypogonadism (55). 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 (56, 57), the evidence prior to our study has been limited.

Our data indicated a central cause of suppression to the gonadotrophic axis, with a decrease in both pituitary LH and gonadal testosterone. Interestingly, we observed a differential impact of the cumulative opioid dose on adrenal and gonadal androgens, respectively, with a significantly delayed recovery of DHEA and DHEAS, but a trend towards faster recovery of gonadal testosterone synthesis in patients with higher cumulative opioid doses. Previous data on opioid effects on adrenal androgen production are very scarce, but our findings with respect to gonadal testosterone biosynthesis contrasted previous studies describing suppressive opioid effects on the HPG axis (41, 42).

Our study revealed a loss of both adrenal and gonadal androgen production in young and middle-aged men after major trauma. This effect was further enhanced by long-lasting suppression of androgen-activating systemic 5 α -reductase activity, as demonstrated by urinary steroid metabolite analysis. Androgens are important in wound healing, erythrocytosis, bone density, and muscle mass (58). 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 injuries (9). A meta-analysis of 15 randomised controlled trials of 806 burn 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 (59). The use of oxandrolone in major trauma was investigated in 2 intensive care studies but no benefit was demonstrated (60, 61).

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 6 months as well as the measurement of circulating (and in a smaller cohort also excreted) steroid hormones by state-of-the-art mass spectrometry assays. Analyzing a young to middle-aged patient cohort has also reduced the confounding effects of age-related comorbidities. Another strength is the unique opportunity our study offered for analysis of the opioid effects on endocrine recovery, facilitated by detailed prospective, longitudinal phenotyping with dedicated software.

Our study was 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 modeled

data. While we measured total cortisol and cortisone by tandem mass spectrometry, we did not measure free cortisol or cortisol-binding globulin. We were only able to measure urinary steroid excretion in a subcohort of patients, as accurate and repeated collection of 24-hour urine proved very challenging under ICU conditions. 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 4 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 6 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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References

1. World Health Organization. *Injuries and violence: the facts 2014*. WHO Library Cataloguing-in-Publication Data; 2014:2.
2. ITF. Road safety annual report 2014. Paris: OECD Publishing; 2014:2. <https://doi.org/10.1787/irtad-2014-en>.
3. National Audit Office. Major trauma care in England: report by the Comptroller and Auditor General. 2010. <http://www.nao.org.uk/wp-content/uploads/2010/02/0910213.pdf>. Accessed January 5, 2016.
4. Moran CG, Lecky F, Bouamra O, et al. Changing the system – major trauma patients and their outcomes in the NHS (England) 2008-17. *Eclinicalmedicine*. 2018;2:13–21.
5. Ministry of Defence. *UK forces: Operations in Afghanistan*. <https://www.gov.uk/guidance/uk-forces-operations-in-afghanistan>.
6. Hodgetts TJ, Mahoney PF. Military pre-hospital care: why is it different? *J R Army Med Corps*. 2009;155(1):4–8.
7. Durham RM, Moran JJ, Mazuski JE, Shapiro MJ, Baue AE, Flint LM. Multiple organ failure in trauma patients. *J Trauma*. 2003;55(4):608–616.
8. Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. *Injury*. 2007;38(12):1336–1345.
9. Wolf SE, Edelman LS, Kemalyan N, et al. Effects of oxandrolone on outcome measures in the severely burned: a multicenter prospective randomized double-blind trial. *J Burn Care Res*. 2006;27(2):131–139; discussion 140.
10. Arbabi S, Ahrns KS, Wahl WL, et al. Beta-blocker use is associated with improved outcomes in adult burn patients. *The J Trauma*. 2004;56(2):265–269.
11. Esteves CL, Verma M, Róg-Zielińska E, et al. Pro-inflammatory cytokine induction of 11 β -hydroxysteroid dehydrogenase type 1 in A549 cells requires phosphorylation of C/EBP β at Thr235. *PLoS One*. 2013;8(9):e75874.
12. Palmer C. Major trauma and the injury severity score – where should we set the bar? *Annu Proc Assoc Adv Automot Med*. 2007;51:13–29.

13. Balogh ZJ, Varga E, Tomka J, Süveges G, Tóth L, Simonka JA. The new injury severity score is a better predictor of extended hospitalization and intensive care unit admission than the injury severity score in patients with multiple orthopaedic injuries. *J Orthop Trauma*. 2003;17(7):508–512.
14. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA*. 1993;270(24):2957–2963.
15. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13(10):818–829.
16. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*. 1992;101(6):1644–1655.
17. *Prescribing Information and Communications System (PICS)*. 2019:1. <https://www.ubh.nhs.uk/birmingham-systems-pics.htm>. Accessed November 14, 2019.
18. Lasheen W, Walsh D, Mahmoud F, et al. The intravenous to oral relative milligram potency ratio of morphine during chronic dosing in cancer pain. *Palliat Med*. 2010;24(1):9–16.
19. Campbell IT, Watt T, Withers D, et al. Muscle thickness, measured with ultrasound, may be an indicator of lean tissue wasting in multiple organ failure in the presence of edema. *Am J Clin Nutr*. 1995;62(3):533–539.
20. Reid CL, Campbell IT, Little RA. Muscle wasting and energy balance in critical illness. *Clin Nutr*. 2004;23(2):273–280.
21. Clark RV, Walker AC, Miller RR, O'Connor-Semmes RL, Ravussin E, Cefalu WT. Creatine (methyl-d3) dilution in urine for estimation of total body skeletal muscle mass: accuracy and variability vs. MRI and DXA. *J Appl Physiol (1985)*. 2018;124(1):1–9.
22. Buckinx F, Landi F, Cesari M, et al. Pitfalls in the measurement of muscle mass: a need for a reference standard. *J Cachexia Sarcopenia Muscle*. 2018;9(2):269–278.
23. Milner EA, Cioffi WG, Mason AD Jr, McManus WF, Pruitt BA Jr. Accuracy of urinary urea nitrogen for predicting total urinary nitrogen in thermally injured patients. *JPEN J Parenter Enteral Nutr*. 1993;17(5):414–416.
24. Ionita IA, Fast DM, Akhlaghi F. Development of a sensitive and selective method for the quantitative analysis of cortisol, cortisone, prednisolone and prednisone in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009;877(8-9):765–772.
25. Kushnir MM, Blamires T, Rockwood AL, et al. Liquid chromatography-tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals. *Clin Chem*. 2010;56(7):1138–1147.
26. O'Reilly MW, Taylor AE, Crabtree NJ, et al. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. *J Clin Endocrinol Metab*. 2014;99(3):1027–1036.
27. Chadwick CA, Owen LJ, Keevil BG. Development of a method for the measurement of dehydroepiandrosterone sulphate by liquid chromatography-tandem mass spectrometry. *Ann Clin Biochem*. 2005;42(Pt 6):468–474.
28. Haring R, Wallaschofski H, Teumer A, et al. A SULT2A1 genetic variant identified by GWAS as associated with low serum DHEAS does not impact on the actual DHEA/DHEAS ratio. *J Mol Endocrinol*. 2013;50(1):73–77.
29. Shackleton CH. Mass spectrometry in the diagnosis of steroid-related disorders and in hypertension research. *J Steroid Biochem Mol Biol*. 1993;45(1-3):127–140.
30. Breslow NE, Clayton DG. Approximate Inference in Generalized Linear Mixed Models. *J Am Stat Assoc*. 2012;88(421):9–25.
31. Meyer MC. Inference using shape-restricted regression splines. *Ann Appl Stat*. 2008;2(3):1013–1033.
32. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*. 1996;15(4):361–387.
33. Foster MA. The endocrine and metabolic response in male survivors of major trauma – supplementary figures. *figshare*. Deposited 27 November 2019:1–3. <https://www.biorxiv.org/content/10.1101/577502v3.full>.
34. Prete A, Yan Q, Al-Tarrah K, et al. The cortisol stress response induced by surgery: a systematic review and meta-analysis. *Clin Endocrinol (Oxf)*. 2018;89(5):554–567.
35. Chapman KE, Coutinho A, Gray M, Gilmour JS, Savill JS, Seckl JR. Local amplification of glucocorticoids by 11beta-hydroxysteroid dehydrogenase type 1 and its role in the inflammatory response. *Ann N Y Acad Sci*. 2006;1088(1):265–273.
36. Ray JA, Meikle AW. Cortisol-cortisone shuttle: a functional indicator of 11beta-HSD activity. In: Esposito A, Bianchi V, eds. *Cortisol Physiology, Regulation and Health Implications*. Nova Science Publishers, Inc; 2012:91–106.
37. Hassan-Smith ZK, Morgan SA, Sherlock M, et al. Gender-specific differences in skeletal muscle 11β-HSD1 expression across healthy aging. *J Clin Endocrinol Metab*. 2015;100(7):2673–2681.
38. Wang P, Ba ZF, Jarrar D, Cioffi WG, Bland KI, Chaudry IH. Mechanism of adrenal insufficiency following trauma and severe hemorrhage: role of hepatic 11beta-hydroxysteroid dehydrogenase. *Arch Surg*. 1999;134(4):394–401.
39. Tiganeşcu A, Tahrani AA, Morgan SA, et al. 11β-Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *J Clin Invest*. 2013;123(7):3051–3060.
40. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med*. 2013;368(16):1477–1488.
41. de Vries F, Bruin M, Lobatto DJ, et al. Opioids and their endocrine effects: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2019. doi: [10.1210/clinem/dgz022](https://doi.org/10.1210/clinem/dgz022).
42. Fountas A, Chai ST, Kourkouti C, Karavitaki N. MECHANISMS OF ENDOCRINOLOGY: endocrinology of opioids. *Eur J Endocrinol*. 2018;179(4):R183–R196.
43. Kim MS, Shigenaga J, Moser A, Grunfeld C, Feingold KR. Suppression of DHEA sulfotransferase (Sult2A1) during the acute-phase response. *Am J Physiol Endocrinol Metab*. 2004;287(4):E731–E738.
44. Foster MA. *SupplTable1_SupplReferences_JCEM_25Nov19*. 2019:1–4. <https://doi.org/10.6084/m9.figshare.8332736.v1>.
45. Arlt W, Hammer F, Sanning P, et al. Dissociation of serum dehydroepiandrosterone and dehydroepiandrosterone sulfate in septic shock. *J Clin Endocrinol Metab*. 2006;91(7):2548–2554.
46. Butcher SK, Killampalli V, Lascelles D, Wang K, Alpar EK, Lord JM. Raised cortisol:DHEAS ratios in the elderly after injury: potential impact upon neutrophil function and immunity. *Aging Cell*. 2005;4(6):319–324.
47. Beishuizen A, Thijs LG, Vermes I. Decreased levels of dehydroepiandrosterone sulphate in severe critical illness: a sign of exhausted adrenal reserve? *Crit Care*. 2002;6(5):434–438.
48. Lindh A, Carlström K, Eklund J, Wilking N. Serum steroids and prolactin during and after major surgical trauma. *Acta Anaesthesiol Scand*. 1992;36(2):119–124.
49. Parker CR Jr, Baxter CR. Divergence in adrenal steroid secretory pattern after thermal injury in adult patients. *J Trauma*. 1985;25(6):508–510.
50. Radford DJ, Wang K, McNelis JC, et al. Dehydroepiandrosterone sulfate directly activates protein kinase C-beta to increase human neutrophil superoxide generation. *Mol Endocrinol*. 2010;24(4):813–821.
51. Folan MM, Stone RA, Pittenger AL, Stoffel JA, Hess MM, Kroboth PD. Dehydroepiandrosterone, dehydroepiandrosterone-sulfate, and cortisol concentrations in intensive care unit patients. *Crit Care Med*. 2001;29(5):965–970.
52. Ilias I, Stamoulis K, Armaganidis A, et al. Contribution of endocrine parameters in predicting outcome of multiple

- trauma patients in an intensive care unit. *Hormones (Athens)*. 2007;6(3):218–226.
53. Brorsson C, Dahlqvist P, Nilsson L, Thunberg J, Sylvan A, Naredi S. Adrenal response after trauma is affected by time after trauma and sedative/analgesic drugs. *Injury*. 2014;45(8):1149–1155.
54. Bergquist M, Huss F, Fredén F, Mass GHC. Altered adrenal and gonadal steroids biosynthesis in patients with burn injury. *Clin Mass Spectrom*. 2016;1:19–26.
55. Berg C, Oeffner A, Schumm-Draeger PM, et al. Prevalence of anterior pituitary dysfunction in patients following traumatic brain injury in a German multi-centre screening program. *Exp Clin Endocrinol Diabetes*. 2010;118(2):139–144.
56. Vogel AV, Peake GT, Rada RT. Pituitary-testicular axis dysfunction in burned men. *J Clin Endocrinol Metab*. 1985;60(4):658–665.
57. Spratt DI, Bigos ST, Beitins I, Cox P, Longcope C, Orav J. Both hyper- and hypogonadotropic hypogonadism occur transiently in acute illness: bio- and immunoactive gonadotropins. *J Clin Endocrinol Metab*. 1992;75(6):1562–1570.
58. Demling RH. Comparison of the anabolic effects and complications of human growth hormone and the testosterone analog, oxandrolone, after severe burn injury. *Burns*. 1999;25(3):215–221.
59. Li H, Guo Y, Yang Z, Roy M, Guo Q. The efficacy and safety of oxandrolone treatment for patients with severe burns: a systematic review and meta-analysis. *Burns*. 2016;42(4):717–727.
60. Bulger EM, Jurkovich GJ, Farver CL, Klotz P, Maier RV. Oxandrolone does not improve outcome of ventilator dependent surgical patients. *Ann Surg*. 2004;240(3):472–478; discussion 478.
61. Gervasio JM, Dickerson RN, Swearingen J, et al. Oxandrolone in trauma patients. *Pharmacotherapy*. 2000;20(11):1328–1334.