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ORIGINAL ARTICLE

Adrenal

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Plasma steroid concentrations reflect acute disease severity and normalise during recovery in people hospitalised with COVID-19

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Abstract

Objective: Endocrine systems are disrupted in acute illness, and symptoms reported following coronavirus disease 2019 (COVID-19) are similar to those found with clinical hormone deficiencies. We hypothesised that people with severe acute COVID-19 and with post-COVID symptoms have glucocorticoid and sex hormone deficiencies.

Design/Patients: Samples were obtained for analysis from two UK multicentre cohorts during hospitalisation with COVID-19 (*International Severe Acute Respiratory Infection Consortium/World Health Organisation [WHO] Clinical Characterization Protocol for Severe Emerging Infections in the UK study*), and at follow-up 5 months after hospitalisation (*Post-hospitalisation COVID-19 study*).

Measurements: Plasma steroids were quantified by liquid chromatography–mass spectrometry. Steroid concentrations were compared against disease severity (WHO ordinal scale) and validated symptom scores. Data are presented as geometric mean (SD).

Results: In the acute cohort ($n = 239$, 66.5% male), plasma cortisol concentration increased with disease severity (cortisol 753.3 [1.6] vs. 429.2 [1.7] nmol/L in fatal vs. least severe, $p < .001$). In males, testosterone concentrations decreased with severity (testosterone 1.2 [2.2] vs. 6.9 [1.9] nmol/L in fatal vs. least severe, $p < .001$). In the follow-up cohort ($n = 198$, 62.1% male, 68.9% ongoing symptoms, 165 [121–192] days postdischarge), plasma cortisol concentrations (275.6 [1.5] nmol/L) did not differ with in-hospital severity, perception of recovery, or patient-reported symptoms. Male testosterone concentrations (12.6 [1.5] nmol/L) were not related to in-hospital severity, perception of recovery or symptom scores.

Conclusions: Circulating glucocorticoids in patients hospitalised with COVID-19 reflect acute illness, with a marked rise in cortisol and fall in male testosterone. These findings are not observed 5 months from discharge. The lack of association between hormone concentrations and common post-COVID symptoms suggests steroid insufficiency does not play a causal role in this condition.

KEYWORDS
adrenal, cortisol, COVID 19, long COVID, testosterone

1 | INTRODUCTION

Secretion and metabolism of steroid hormones is altered during acute illness. Glucocorticoids rise through activation of the hypothalamic–pituitary–adrenal (HPA) axis, however up to 20% of critically unwell patients may develop 'critical illness-related corticosteroid insufficiency' (CIRCI).[1] The potent glucocorticoid, dexamethasone, is itself an effective treatment for severe coronavirus disease 2019 (COVID-19).[2] In males, downregulation of the hypothalamic–pituitary–gonadal (HPG) axis results in low circulating testosterone.[3]

Previous studies reporting glucocorticoid responses to acute COVID-19 in-hospital have conflicting results, reporting both cortisol elevation[4] and insufficiency.[5–7] However, severe disease was underrepresented, particularly in those studies reporting hypocortisolism.[5,6]

Studies following hospitalised patients report that most individuals do not feel fully recovered following COVID-19.[8] The 'post-COVID-19 condition' (or 'long-COVID') has been defined as symptoms including fatigue, breathlessness, and cognitive dysfunction persisting 3 months after infection.[9] It is plausible that endocrine abnormalities persist after acute infection, given similar symptoms occur in endocrinopathies like adrenal insufficiency. There is some overlap between these persistent symptoms and those described by patients with chronic fatigue syndrome, in which hypocortisolism occurs.[10] Whilst clinically overt adrenal failure was

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excluded in a cohort of patients recovering from COVID-19,[11] more subtle abnormalities in the HPA axis have not been explored.

We hypothesised that patients hospitalised with severe COVID-19 and those with ongoing fatigue and neurocognitive symptoms 5 months since hospitalisation have lower circulating glucocorticoids than those who had less severe disease, and those who felt recovered, respectively. Secondary hypotheses were that severe COVID-19, and ongoing symptoms post-COVID, are associated with male androgen deficiency. We quantified the plasma profile of circulating glucocorticoid and sex steroid hormones, precursors and metabolites in two study cohorts of adults admitted to hospitals in the United Kingdom with COVID-19—one representing acute illness and the other following patients after hospital discharge.

2 | METHODS

2.1 | Study design

2.1.1 | International Severe Acute Respiratory Infection Consortium (ISARIC)/World Health Organisation (WHO) Clinical Characterization Protocol for Severe Emerging Infections in the UK (CCP-UK)

Plasma samples during acute illness were obtained from patients recruited between 12 March and 17 June 2020 to the ISARIC/WHO CCP-UK study. Patients were recruited before dexamethasone became the standard of care for treatment of severe disease.[2] This prospective cohort study recruited patients from 306 UK hospitals and was delivered by the ISARIC Coronavirus Clinical Characterisation Consortium (ISARIC4C) investigators. The protocol, revision history, case report form, patient information leaflets, consent forms and details of the Independent Data and Material Access Committee are available at isaric4c.net. The study was registered at https://www.isrctn.com/ISRCTN66726260 and designated an Urgent Public Health Research Study by the National Institute for Health Research UK. Ethical approval was given by the South Central—Oxford C Research Ethics Committee in England (Ref 13/SC/0149), Scotland A Research Ethics Committee (Ref 20/SS/0028), and WHO Ethics Review Committee (RPC571 and RPC572, 25 April 2013). The requirement for consent for data collection was waived in view of the public health emergency.

2.1.2 | Posthospitalisation COVID-19 study (PHOSP-COVID)

Plasma samples taken at the first scheduled follow-up visit after hospitalisation with COVID-19 were obtained from patients recruited to the PHOSP-COVID study (discharged between 17 March 2020 and 18 January 2021). This prospective, longitudinal cohort study follows adults previously hospitalised with COVID-19, recruiting from 53 UK hospitals. The PHOSP-COVID study was registered at https://www.isrctn.com/ISRCTN10980107 and approved by the Leeds West Research Ethics Committee (20/YH/0225). All patients gave written informed consent.

2.2 | Participants, data collection, and samples

2.2.1 | ISARIC/WHO CCP-UK

A prespecified case report form was used to collect demographic and clinical data. Comorbidities were defined using a modified Charlson comorbidity index and obesity was clinician-defined.

A random selection of plasma samples obtained on the day of recruitment was analysed. Samples which could not be matched to patient data, those from pregnant women, patients aged <16 years or with unknown drug history were excluded from subsequent data analyses (Supporting Information S1: Figure 1).

2.2.2 | PHOSP-COVID

Patients were recruited at discharge and invited to attend a research visit 4–7 months later (the '5-month visit'), when blood samples were obtained and participants invited to complete validated questionnaires to document post-COVID health status.[8] In this analysis we included scores for fatigue (Functional Assessment of Chronic Illness Therapy [FACIT]), breathlessness (Dyspnoea-12), depression (Patient Health Questionnaire-9 [PHQ-9]), anxiety (Generalised Anxiety Disorder 7-item scale [GAD-7]) and posttraumatic stress (Post Traumatic Stress Disorder Checklist [PCL-5]). Patients were asked 'Do you feel fully recovered?'. Data relating to demographics and hospital admission was extracted from hospital notes by site investigators. Body mass index (BMI) was calculated and obesity defined as BMI ≥ 30 kg/m^2.

We excluded participants prescribed medications likely to affect circulating glucocorticoid concentrations (oral, inhaled, or topical glucocorticoids, azole antifungals, metyrapone, mitotane), or sex steroids (hormonal contraceptives, oestrogen, or testosterone replacement therapy and gonadotropin-releasing hormone agonists). We also excluded patients with active malignancy, cirrhosis and end-stage renal disease (Supporting Information S1: Figure 1).

2.3 | Laboratory analyses

Steroid hormones, precursors and metabolites were quantified simultaneously by liquid chromatography tandem mass spectrometry (LC–MS/MS see Supporting Information methods). These included plasma glucocorticoids (cortisol and corticosterone), glucocorticoid metabolites (cortisone, 20β-dihydrocortisol, 11-dehydrocorticosterone) and precursors (11-deoxycortisol, 21-deoxycortisol, 17-hydroxyprogesterone), androgens (androstenedione, testosterone, 5α-dihydrotestosterone) oestrogens (oestradiol, oestriol and oestrone), and 11-oxyandrogens (11-ketotestosterone, 11β-hydroxyandrostenedione, 11-ketoandrostenedione).

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2.4 | Statistical analysis

2.4.1 | Laboratory data handling

Where a steroid could not be measured, or was measured below the lower limit of quantitation (LLOQ), a value equal to 1/3 of the LLOQ was substituted. The full range of oestrogen concentrations in the male or postmenopausal female range were not spanned with this method, so these results are not included. Statistical analysis was not undertaken for compounds where ≥20% of samples were below the LLOQ. In the ISARIC4C cohort this included 11-deoxycortisol and (in females) 5α-dihydrotestosterone. In the PHOSP cohort this included 21-deoxycortisol and (in females), 5α-dihydrotestosterone and androstenedione. The ratio of cortisol:cortisone was used to indicate relative activities of the 11β-hydroxysteroid isoenzymes.

Continuous variables with a right skew were $\log_2$ transformed and presented as geometric mean (GM) and geometric standard deviation (GSD). Other continuous variables are shown as mean (standard deviation) or median (interquartile range), and categorical variables as number (percentage). Patients were stratified into five groups based on their peak illness severity at any point during their illness according to the WHO COVID-19 ordinal scale for clinical improvement.[12] For univariate comparisons, we used Student's *t*-test or analysis of variance for normally distributed and transformed continuous variables, Kruskal–Wallis test for nonnormally distributed continuous variables and χ^2 tests for categorical variables. Correlation tests were performed using Spearman's method.

As samples were obtained from the ISARIC4C cohort before exclusions for medications being applied, patients from this cohort prescribed medications likely to affect circulating concentrations of glucocorticoids or sex steroids were excluded from the analysis of those steroids (Supporting Information Figure S1). Two patients were excluded from the analysis of glucocorticoids in the PHOSP cohort due to suspicion of steroid treatment based on LC–MS/MS identification (Supporting Information Figure S1).

Participants were stratified by sex for sex steroid analysis. In males, testosterone concentrations were compared with haematocrit and prevalence of erectile dysfunction.

Multivariable logistic regression was undertaken with in-hospital mortality as the dependent variable, using cortisol or testosterone concentration as independent variables together with predictors from the ISARIC4C mortality score.[13] These included age, number of comorbidities, respiratory rate, urea, C-reactive protein (CRP), oxygen saturation and illness duration. For cortisol we also included sex, and for testosterone, obesity.

A sensitivity analysis was undertaken on patient samples in the PHOSP cohort collected fasted and before 10 AM to assess the impact of timing of blood sampling on cortisol and testosterone concentrations in this group.

Healthy population ranges derived from LC–MS/MS and published by the Laboratory Corporation of America are used for reference.[14]

We used R (R Core Team version 4.0.3) for data analysis. *p*-Values were adjusted for multiple comparisons using the Benjamini–Hochberg method, and a value <.05 considered statistically significant.

3 | RESULTS

3.1 | Demographics

The characteristics of the 239 hospitalised patients with acute COVID-19 (median [interquartile range] 11 [6–16.5] days from symptom onset), and 198 patients at the 5-month follow-up visit discharge (165 [121–192] days) are presented in Table 1. Eight patients were enrolled in both cohorts.

3.2 | Clinical outcomes

The in-hospital mortality rate for this ISARIC4C cohort was 19.7% and was higher in males than females (23.9% vs. 11.2%, $p = .020$). Those who died were older (mean age: 69.7 [12.4] years) and those who received invasive mechanical ventilation were younger (52.9 [13.8] years) than all other groups (64.8 [20.3] years, 66.1 [15.6] years and 61.9 [13.4] years in order of severity, $p < .001$). Patients with more severe disease had higher CRP and neutrophil counts and lower lymphocyte counts (Supporting Information S1: Table 2).

In this PHOSP subset of hospital survivors, 20.2% ($n = 40/198$) had received invasive mechanical ventilation. At the 5-month follow-up visit 31.1% ($n = 50/161$) of patients stated that they felt fully recovered from acute COVID-19. There was no association between patient-reported recovery and age ($p = .639$), sex ($p = .103$), number of comorbidities ($p = .503$) or inpatient treatment with glucocorticoids ($p = .977$) in this subset. The association with inpatient severity ($p = .031$) was not significant when adjusted for multiple testing ($p = .155$).

4 | GLUCOCORTICOIDS

4.1 | Acute COVID-19

The GM cortisol concentration during hospital admission was 580.2 (1.7) nmol/L ($n = 189$). For reference, 62% ($n = 114$) of patients would fall above the Labcorp healthy population morning LC–MS/MS reference range (221–524 nmol/L). The maximal measured concentration was 3109 nmol/L. Concentrations rose incrementally across the severity groups and were 1.8× higher in those with fatal disease ($p < .001$), and 1.4× higher in those requiring supplemental oxygen when compared to patients breathing room air ($p = .040$) (Figure 1). Thirteen (7%) patients had a cortisol concentration <276 nmol/L of which six were in the

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TABLE 1 Demographic and clinical features of two study cohorts of patients during and after acute hospitalisation with COVID-19.

	n	Acute cohort	*n*	Follow-up cohort
Age in years at admission, median (IQR)	237	65.0 (54.0–75.0)	198	58.0 (49·0–65·0)
Male sex, *N* (%)	239	159 (66.5)	198	123 (62.1)
Ethnicity, *N* (%)	228		193	
White	–	185 (81.1)	–	145 (75.1)
Black	–	14 (6.1)	–	10 (5.2)
South Asian	–	7 (3.1)	–	30 (15.5)
East Asian	–	–	–	–
Other ethnic minority	–	21 (9.2)	–	8 (4.1)
No. of days between symptom onset and sample, median (IQR)	231	10.0 (6.0-16.6)	–	–
No. of days between discharge and sample, median (IQR)	N/A	N/A	182	165 (121–192)
Comorbidities, *N* (%)				
Diabetes mellitus	239	70 (29.3)	198	27 (13.6)
Chronic cardiac disease	239	58 (24.3)	196	74 (37.8)
Obesity	239	39 (16.3)	166	90 (54.2)
Asthma	234	39 (16.7)	198	34 (17.2)
Chronic lung disease, not asthma	239	40 (16.7)	197	27 (13.7)
Chronic kidney disease	239	25 (10.5)	198	0 (0.0)
Neoplasia	232	13 (5.6)	198	0 (0.0)
Chronic haematological disease	232	7 (3.0)	196	–
Dementia	239	7 (2.9)	197	0 (0.0)
Moderate or severe liver disease	239	–	198	0 (0.0)
Chronic rheumatological disease	239	21 (8.8)	198	25 (12.6)
Laboratory parameters at admission to hospital, median (IQR)				
CRP (mg/L)	214	105.0 (59.3–210.0)	184	89.0 (45.5–153)
Neutrophils (10^9/L)	228	5.5 (3.5–7.9)	190	5.3 (3.7–7.0)
Lymphocytes (10^9/L)	226	0.9 (0.6–1.2)	191	1.0 (0.7–1.4)
WHO ordinal severity scale	239		198	
No supplemental oxygen	–	38 (15.9)	–	42 (21.2)
Supplemental oxygen	–	63 (26.4)	–	85 (42.9)
NIV/HFNO	–	61 (25.5)	–	31 (15.7)
IMV	–	30 (12.6)	–	40 (20.2)
Fatal	–	47 (19.7)	–	–

Note: 'Other ethnic minority' group includes: Arab, Latin American, Aboriginal/First Nations, West Asian, Mixed ethnicity and other.

Obesity was 'clinician defined' in the acute cohort and measured in the follow-up cohort. $N = 8$ patients were enrolled in both cohorts. Results not reported where *n* is between 0 and 5.

Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-reactive protein; HFNO, high flow nasal oxygen; IMV, invasive mechanical ventilation; IQR, interquartile range; NIV, noninvasive ventilation; WHO, World Health Organization.

lowest severity group and there was no correlation between plasma cortisol and duration of illness at time of sample ($p = .090$)

Supporting Information S1: Table 3 details the plasma concentrations of glucocorticoids, metabolites and precursors according to maximal disease severity. The cortisol precursor 21-deoxycortisol, and metabolite 20β-dihydrocortisol increased with disease severity, as did the cortisol:cortisone ratio, as shown in Figure 1B ($p < .001$).

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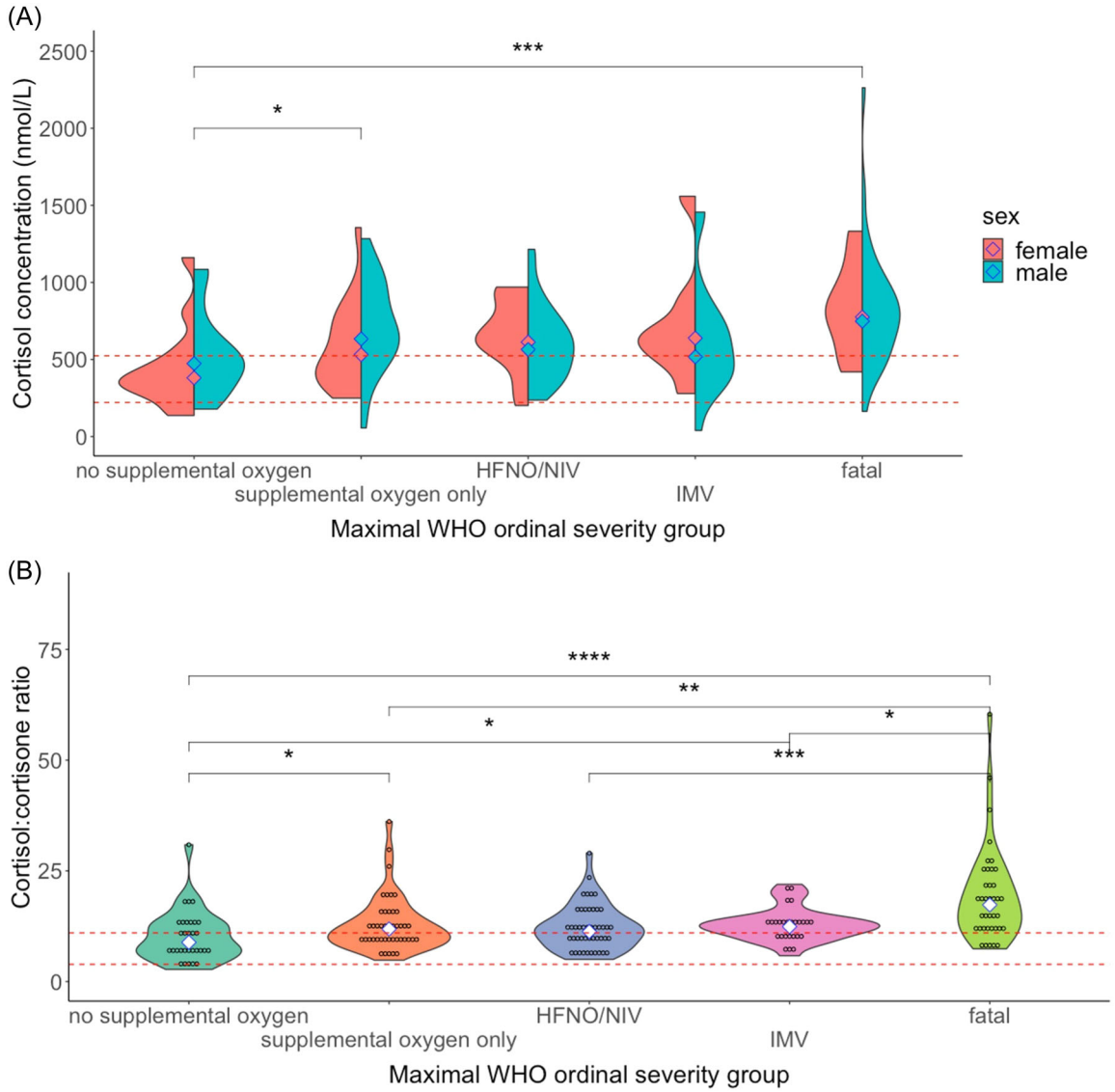

FIGURE 1 Plasma cortisol and cortisol: cortisone ratio is higher in fatal COVID-19 disease. (A) Split violin plot representation of cortisol concentration by severity group and sex during acute illness ($n = 127$ males, $n = 57$ females). (B) Violin plot representation of cortisol:cortisone ratio by severity group with dot-plot overlay. Geometric mean indicated by diamond point, normal healthy am reference range indicated by red dotted line (Labcorp). p Values taken from ANOVA of $\log_2$cortisol or $\log_2$(cortisol:cortisone) against severity, with post-hoc Benjamini–Hochberg p-value adjustment ($N = 184$). ANOVA, analysis of variance; COVID-19, coronavirus disease 2019; HFNO, high-flow nasal oxygen; IMV, invasive mechanical ventilation; NIV, noninvasive ventilation; WHO, World Health Organization. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$, else insignificant.

Cortisol concentrations were not associated with in-hospital mortality in multivariate analysis including age, sex, duration of illness, respiratory rate, oxygen saturation, urea and CRP (Supporting Information S1: Table 4).

4.2 | 5-Month follow-up

In the follow-up cohort, the GM cortisol concentration (275.6 [1.5] nmol/L) was within the reference range, and did not differ with in-hospital severity ($p = .944$), or in-hospital glucocorticoid therapy during initial illness (284.7 [1.4] vs. 276.4 [1.5] nmol/L, glucocorticoid therapy vs. no therapy, $p = .612$). The cortisol:cortisone ratio was normal (GM: 6.1 [1.3]) and did not differ across severity groups ($p = .986$). Full results of glucocorticoid concentrations by WHO ordinal group are displayed in Supporting Information S1: Table 5.

Cortisol concentrations in patients stratified by patient-reported questionnaire scores, and by CRP (measured at the same follow-up visit) are shown in Figure 2 (detailed in Supporting Information S1: Table 6).

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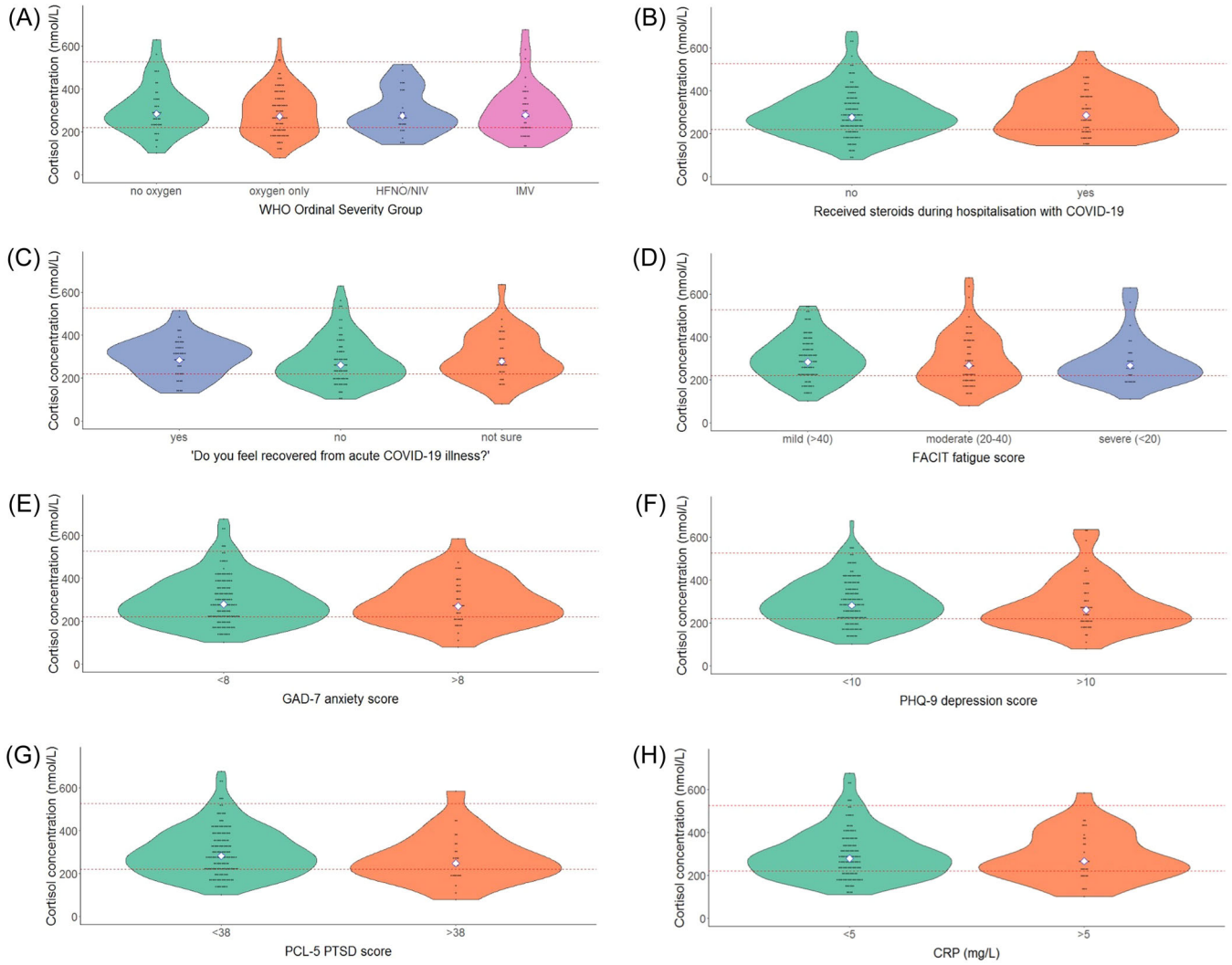

FIGURE 2 Cortisol concentration is not related to validated symptom scores. Violin plot representation of cortisol concentration by symptom score with dot-plot overlay. Geometric mean indicated by diamond point, normal healthy am reference range indicated by red dotted line (Labcorp). *p* Values taken from ANOVA of $\log_2$(cortisol) against symptom score, with post-hoc Benjamini–Hochberg *p*-value adjustment ($N = 196$). ANOVA, analysis of variance; CRP, C-reactive protein; FACIT, functional assessment of chronic illness therapy, GAD-7, Generalized Anxiety Disorder 7-item scale; PHQ-9, Patient Health Questionnaire-9; PCL-5, Post Traumatic Stress Disorder Checklist; WHO, World Health Organisation. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$, else insignificant.

There was no relationship between plasma cortisol concentration and perception of recovery (282.5 [1.4] vs. 260.0 [1.5] nmol/L, $p = 1.000$ *recovered vs. not recovered*), or patient-reported symptoms of fatigue (FACIT-F score), depression (PHQ-9 score), anxiety (GAD-7 score), posttraumatic stress (PCL-5 score) or breathlessness (Dyspnoea-12 score) (all $p > .05$).

A sensitivity analysis was performed in the 66 patients who had plasma obtained before 10 AM to account for differences in sample time. The GM cortisol level was slightly higher in this subgroup (303.0 [1.4] vs. 262.7 [1.5] nmol/L in those sampled after 10 AM, $p = .012$), but there remained no significant difference in cortisol concentration between in-hospital severity groups, or by patient-reported symptoms ($p > .05$ for all).

5 | ANDROGENS

5.1 | Acute COVID-19

Table 2 shows adrenal and gonadal androgens in men during hospitalisation with acute COVID-19. In males, the GM (GSD) testosterone concentration was 2.6 (3.0) nmol/L and only 12% of patients (18/151) had testosterone concentrations within the healthy reference range (9–32 nmol/L).[15] There was no correlation between testosterone and age ($r = -.029$, $p = .721$) or illness duration ($r = -.063$, $p = .451$), and a reduction in testosterone concentrations in those with clinician-identified obesity did not

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TABLE 2 Androgen concentrations by maximal WHO ordinal severity rating in male patients during acute hospitalisation with COVID-19.

	WHO ordinal scale for clinical improvement									
	No supplemental oxygen		Oxygen only		HFNO/NIV		IMV		Fatal	
	GM	GSD	GM	GSD	GM	GSD	GM	GSD	GM	GSD
Testosterone (nmol/L)	6.9[c]	1.9	4.1[b,c]	3.0	3.3[b]	2.1	1.0[a]	3.0	1.2[a]	2.2
5a Dihydrotestosterone (nmol/L)	0.9[b]	2.4	0.4[a,b]	3.6	0.3[a]	4.0	0.2[a]	4.8	0.3[a]	3.3
11 Ketotestosterone (nmol/L)	0.5[a]	1.9	0.3[a]	3.0	0.4[a]	3.1	0.3[a]	2.4	0.4[a]	2.2
Androstenedione (nmol/L)	1.9[a]	1.7	1.6[a]	2.0	2.0[a]	1.9	1.9[a]	2.4	1.7[a]	2.2
11β Hydroxyandrostenedione (nmol/L)	5.5[a]	1.6	5.4[a]	2.7	7.0[a]	2.6	6.5[a]	3.1	9.4[a]	2.6
11 Ketoandrostenedione (nmol/L)	0.4[a]	1.9	0.3[a]	2.6	0.5[a]	2.7	0.4[a]	3.3	0.5[a]	3.2

Note: Results presented as GM and GSD. $N = 151$.

Abbreviations: ANOVA, analysis of variance; COVID-19, coronavirus disease 2019; GM, geometric mean; GSD, geometric standard deviation; HFNO, high flow nasal oxygen; IMV, invasive mechanical ventilation; NIV, noninvasive ventilation; WHO, World Health Organisation.

[a–c]Means in a row without a common superscript letter differ ($p < .05$), as analysed by one-way ANOVA with post-hoc Benjamini–Hochberg p-value adjustment.

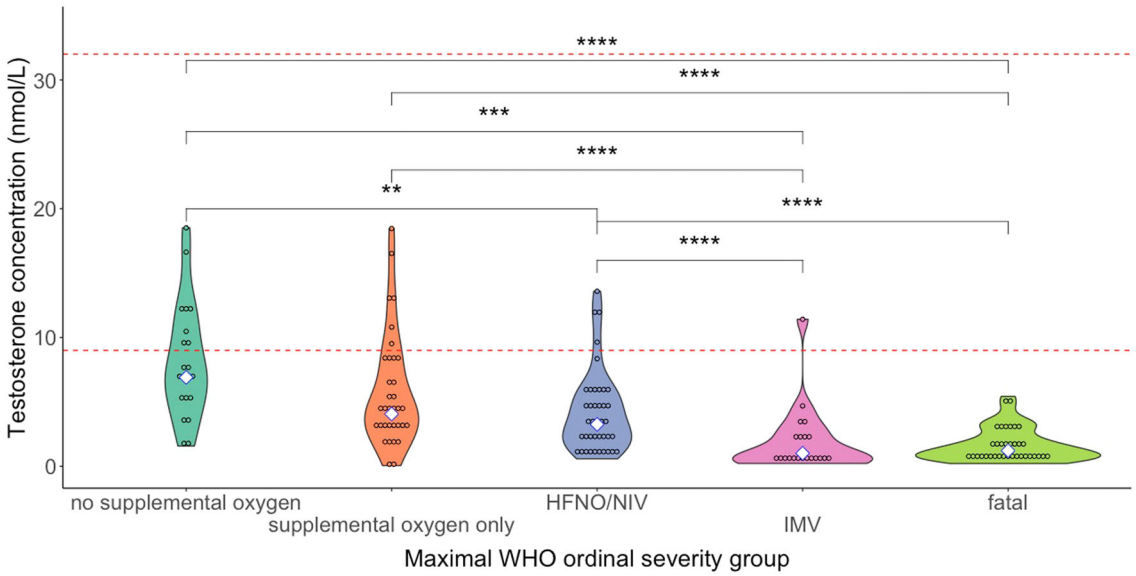

FIGURE 3 Testosterone concentration in males is inversely scaled with COVID-19 severity during acute illness. Violin plot representation of testosterone concentration by severity group with dot-plot overlay. Geometric mean indicated by diamond point, normal healthy am reference range indicated by red dotted line (Labcorp). p Values taken from ANOVA of $\log_2$(testosterone) against severity, with post-hoc Benjamini–Hochberg p-value adjustment ($N = 151$). ANOVA, analysis of variance; COVID-19, coronavirus disease 2019; HFNO, high-flow nasal oxygen; IMV, invasive mechanical ventilation; NIV, noninvasive ventilation; WHO, World Health Organisation. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$, else insignificant.

reach statistical significance (1.5 [3.9] vs. 2.8 [2.8] nmol/L, $p = .054$).

Figure 3 shows that testosterone concentrations in males were inversely associated with acute disease outcomes. Those with fatal disease had testosterone levels almost 6x lower than those in the lowest severity group (1.2 [2.2] vs. 6.9 [1.9] nmol/L, $p < .001$). Similarly, there was a threefold difference in 5α-dihydrotestosterone concentration between these groups (0.3 [3.3] vs. 0.9 [2.4] nmol/L, $p < .001$).

Multivariable analysis highlighted lower testosterone concentrations as an independent predictor of male mortality (Table 3). There were no differences in keto-or hydroxylated androgens, nor of androstenedione between severity groups. An increase in 11β-hydroxyandrostenedione with disease severity did not reach statistical significance (9.4 [2.6] nmol/L in fatal disease vs. 5.5 [1.6] nmol/L in patients not requiring supplemental oxygen, or 5.4 [2.7] nmol/L in patients requiring oxygen only, $p = .070$ and $p = .064$, respectively).

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TABLE 3 Association between testosterone concentration and in-hospital mortality from COVID-19 in males.

Variable	Adjusted odds ratio (95% confidence interval)	Adjusted *p*-Value
Testosterone	0.65 (0.44–0.88)	0.01
Comorbidity count	1.22 (0.65–2.33)	0.54
Day of illness	0.97 (0.91–1.03)	0.37
Age	1.06 (1.01–1.12)	0.02
Urea	1.16 (1.02–1.34)	0.03
Respiratory rate	1.11 (1.00–1.23)	0.05
SpO_2	0.58 (0.25–1.22)	0.17
CRP	1.00 (1.00–1.01)	0.32
Obesity	1.83 (0.29–10.65)	0.50

Note: Disease severity was not related to androgen levels in females (Supporting Information S1: Table 7).

5.2 | 5-month follow-up

At follow-up, the GM (GSD) male testosterone concentration was 12.6 (1.5) nmol/L and was within the reference range in 74.0% of men (n = 91/123). Thirteen (10.6%) men had a very low testosterone (<7 nmol/L). Follow-up testosterone concentrations were not related to in-hospital severity, patient-reported questionnaire scores or CRP (Supporting Information S1: Tables 8 and 9). The proportion of males with obesity (73.1% vs. 46.7%, p = .020) was statistically greater in the low testosterone group, but the association with diabetes mellitus was not significant (25.0% vs. 11.2%, p = .054).

A sensitivity analysis was performed in the 39 men who had plasma obtained before 10 AM and in the fasting state to determine if differences in time of sampling influenced testosterone levels. There was a similar plasma testosterone concentration (11.7 [1.5] nmol/L) and proportion with testosterone <9.2 nmol/L (n = 12/39 [30.8%]) in this subgroup. There remained no significant difference in testosterone concentration between WHO ordinal severity groups, or by questionnaire scores (p > .05 for all). In this group, there was a correlation between testosterone levels and clinical features of hypogonadism. 50% of men with testosterone <9.2 nmol/L reported erectile dysfunction (vs. 5%; χ^2 8.31, p = .004) and 25% had a haematocrit <0.40 (vs. 0%; χ^2 4.49, p = .030).

There remained no relationship between female testosterone (1.5 [1.4] nmol/L] and disease severity at 5 months from discharge (p = .303).

6 | DISCUSSION

In this study of two UK cohorts of adults hospitalised with COVID-19 we demonstrated widespread changes in steroid hormones, precursors and metabolites during acute illness, but not in most patients 5 months from discharge. Glucocorticoid hormones were acutely elevated, and correlated with maximal disease severity, whilst the inverse was true for male testosterone. Glucocorticoid and androgen levels were not clinically abnormal at follow-up in most patients, nor were they associated with ongoing symptoms, suggesting steroid insufficiency is not a causal factor for this symptomatology.

During hospitalisation, most patients sampled had cortisol levels above those typically seen in health (221–524 nmol/L),[14] with a 1.8-fold difference between the lowest and highest severity groups. Higher cortisol levels have been associated with mortality in severe sepsis and pneumonia.[16,17] Our results are in accord with at least some other studies[4] which have shown similarly high cortisol levels in hospitalised COVID-19 patients, and correlation with mortality. Through analysis of glucocorticoid metabolites and precursors we found a shift in the balance between peripheral inactivation and reactivation of cortisol, as reflected in a marked elevation (up to 2.3× normal) in the cortisol:cortisone ratio. Cortisol is converted to cortisone by the 11 beta-hydroxysteroid dehydrogenase (11β-HSD) type 2 enzyme, whilst 11β-HSD1 regenerates cortisol from cortisone, and is stimulated by inflammatory cytokines.[18] The cortisol:cortisone ratio reflects systemic 11β-HSD balance, and is elevated both in septic shock,[19] and in response to an acute corticotrophin (ACTH) stimulus.[20]

We found no evidence of glucocorticoid insufficiency during acute COVID-19, with only 7% of the sampled ISARIC4C cohort having cortisol concentrations <276 nmol/L—the suggested 'CIRCI' threshold.[21] Most of these had milder disease which may not have elicited a cortisol response. Rescue from hypocortisolism is, therefore unlikely to explain the therapeutic effect of dexamethasone in severe disease, consistent with the accepted mechanism of action being suppression of myeloid-driven immunopathology.[22] Other studies reporting adrenal insufficiency in patients with COVID-19 over-represent mild disease: one small cohort of 28 patients had a single fatality,[5] while 20% of patients in the 'moderate-severe disease' group of another study were in fact asymptomatic and stratified as such only on the basis of background comorbidity.[6] In an intensive care series, 6/9 patients had morning cortisol levels <276 nmol/L, however there was a lack of control for confounders (e.g., recent glucocorticoid treatment).[7]

Hypoadrenalism during the recovery phase following acute illness is a possible mechanism for post-COVID symptoms and was identified at 3 months posthospitalisation in 24 of 61 severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) survivors in 2005.[23] In our follow-up cohort 5 months from discharge, cortisol concentrations were within the expected reference range and were not associated with illness severity, glucocorticoid therapy during acute illness, or ongoing symptoms. In a follow-up study of 70 patients hospitalised with COVID-19 ≥3 months earlier, all patients achieved a cortisol level of >450 nmol/L after administration of 250 μg tetracosactide (ACTH 1–24), excluding clinically overt adrenal insufficiency.[11] Another study of patients attending a long-COVID clinic showed a weak but positive correlation between plasma cortisol and the Fatigue Assessment Scale at 3 months from discharge (r = .173, p = .018).[24] Conversely, lower cortisol levels were found at 3 months after acute illness in patients with respiratory

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symptoms, and those with ≥4 symptoms, in another posthospitalisation cohort.[25] Importantly, the potential for interference from exogenous steroids was not considered in these patients.

Testosterone was profoundly suppressed in males during acute COVID-19, as has been reported previously.[26] A total of 88% of men had a testosterone concentration below the Endocrine Society reference range.[15] There was a clear inverse correlation with disease severity, with an 80% drop in total testosterone between the least and most severely affected groups (6.9 vs. 1.2 nmol/L). Low testosterone in this context is most likely an acute illness response, as is well recognised in other severe inflammatory states such as in sepsis,[27] but could also be explained by existing untreated hypogonadism or direct viral injury to the HPG axis. The testis is considered vulnerable to infection by the SARS-CoV-2 virus on account of expression of angiotensin-converting enzyme 2 and the TMPRSS2 coreceptor,[28] and cases of orchitis have been reported.[29]

These findings raise concerns about long-term hypogonadism. Using a relatively conservative limit of >9.2 nmol/L, we demonstrated normal testosterone levels in 74% of men in our follow-up cohort. The majority who fell below this limit had a borderline result (7–9.2 nmol/L), therefore, in the absence of sex hormone-binding globulin or free testosterone values, it is difficult to conclude whether they were truly hypogonadal. However, the link demonstrated between low testosterone and clinical markers of hypogonadism (low haematocrit and erectile dysfunction) in our subgroup sensitivity analysis gives weight to this finding. Erectile dysfunction has been frequently reported in men after COVID-19,[30] and a similar proportion of (predominantly hypogonadotropic) hypogonadism was reported in 28.7% of males 11 weeks posthospital discharge with COVID-19 in Spain,[31] and in 30% at 1 year in an Italian cohort.[32] Even with its strict cut-off of 11 nmol/L, the 2010 European Male Ageing Study found the community prevalence of biochemical hypogonadism in men aged 40–79 years to be considerably lower than this at 17%.[33] Whether COVID-19 is causative, or simply exposes a more at-risk population, is unknown, but men with sexual dysfunction after COVID-19 should undergo appropriate biochemical testing for hypogonadism.

6.1 | Strengths and limitations

This study has several strengths. We sampled a spectrum of disease severity, and present profiles reflecting both the acute illness period, and recovery, capturing a cohort with a high prevalence of ongoing symptoms. We have used the gold standard approach to steroid quantification, and used a range of validated patient reporting measures to compare steroid concentrations with ongoing symptoms.

One clear limitation is that populations were not nested, so this is not a longitudinal analysis. Plasma samples were not collected via dynamic testing, so clinically definitive endocrine sufficiency or deficiency cannot be determined. Furthermore, details of sample collection timing and conditions were not recorded in the ISARIC4C cohort, so results cannot be adjusted for time of day. Observations that cortisol rhythmicity is lost in patients with COVID-19 suggest this is unlikely to affect these findings.[4] We do not have synchronous measurements of tropic hormones or binding proteins which may help determine the level of the underlying response.

In conclusion, during acute illness, patients hospitalised with COVID-19 show a range of plasma steroid responses dependent on illness severity, including an increase in circulating glucocorticoids, a shift in peripheral glucocorticoid metabolism, and, in males, a marked decline in circulating testosterone. Both glucocorticoids and androgens appear to normalise in the months after acute infection in most patients, however, the consistent finding of low testosterone levels in a proportion of men highlights this as a group worthy of further exploration in post-COVID research and practice.

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REFERENCES

1. Marik PE, Pastores SM, Annane D, et al. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an International Task Force by the American College of Critical Care Medicine. *Crit Care Med*. 2008;36(6):1937-1949.
2. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in hospitalized patients with Covid-19. *N Engl J Med*. 2021;384(8):693-704.
3. 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.
4. Tan T, Khoo B, Mills EG, et al. Association between high serum total cortisol concentrations and mortality from Covid-19. *Lancet Diabetes Endocrinol*. 2020;8(8):659-660.
5. Alzahrani AS, Mukhtar N, Aljomaiah A, et al. The impact of Covid-19 viral infection on the hypothalamic–pituitary–adrenal axis. *Endocr Pract*. 2021;27(2):83-89.
6. Das L, Dutta P, Walia R, et al. Spectrum of endocrine dysfunction and association with disease severity in patients with Covid-19: insights from a cross-sectional, observational study. *Front Endocrinol*. 2021;12:645787.
7. Mao Y, Xu B, Guan W, et al. The adrenal cortex, an underestimated site of SARS-CoV-2 infection. *Front Endocrinol*. 2021;11(1060):593179.
8. Evans RA, McAuley H, Harrison EM, et al. Physical, cognitive, and mental health impacts of Covid-19 after hospitalisation (PhospCovid): a uk multicentre, prospective cohort study. *Lancet Respir Med*. 2021;9(11):1275-1287.
9. Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV. A clinical case definition of post-Covid-19 condition by a Delphi consensus. *Lancet Infect Dis*. 2022;22(4):e102-e107.
10. Papadopoulos AS, Cleare AJ. Hypothalamic-pituitary-adrenal axis dysfunction in chronic fatigue syndrome. *Nat Rev Endocrinol*. 2011;8(1):22-32.
11. Clarke SA, Phylactou M, Patel B, et al. Normal adrenal and thyroid function in patients who survive Covid-19 infection. *J Clin Endocrinol Metab*. 2021;106(8):2208-2220.
12. Marshall JC, Murthy S, Diaz J, et al. A minimal common outcome measure set for Covid-19 clinical research. *Lancet Infect Dis*. 2020;20(8):e192-e197.

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13. Knight SR, Ho A, Pius R, et al. Risk stratification of patients admitted to hospital with Covid-19 using the isaric who clinical characterisation protocol: development and validation of the 4c mortality score. *BMJ*. 2020;370:m3339.
14. Laboratory Corporation of America® Holdings. Endocrinology Expected Values and S.I. Unit Conversion Tables. 2021. Accessed June 20, 2023. https://specialtytesting.labcorp.com/sites/default/files/2021-07/L5167-0421-18%20Endocrine%20Expected%20Values_0.pdf
15. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2018;103(5):1715-1744.
16. Remmelts HHF, Meijvis SCA, Kovaleva A, Biesma DH, Rijkers GT, Heijligenberg R. Changes in serum cortisol levels during community-acquired pneumonia: the influence of dexamethasone. *Respir Med*. 2012;106(6):905-908.
17. Sam S, Corbridge TC, Mokhlesi B, Comellas AP, Molitch ME. Cortisol levels and mortality in severe sepsis. *Clin Endocrinol*. 2004;60(1):29-35.
18. Chapman KE, Coutinho AE, Zhang Z, Kipari T, Savill JS, Seckl JR. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. *J Steroid Biochem Mol Biol*. 2013;137(100):82-92.
19. Cohen J, Smith ML, Deans RV, et al. Serial changes in plasma total cortisol, plasma free cortisol, and tissue cortisol activity in patients with septic shock: an observational study. *Shock*. 2012;37(1):28-33.
20. Walker BR, Campbell JC, Fraser R, Stewart PM, Edwards CRW. Mineralocorticoid excess and inhibition of 11 β-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. *Clin Endocrinol*. 1992;37(6):483-492.
21. Annane D, Pastores SM, Rochwerg B, et al. Guidelines for the diagnosis and management of critical illness-related corticosteroid insufficiency (Circi) in critically ill patients (Part I): Society of Critical Care Medicine (SCCM) and European Society of Intensive Care Medicine (ESICM) 2017. *Intensive Care Med*. 2017;43(12):1751-1763.
22. Dorward DA, Russell CD, Um IH, et al. Tissue-specific immunopathology in fatal Covid-19. *Am J Respir Crit Care Med*. 2021;203(2):192-201.
23. Leow MK-S, Kwek DS-K, Ng AW-K, Ong K-C, Kaw GJ-L, Lee LS-U. Hypocortisolism in survivors of severe acute respiratory syndrome (SARS). *Clin Endocrinol*. 2005;63(2):197-202.
24. Sunada N, Honda H, Nakano Y, et al. Hormonal trends in patients suffering from long Covid symptoms. *Endocr J*. 2022;69:1173-1181.
25. Su Y, Yuan D, Chen DG, et al. Multiple early factors anticipate post-acute Covid-19 sequelae. *Cell*. 2022;185(5):881-895.
26. Dhindsa S, Zhang N, McPhaul MJ, et al. Association of circulating sex hormones with inflammation and disease severity in patients with Covid-19. *JAMA Netw Open*. 2021;4(5):e2111398.
27. Fourrier F, Jallot A, Leclerc L, et al. Sex steroid hormones in circulatory shock, sepsis syndrome, and septic shock. *Circ Shock*. 1994;43(4):171-178.
28. Liu X, Chen Y, Tang W, et al. Single-cell transcriptome analysis of the novel coronavirus (SARS-CoV-2) associated gene Ace2 expression in normal and non-obstructive azoospermia (NOA) human male testes. *Science China Life Sciences*. 2020;63(7):1006-1015.
29. Pan F, Xiao X, Guo J, et al. No evidence of severe acute respiratory syndrome-coronavirus 2 in semen of males recovering from coronavirus disease 2019. *Fertil Steril*. 2020;113(6):1135-1139.
30. Chu KY, Nackeeran S, Horodyski L, Masterson TA, Ramasamy R. Covid-19 infection is associated with new onset erectile dysfunction: insights from a National Registry. *Sex Med*. 2022;10(1):100478-1.
31. Moreno-Perez O, Merino E, Alfayate R, et al. Male pituitary-gonadal axis dysfunction in post-acute Covid-19 syndrome-prevalence and associated factors: a Mediterranean case series. *Clin Endocrinol*. 2022;96(3):353-362.
32. Salonia A, Pontillo M, Capogrosso P, et al. Testosterone in males with Covid-19: a 12-month cohort study. *Andrology*. 2023;11(1):17-23.
33. Wu FCW, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *N Engl J Med*. 2010;363(2):123-135.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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