

The ultra-acute steroid response to traumatic injury: a cohort study

Conor Bentley,^{1,2,3,†} Jon Hazeldine,^{1,2,†} Laura Bravo,⁴ Angela E. Taylor,⁵ Lorna C. Gilligan,⁵ Fozia Shaheen,⁵ Animesh Acharjee,^{2,4,6} George Gkoutos,^{2,4,6,7} Mark A. Foster,^{1,2,8} Wiebke Arlt,^{5,9,*} and Janet M. Lord^{1,2,9}

¹Institute of Inflammation and Ageing, University of Birmingham, Birmingham B15 2TT, United Kingdom

²National Institute for Health Research Surgical Reconstruction and Microbiology Research Centre, Queen Elizabeth Hospital Birmingham, Birmingham B15 2TT, United Kingdom

³School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

⁴Institute of Cancer and Genomic Sciences, Centre for Computational Biology, University of Birmingham, Birmingham B15 2TT, United Kingdom

⁵Institute of Metabolism and Systems Research, University of Birmingham, Birmingham B15 2TT, United Kingdom

⁶Institute of Translational Medicine, University Hospitals Birmingham NHS Foundation Trust, Birmingham B15 2TT, United Kingdom

⁷Medical Research Council Health Data Research UK (HDR), United Kingdom

⁸Royal Centre for Defence Medicine, Birmingham Research Park, Birmingham B15 2SQ, United Kingdom

⁹National Institute for Health Research Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham B15 2GW, United Kingdom

*Corresponding author: Wiebke Arlt, Medical Research Council London Institute of Medical Sciences, Imperial College Hammersmith Campus, London, W12 0NN, UK. Email: w.ahrt@lms.mrc.ac.uk

Abstract

Objective: Trauma-induced steroid changes have been studied post-hospital admission, resulting in a lack of understanding of the speed and extent of the immediate endocrine response to injury. The Golden Hour study was designed to capture the ultra-acute response to traumatic injury.

Design: We conducted an observational cohort study including adult male trauma patients <60 years, with blood samples drawn ≤1 h of major trauma by pre-hospital emergency responders.

Methods: We recruited 31 adult male trauma patients (mean age 28 [range 19–59] years) with a mean injury severity score (ISS) of 16 (IQR 10–21). The median time to first sample was 35 (range 14–56) min, with follow-up samples collected 4–12 and 48–72 h post-injury. Serum steroids in patients and age- and sex-matched healthy controls (HCs) ($n = 34$) were analysed by tandem mass spectrometry.

Results: Within 1 h of injury, we observed an increase in glucocorticoid and adrenal androgen biosynthesis. Cortisol and 11-hydroxyandrostenedione increased rapidly, whilst cortisone and 11-ketoandrostenedione decreased, reflective of increased cortisol and 11-oxygenated androgen precursor biosynthesis by 11 β -hydroxylase and increased cortisol activation by 11 β -hydroxysteroid dehydrogenase type 1. Active classic gonadal androgens testosterone and 5 α -dihydrotestosterone decreased, whilst the active 11-oxygenated androgen 11-ketotestosterone maintained pre-injury levels.

Conclusions: Changes in steroid biosynthesis and metabolism occur within minutes of traumatic injury. Studies that address whether ultra-early changes in steroid metabolism are associated with patient outcomes are now required.

Keywords: major trauma, steroids, glucocorticoids, 11-oxygenated androgens, mass spectrometry

Significance

To date, trauma-induced steroid changes were only studied in samples drawn post-hospital admission, several hours after injury with increasing likelihood of drug administration impacting on steroids. In this study, blood samples from 31 male survivors of major trauma were drawn by first responders upon arrival at the scene, allowing for analysis of the ultra-acute steroid response to major injury. We carried out multi-steroid profiling by reference standard tandem mass spectrometry, also including the adrenal-derived 11-oxygenated androgens not previously measured in the context of trauma. Results reveal a unique insight into the ultra-early steroid response to trauma, with increases in cortisol generation and activation, and loss of gonadal but not 11-oxygenated androgens. Future research should clarify whether interindividual differences are of prognostic value.

[†] These authors contributed equally to this work.

Introduction

Traumatic injury triggers a systemic stress response, central to which is activation of the hypothalamic-pituitary-adrenal (HPA) axis and the secretion of adrenocorticotrophic hormone (ACTH) by the pituitary which activates the adrenal gland resulting in increased secretion of cortisol. As a mediator of the endocrine and metabolic response to stress, cortisol facilitates host survival by promoting energy mobilisation and cell metabolism but also has potentially detrimental immune suppressive properties.¹ Exaggerated stress responses that result in prolonged hypercortisolaemia drive a state of systemic catabolism that is associated with the loss of lean muscle mass, poor wound healing, and increased susceptibility to infection and multiple organ dysfunction syndrome (MODS). In the setting of traumatic brain injury (TBI),²⁻⁶ blunt/penetrative trauma,^{7,8} thermal injury,⁹⁻¹³ and blast injury,^{8,14,15} prospective observational-based cohort studies have demonstrated marked trauma-induced alterations in adrenal and gonadal steroid synthesis. Serum concentrations of the adrenal-derived androgen precursor dehydroepiandrosterone (DHEA), its sulphate ester DHEAS, and androstenedione are all markedly reduced within hours of trauma, with circulating DHEAS remaining below the reference range of healthy controls (HCs) for up to 6 months post-injury.^{9,10,16-18} Furthermore, a state of temporary hypogonadism has been documented in male trauma patients, with serum testosterone concentrations falling below the range observed in HC for up to 8 weeks post-injury.^{2,3,6,8,9,13-15}

The impact of severe injury on the production of corticosteroids is controversial. Whilst some studies have described post-injury increases in circulating cortisol,^{4,7,10,11,17,18} others have observed no effect^{8,13} or described a state of critical illness-related corticosteroid insufficiency, detecting cortisol concentrations at the lower end of the HC concentration range at the time of hospital admission and in the days following injury.^{5,6,19-21} Mechanistically, studies that have reported critical illness-induced adrenal insufficiency and temporary hypogonadism suggest a number of factors may be involved such as trauma-induced damage to the HPA axis,²²⁻²⁴ acute suppression of luteinising hormone synthesis,⁸ tissue glucocorticoid resistance,²⁵ and inflammation-driven inhibition or activation of steroidogenic enzymes.^{13,24}

To date, all studies that have investigated trauma-induced changes in adrenal and gonadal steroid synthesis have analysed blood samples obtained post-hospital admission. Consequently, significant inter-study variation exists in the time elapsed between injury and sample acquisition. Recently, via the analysis of blood samples obtained in the pre-hospital setting, we have examined the ultra-early (≤ 1 h) immune and inflammatory response to trauma, demonstrating evidence of concomitant immune activation and suppression as well as endothelial dysfunction within minutes of injury.^{7,26} However, except for our previous description of elevated serum cortisol concentrations,⁷ no data currently exist on steroid metabolism in the immediate aftermath of traumatic injury. Moreover, very little information exists on the impact of trauma on the synthesis of other adrenal-derived steroids, particularly the adrenal-derived 11-oxygenated androgens,²⁷ which include 11 β -hydroxyandrostenedione (11OHA4), 11-ketoandrostenedione (11KA4), and 11-ketotestosterone (11KT). The two latter represent androgen receptor agonists, with 11KT binding and activating the androgen receptor with similar potency and efficacy to testosterone.²⁸

Here we undertook a comprehensive examination of steroid biosynthesis and metabolism in the pre-hospital setting in the minutes following injury to provide novel insights into the dynamics of the immediate endocrine stress response to major trauma.

Materials and methods

Study design and setting

Data presented in this manuscript have been derived from samples acquired from patients enrolled into the Brain Biomarkers After Trauma Study (BBATS, also termed the Golden Hour study; REC reference: 13/WA/0399), a prospective longitudinal observational study conducted at a single major trauma centre site, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.

Participants

We included patients with a predicted injury severity score (ISS) ≥ 8 and aged ≥ 18 years and no evidence of TBI. They were enrolled on a 24/7 basis by pre-hospital emergency care teams within 1 h of injury. A screening log of all trauma patients was prospectively maintained. No patients were included that had an ISS < 8 and received pre-hospital blood products or steroids before the collection of the first study sample. For detailed information relating to study inclusion/exclusion criteria, patient consent, and pre-hospital enrolment, see Hazeldine *et al.*⁷

For comparison, we recruited a control cohort comprising 34 adult men who were not taking regular medication, or suffering from any acute, or chronic illness; their blood samples were collected in the non-fasted state between 07:00 and 10:00 h, that is, close to peak time of steroids with diurnal secretion pattern. Written informed consent was obtained from all participants prior to their enrolment into the study, which was approved by the local research ethics committee (West Midlands Research Ethics Committee number 10/H1208/48).

Blood sampling and preparation

All patients underwent venepuncture or placement of a peripherally inserted cannula to obtain blood samples for research purposes using Vacutainers[®] (Becton Dickinson, Oxford, United Kingdom) that contained z-serum clotting activator. Following collection in the pre-hospital setting, blood samples were stored at room temperature in the transporting ambulance or helicopter, before being placed in a study-specific refrigerator located in the accident and emergency department. Laboratory research personnel undertook sample preparation and storage within 1 h of sample deposition. In addition to pre-hospital samples, blood was obtained at two additional post-injury time points, 4-12 and 48-72 h. Blood samples were kept at room temperature for 30 min prior to centrifugation at 1620 $\times g$ for 10 min. Serum was aliquoted and stored at -80°C until analysed.

Clinical data collection

Clinical and demographic data were obtained from electronic medical hospital records. Data regarding mortality, length of intensive care unit (ICU) and hospital stay, ISS, new ISS (NISS), Glasgow Coma Scale (GCS), and abbreviated injury scale (AIS) scores were obtained from the Trauma Audit Research Network (TARN).

Steroid analysis

Serum steroid concentrations were quantified using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) multi-steroid profiling method.^{8,29,30} In brief, analysis was carried out on a Waters Xevo-XS, with Acquity uPLC, using a water/methanol (0.1% formic acid) gradient system and a HSS T3, 1.8 μ m, 1.2 $\times$ 50 mm column.⁸ An internal standard mixture was added to the serum and the proteins crashed with 100 μ L of acetonitrile. Steroids were then extracted via liquid-liquid extraction using 1 mL methyl tert-butyl ether (MTBE). The MTBE layer containing steroids was transferred and evaporated under nitrogen and then reconstituted in 125 μ L of 50/50 methanol/water before analysis. Steroids were quantified against a calibration series ranging from 0.05 to 250 ng/mL.

Serum DHEAS was measured separately as described by Chadwick et al. and Foster et al.^{8,31} Internal standard (DHEAS-d2) was added to 20 μ L of serum, followed by 20 μ L of ZnSO₄ and 100 μ L of acetonitrile. The samples were then centrifuged, and 100 μ L of the solution was transferred to a new vial, dried, and reconstituted in 200 μ L of methanol/water prior to LC-MS/MS analysis.³¹ DHEAS was quantified against a calibration series ranging from 250 to 8000 ng/mL.

Statistical analyses

Comparisons between steroid concentrations in HCs and at the three post-trauma time points were made using two-sample Wilcoxon test for non-normal data with *P*-value adjusted through the Holm-Bonferroni method. Pairwise analysis between time points in trauma patients was also performed through Wilcoxon test. Analysis was carried out using R (v3.5.2) with packages *ggpubr* and *Metrics*. A *P* value < .05 was considered statistically significant. All analysis is accessible in github/InFlamUOB/TraumaSteroids.

Results

Patient enrolment and demographics

Between January 2015 and June 2019, 31 patients with a predicted ISS $\geq$ 8 and aged $\geq$ 18 years were enrolled. The mean age of the 34 HCs (30 years [range 19-59 years]) was similar to that of the trauma patient cohort (Table 1).

Figure 1 depicts patient enrolment into the Golden Hour study. Thirty-one male patients within the extracranially injured cohort (mean age 28 (range 19-59) years) were selected for steroid analysis who all had blood samples acquired $\leq$ 1 h post-injury (mean time to sample 35 min [range 14-56]) (Table 1). Due to various logistical and clinical reasons, not all study participants had post-hospital admission blood samples collected at the 4-12 or 48-72 h post-injury time points (Figure 1). Recruited patients had a median ISS of 16 (IQR 10-21), with 48% of subjects eligible as a result of a road traffic collision. Forty-two percent of pre-hospital samples were collected in the afternoon (12:01 h-18:00 h), with 46% shared equally between the evening (18:01 h-00:00 h) and the night (00:01 h-06:00 h) and only 12% between 06:01 h and 12:00 h. There was no relationship between time of injury and injury severity ($n = 31$, $R = -0.034$, $P = .86$).

Glucocorticoid and mineralocorticoid biosynthesis

Within the first hour after trauma, we observed a significant upregulation of the biosynthesis of precursors of the core

Table 1. Patient demographics.

Demographics		
Healthy controls (<i>n</i>)	34	
Healthy controls-age, years-mean (range)	30.6	(19-59)
Trauma patients (<i>n</i>)	31	
Trauma patient-age, years (mean [range])	28.1	(19-59)
Time to pre-hospital sample, minutes post-injury (mean [range])	35	(14-56)
Injury severity score (ISS) (median [IQR])	16	(10-21)
New ISS (NISS) (median [IQR])	22	(14-29)
Glasgow Coma Scale (GCS) (range)		(3-15)
Surgical intervention at scene, <i>n</i> (%)	1	3
Blood product administered at scene, <i>n</i> (%)	2	6
Sedation at scene, <i>n</i> (%)	6	19
Mechanism of injury		
Fall, <i>n</i> (%)	1	3
Assault, <i>n</i> (%)	1	3
Penetrating, <i>n</i> (%)	8	26
A/P, <i>n</i> (%)	4	13
Road traffic collision, <i>n</i> (%)	15	48
Blunt impact, <i>n</i> (%)	2	6
Alcohol involved, <i>n</i> (%)	5	16
Outcomes		
ICU admission, <i>n</i> (%)	15	48
ICU length of stay (day [range])	4	(1-21)
Organ failure, <i>n</i> (%)	14	45
Hospital length of stay (day [range])	14.1	(1-60)

Abbreviations: ISS, injury severity score; IQR, interquartile range; GCS, Glasgow Coma Scale; ICU, intensive care unit.

steroid precursors progesterone and 17-hydroxyprogesterone as well as of dedicated precursors of mineralocorticoids (11-deoxycorticosterone, corticosterone) and glucocorticoids (11-deoxycortisol) (Figure 2). This was generally maintained at 4-12 h after trauma but attenuated down to concentrations similar to HCs at 48-72 h (Figures 2 and 3).

Compared to HCs, serum concentrations of the active glucocorticoid cortisol increased immediately after trauma and remained increased at 4-12 h, followed by a decrease to levels at or slightly below those in HCs at 48-72 h post-injury (Figure 4). By contrast, circulating cortisone concentrations decreased shortly after trauma, with an even more significant drop observed 48-72 h post-injury, which resulted in a significant increase in the serum cortisol over cortisone ratio (Figure 4).

Classic and 11-oxygenated androgen biosynthesis

Serum concentrations of the adrenal androgen precursors DHEA and androstenedione increased within the first hour after trauma, reflective of immediate hypothalamic-pituitary-adrenal axis activation. However, this was followed by a decrease in serum DHEA and androstenedione to concentrations below those observed in HCs at 48-72 h post-injury (Figures 2 and 5). The sulphate ester of DHEA, DHEAS, also increased shortly post-injury but remained slightly increased, resulting in a significant reduction in the serum DHEA over DHEAS ratio at 48-72 h.

By contrast, circulating concentrations of the active classic male gonadal androgens testosterone (T) and 5 α -dihydrotestosterone (DHT) started to drop immediately after trauma, decreasing to even lower levels by 48-72 h post-injury (Figures 2 and 5).

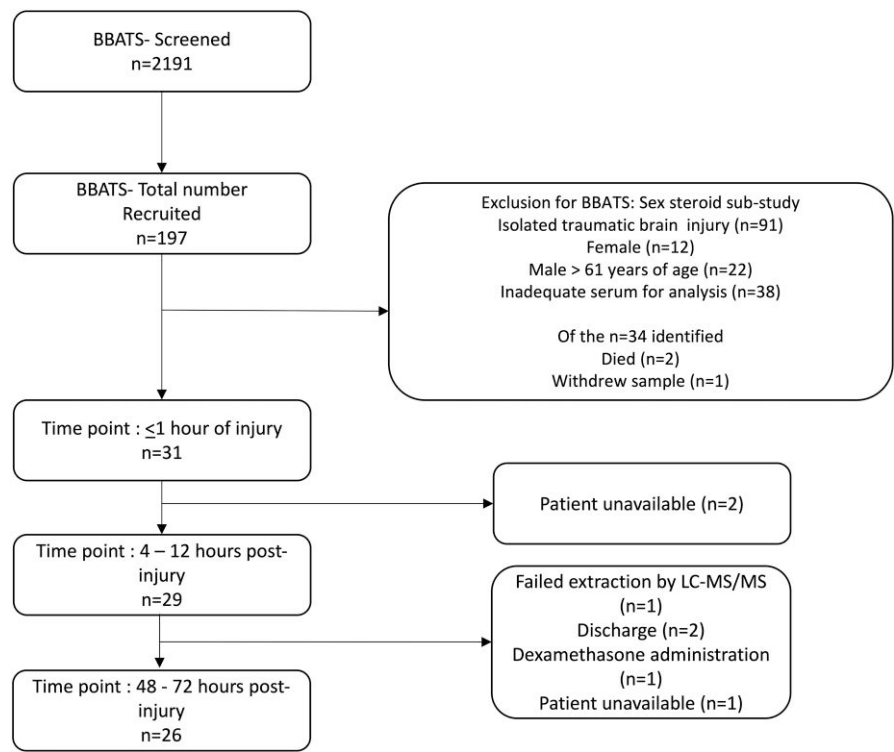


Figure 1. Consort flow diagram showing recruitment and analysis of study subjects into the Golden Hour study (Brain Biomarkers After Trauma Study, BBATS). Flow diagram of patient enrolment and participation at each stage of analysis. The samples available were those who met the initial criteria of non-isolated brain injury patients, male, under 61 years of age, and sufficient serum available for steroid analysis. By liquid chromatography-tandem mass spectroscopy.

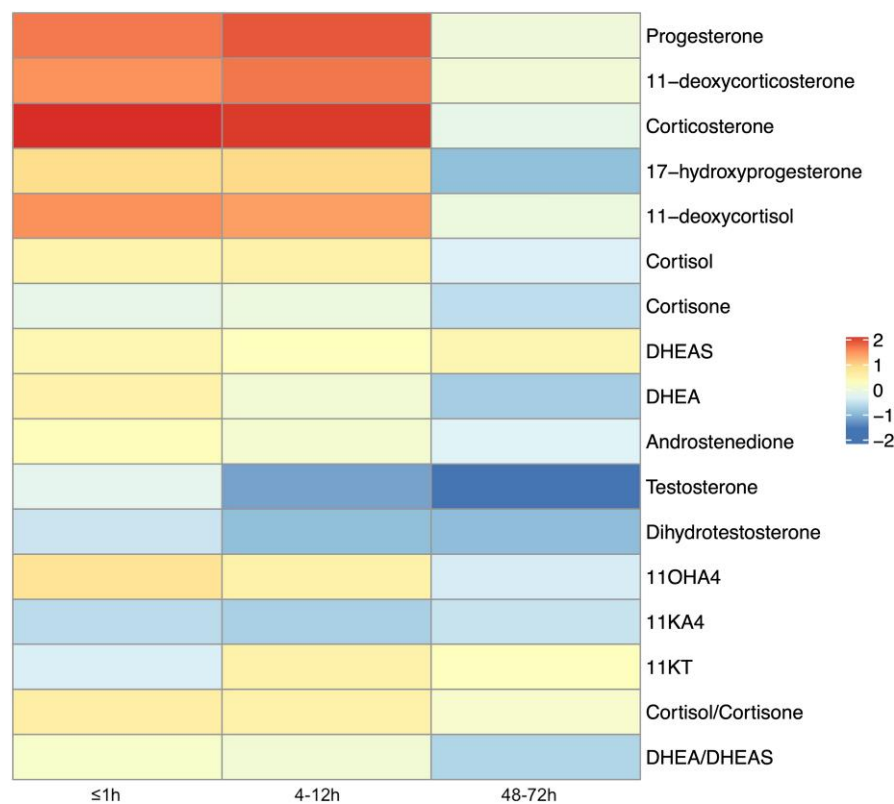


Figure 2. A heat map showing average steroid concentrations in trauma patients ≤1 h ($n = 31$), 4-12 h ($n = 29$), and 48-72 h ($n = 26$) post-injury vs healthy controls ($n = 34$). Colours correspond to the logarithms of the ratio between mean steroid values per time point and the mean healthy controls. A value of 2 will therefore correspond to a 10-fold increase with respect to healthy controls, whereas a value of 0 would correspond to no change at all.

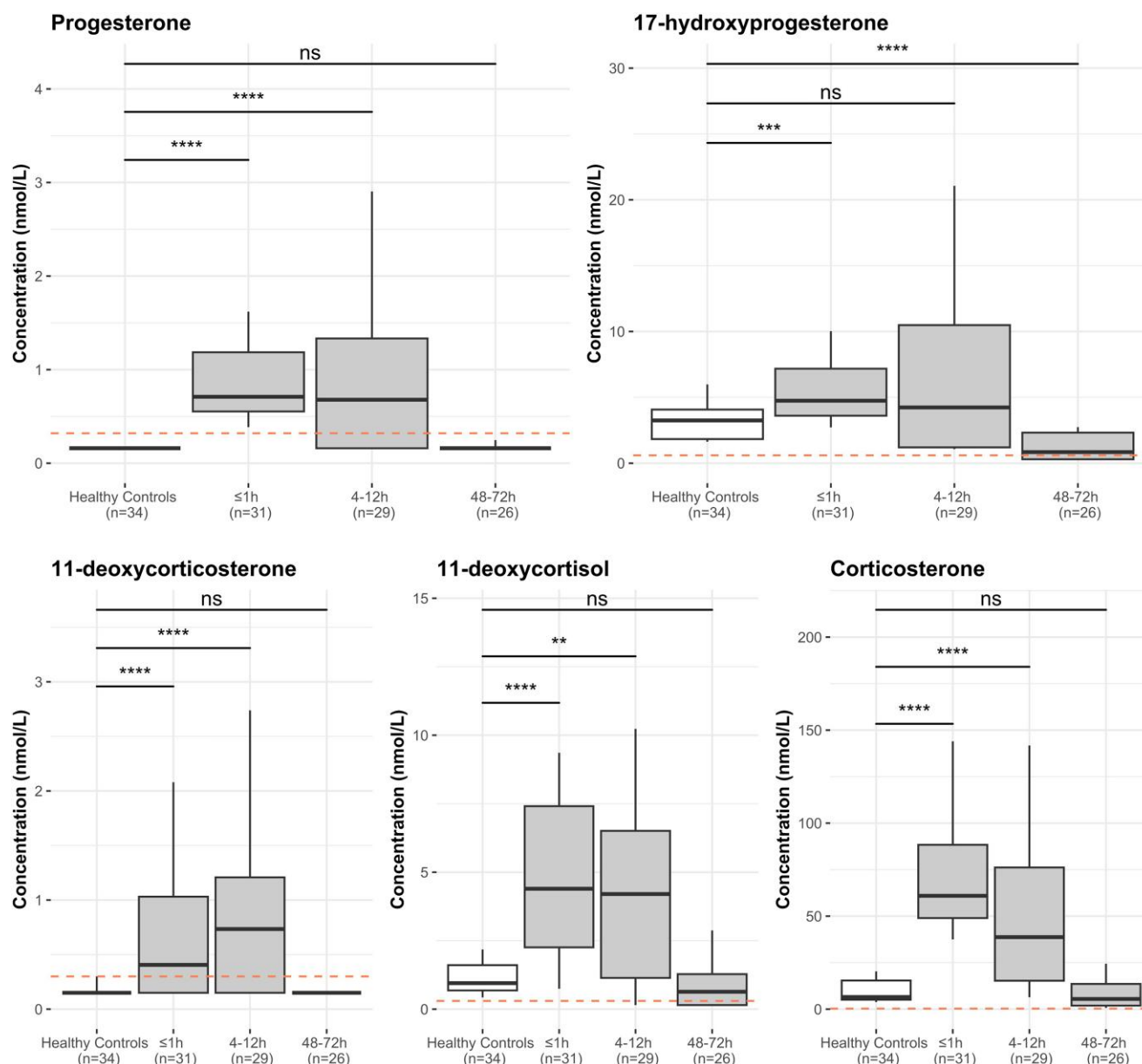


Figure 3. Steroid precursor concentrations in healthy controls ($n = 34$) and in trauma patients ≤ 1 h ($n = 31$), 4-12 h ($n = 29$), and 48-72 h ($n = 26$) post-injury. Serum concentrations of progesterone, 17-hydroxyprogesterone, 11-deoxycorticosterone, corticosterone, and 11-deoxycortisol were quantified by LC-MS/MS; red dotted line represents the analytical lower limit of quantification, progesterone = 0.32, 17OHP = 0.6 nmol/L. With boxplots defined following the quantiles 0.1, 0.25, 0.5, 0.75, and 0.9. * $P < .05$ vs HCs, ** $P < .01$ vs HCs, *** $P < .001$ vs HCs, **** $P < .0001$ vs HCs. HCs, healthy controls; ns, non-significant; LC-MS/MS, liquid chromatography-tandem mass spectroscopy.

The circulating concentrations of the 11-oxygenated androgen precursor 11-hydroxyandrostenedione (11OHA4) increased significantly immediately after trauma but then gradually decreased to concentrations significantly lower than HCs at 48-72 h (Figure 6). Concentrations of 11-ketoandrostenedione (11KA4), the intermediate between 11OHA4 and the active 11-oxygenated androgen 11-ketotestosterone (11KT), decreased immediately after trauma and remained lower than HCs throughout the study period (Figure 6). Interestingly, despite the drop in the two precursors, 11KT stayed unchanged within the first hour after trauma and then increased at 4-12 h and 48-72 h, although this did not reach significance (Figures 2 and 6). 11-Hydroxytestosterone concentrations were very close to the limit of quantification within the first hour post-injury and undetectable thereafter (data not shown).

Discussion

Here, via the analysis of pre-hospital blood samples acquired from 31 male trauma patients within 1 h of injury, we have performed for the first time a detailed analysis of steroid metabolism within min of major injury. Our data demonstrate immediate and significant increases in the circulating concentrations of steroid precursors as well as gluco- and mineralocorticoids. Conversely, gonadal-derived classic androgen biosynthesis was disrupted, whereas the adrenal-derived active 11-oxygenated androgen pool was largely maintained. These findings add for the first time steroid data to the growing body of literature derived from the field of pre-hospital research, where we and others have reported changes in immune function,^{32,33} inflammatory profiles,^{7,34} and coagulation^{26,35,36} in the immediate aftermath of injury.

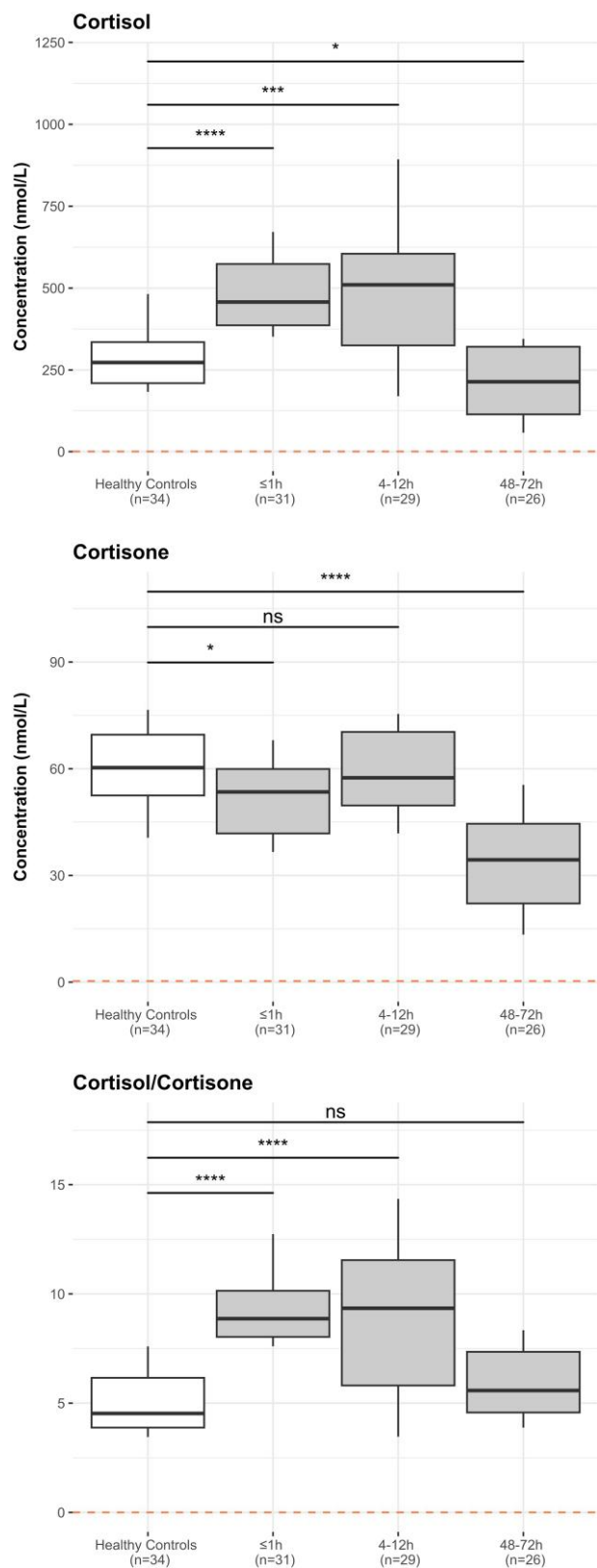


Figure 4. Glucocorticoid concentrations in healthy controls ($n=34$) and in trauma patients ≤ 1 h ($n=31$), 4–12 h ($n=29$), and 48–72 h ($n=26$) post-injury. Serum cortisol, cortisone, and their ratio quantified by LC-MS/MS, where applicable; dotted line is the analytical lower limit of quantification, cortisol and cortisone = 0.3 nmol/L. * $P < .05$ vs HCs, ** $P < .01$ vs HCs, *** $P < .001$ vs HCs, **** $P < .0001$ vs HCs. HCs, healthy controls; ns, non-significant; LC-MS/MS, liquid chromatography-tandem mass spectroscopy.

Within 60 min of injury, serum concentrations of the central steroid precursors progesterone and 17OHP increased to levels significantly higher than those in HCs. Elevated concentrations of pregnenolone, 17-hydroxypregnenolone, and progesterone have previously been reported in hospital admission blood samples acquired from thermally injured males, with concentrations correlating positively with burn size.¹³ 17OHP concentrations were significantly lower in patients by 48 h post-injury, whereas the elevated concentrations of progesterone persisted until 12 h post-injury. Previously, progesterone replenishment has been explored in TBI due to its ability to reduce the permeability of blood-brain barrier, aquaporin-4 expression, and intracranial pressure, whilst increasing cerebral perfusion pressure and reducing inflammation by decreasing serum intercellular, molecule-1 adhesion.^{37,38} However, such interventions have been unsuccessful in improving recovery outcomes in terms of disability and handicap.³⁹

We observed ultra-rapid increase in the glucocorticoid precursor 11-deoxycortisol and the mineralocorticoid precursor 11-deoxycorticosterone. Corticosterone and 11-deoxycortisol have previously been reported as elevated in hospital admission blood samples obtained from thermally injured subjects, with concentrations positively correlating with area of burn injury.¹³ Furthermore, although not recorded in the current study, yet present in burn injury⁴⁰ and TBI,⁴¹ ACTH concentrations were likely to have been raised in this cohort and underlie the rapid changes in a range of steroid precursors. Thus, inducing steroidogenic enzyme activity and subsequent conversion to mineral and glucocorticoid precursors⁹ is a rapid and prioritised response after critical injury.

Post-admission blood sampling in burns patients has previously shown concentrations of cortisol, testosterone, androstenedione, and DHEA to be highest upon arrival to the emergency department.¹³ Of these, cortisol appears to be central in predicting patient outcomes, particularly for the development of MODS.^{42–45} In line with the current literature,^{7,13} we found cortisol concentrations rapidly increased post-injury, reflective of enhanced cortisol biosynthesis via the adrenal enzyme 11 β -hydroxylase, whilst cortisone decreased. This resulted in an increased cortisol over cortisone ratio, indicative of increased systemic glucocorticoid activation by the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1); its expression is modulated in part by pro-inflammatory cytokines.⁴⁶ The previously described rapid post-injury release of the pro-inflammatory cytokine tumour necrosis factor- α (TNF- α)⁴⁷ may explain the rapid increase in cortisol observed after injury via the induction of 11 β -HSD1,^{8,46} together with an increase in cortisol biosynthesis. Increased glucocorticoid activation after trauma is likely to be a homeostatic mechanism to dampen the inflammatory response but could increase risk of infection if excessive.^{48–50} In the first longitudinal study of steroids in traumatic injury and recovery,⁸ the cortisol-to-cortisone ratio peaked 2 weeks post-injury and returned to normal at around 8 weeks. In this study, we observed a cortisol-to-cortisone ratio comparable to HCs by 48–72 h. Differences in the patient cohorts could explain these discordant observations, with those enrolled in the previous study more severely injured and critically unwell, as demonstrated by a higher ISS (25 vs 16) and day 1 SOFA score of 9.⁸

One of the novel and unexpected findings of our study is that within 60 min of traumatic injury, circulating

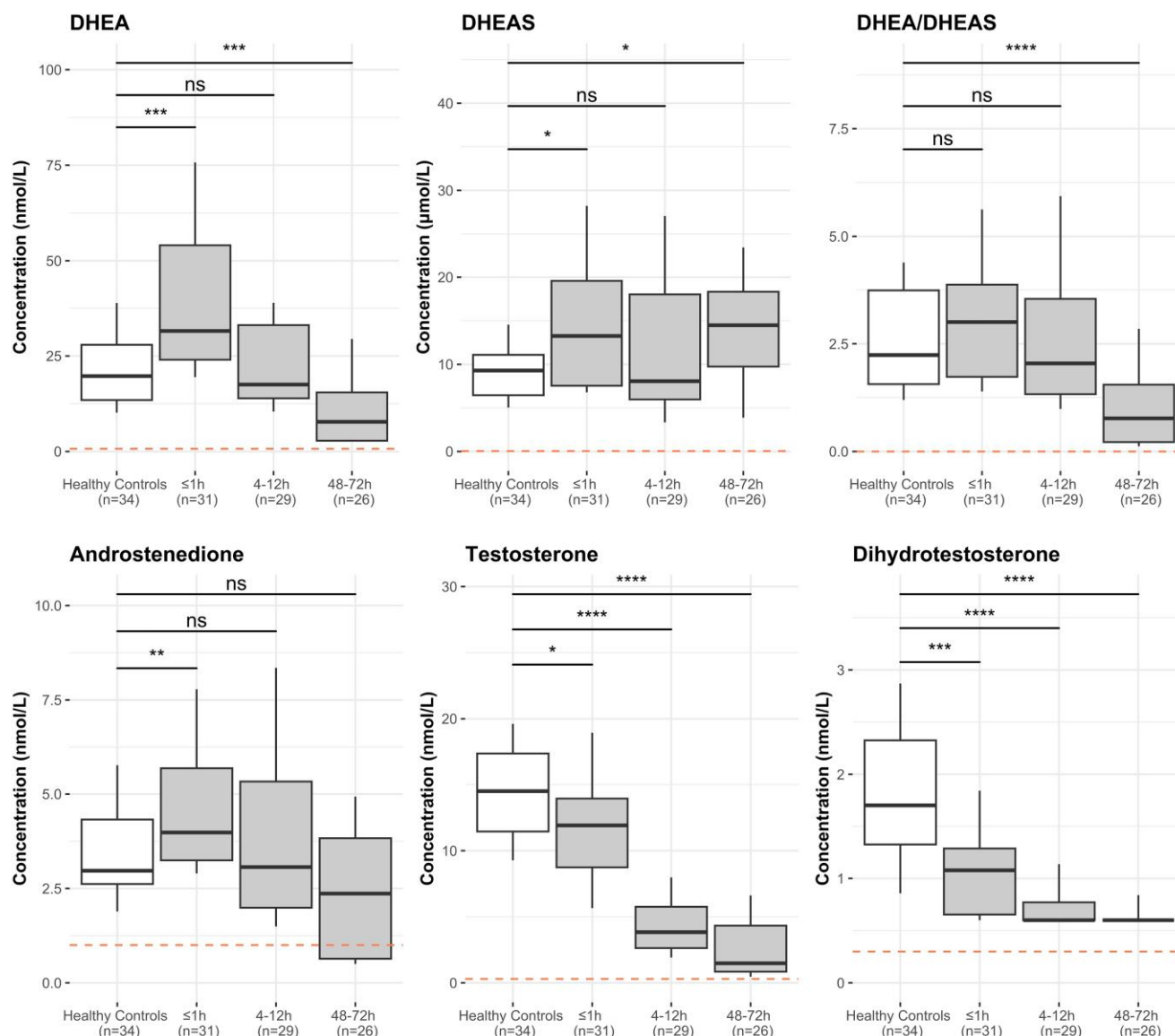


Figure 5. Classic androgen pathway steroid concentrations in healthy controls ($n = 34$) and in trauma patients ≤ 1 h ($n = 31$), 4–12 h ($n = 29$), and 48–72 h ($n = 26$) post-injury. Steroids were quantified by LC-MS/MS; dotted line is the analytical lower limit of quantification, DHEA = 0.7, androstenedione = 1, testosterone and DHT = 0.3 nmol/L, and DHEAS = 0.05 μ mol/L. * $P < .05$ vs HCs, ** $P < .01$ vs HCs, *** $P < .001$ vs HCs, **** $P < .0001$ vs HCs. HCs, healthy controls; ns, non-significant; LC-MS/MS, liquid chromatography-tandem mass spectroscopy.

concentrations of the androgen precursor DHEA significantly increased. An increase in DHEA, alongside androstenedione, has previously been reported in burns patients at the time of hospital admission, with higher concentrations of both recorded in non-survivors.¹³ Conversely, studies in major trauma have reported lower circulating concentrations of DHEA,⁸ which may reflect the acquisition of samples at later post-injury time points. Elevated concentrations of both DHEA and cortisol post-injury suggest rapid activation of the HPA axis post-injury. We also observed an increase in DHEAS, which suggests that the previously reported decrease in circulating DHEAS, linked to downregulation of DHEA sulfotransferase (SULT2A1) activity during the acute response to injury,⁵¹ is likely a later event.

In the current study, we observed a rapid onset of major disruption of gonadal classic androgen synthesis, with testosterone and DHT decreasing within 60 min after trauma, which has been previously shown to persist for several

months⁸ and is likely due to the downregulation of hypothalamic-pituitary-gonadal axis activity and potentially also the suppressive effect of pro-inflammatory cytokines on testicular Leydig cells.⁵² This reduction in testosterone may be a beneficial response. Zolin et al. observed that rising testosterone concentrations after major trauma, in a mixed gender cohort, accompanied by an exaggerated inflammatory response, were associated with a 5-fold increase in MODS and a 2-fold increase in nosocomial infection.⁵³ Other more extensive trauma registry studies have indicated the protective effect that the female gender may play post-injury.^{54,55} However, in men, serum testosterone concentrations have also been shown to correlate inversely with ventilator days, ICU length of stay,⁵⁶ and SOFA score.⁸ In animal burn models, oestradiol administration had a protective effect by attenuating the hypermetabolic state,⁵⁷ with castration shown to protect against heat-induced MODS and lethality.⁵⁸ Furthermore, immunologic impairment, as a direct effect of

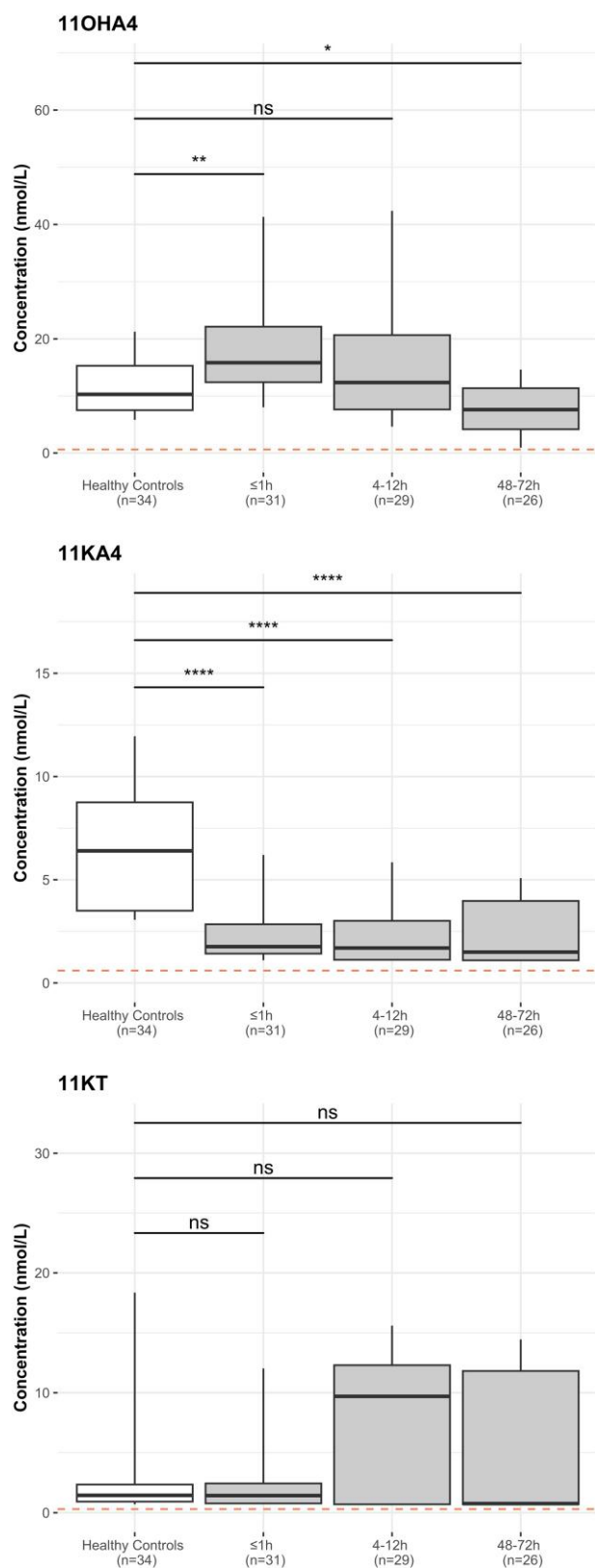


Figure 6. 11-Oxygenated androgen concentrations in healthy controls ($n = 34$) and in trauma patients ≤ 1 h ($n = 31$), 4–12 h ($n = 29$), and 48–72 h ($n = 26$) post-injury. Steroids were quantified by LC-MS/MS; dotted line represents the analytical lower limit of quantification, 11OHA4 = 0.3, 11KA4 and 11KT = 0.6 nmol/L. * $P < .05$ vs HCs, ** $P < .01$ vs HCs, *** $P < .001$ vs HCs, **** $P < .0001$ vs HCs. HCs, healthy controls; ns, non-significant; LC-MS/MS, liquid chromatography-tandem mass spectroscopy.

elevated testosterone concentrations, may alter cell-mediated immunity and cytokine expression⁵⁹ increasing susceptibility to infection.

Whilst it has been known for decades that the adrenal can convert androstenedione to 11-hydroxyandrostenedione, catalysed by the cortisol biosynthesis enzyme, 11 β -hydroxylase, the latter steroid was previously thought to be an irrelevant, terminal metabolite. However, in recent years, the significance of this steroid as the first step into the 11-oxygenated androgen pathway has been revealed,^{60,61} including the demonstration that the 11-oxygenated androgen 11KT has similar androgenic potency to testosterone.⁶² We observed a decrease in 11KA4 and an increase in 11OHA4 during the first hour post-injury, which likely reflects the increased activity of 11 β -hydroxylase as well as of 11 β -HSD1, which not only activates cortisone to cortisol but also inactivates 11KA4 to 11OHA4.⁶⁰ Curiously, we found that serum 11KT remained unchanged or even appeared to slightly increase; the clinical relevance of this remains to be determined, and further studies should investigate the longitudinal course of circulating 11-oxygenated androgen concentrations beyond 3 days post-injury.

The strengths of our study include its novelty as the first study to look at the ultra-acute steroid response within 1 h of injury, its prospective nature, and steroid analysis using reference standard LC-MS/MS. Analysing a young to middle-aged male patient cohort has also reduced the confounding effects of age-related comorbidities, age-related hormonal decline, and gender-related differences in outcomes post-injury.⁵³ However, the study is not without its limitations. Pre-hospital crews must prioritise dealing with any acute life-threatening injuries before blood sample retrieval and provision of fluids for resuscitation. As a result, only those patients who had viable peripheral vein access after immediate stabilisation of injuries were included in the study. Those who required intraosseous administration of fluids or had catastrophic injuries in the first hour were excluded, resulting in a potential bias against those patients with higher ISS. The patients included in this study had no evidence of TBI on trauma CT to avoid confounding issues of primary damage to the hypothalamus or pituitary. Repeating such work in those with a TBI would be warranted, due to higher post-injury incidence of MODS in this group.⁷ The complete avoidance of pain relief medication at both 4–12 and 48–72 h post-injury is both unavoidable and unethical to establish a true sex steroid response profile. Another limitation of the study is the relatively small sample size. As a result, the data should be interpreted with caution, with inflection point studies based on low patient numbers with wide confidence intervals in some steroids highlighting this uncertainty. Furthermore, future work should aim to include pre- and post-menopausal females, mild TBI, adults aged over 60, and patients with higher injury severity, which due to their lower prevalence, will require multi-centre studies. With bigger sample sizes and patient diversity, we would be able to examine whether ultra-early changes in steroid biosynthesis and metabolism are associated with poor patient outcome.

In summary, studying the ultra-early phase post-injury provides a unique snapshot of the physiological response to trauma. We have shown here that changes in circulating steroid concentrations occur within minutes, with stimulation of

adrenal steroid biosynthesis, upregulation of 11 β -hydroxylase and 11 β -HSD1 activity, and disruption of gonadal androgen biosynthesis.

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