



Gibbison, B., Keenan, D. M., Roelfsema, F., Evans, J., Phillips, K., Rogers, C. A., Angelini, G. D., & Lightman, S. L. (2020). Dynamic pituitary-adrenal interactions in the critically ill after cardiac surgery. *The Journal of clinical endocrinology and metabolism*, 105(5), [dgz206]. <https://doi.org/10.1210/clinem/dgz206>

Peer reviewed version

Link to published version (if available):
[10.1210/clinem/dgz206](https://doi.org/10.1210/clinem/dgz206)

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Dynamic pituitary-adrenal interactions in the critically ill after cardiac surgery

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Key Words: Adrenal / ACTH / cortisol / critical illness

Funding

This study was supported by a project grant from the British Heart Foundation (PG/14/72/31080) and the UK NIHR Biomedical Research Centre at the University Hospitals Bristol NHS Foundation Trust and the University of Bristol. The views expressed in this publication are those of the review authors and not necessarily those of the NHS, the NIHR or the UK Department of Health.

Competing Interests / Disclosure

There are no competing interests.

Abstract

Context: Patients with critical illness are thought to be at risk of adrenal insufficiency. There are no models of dynamic hypothalamic-pituitary-adrenal (HPA) axis function in this group of patients and thus current methods of diagnosis are based on aggregated, static models.

Objective: To characterise the secretory dynamics of the HPA axis in the critically ill after cardiac surgery.

Design: Mathematical modelling of cohorts.

Setting: Cardiac critical care unit

Patients/Subjects: 20 male patients critically ill (CI) at least 48 hours after cardiac surgery and 19 healthy (H) male volunteers.

Interventions: None

Main Outcome Measures: Measures of hormone secretory dynamics were generated from serum adrenocorticotrophic hormone (ACTH) sampled every hour and total cortisol every 10-minutes for 24-hours.

Results: All critically ill patients had pulsatile ACTH and cortisol profiles. Critically ill patients had similar ACTH secretion (1036.4(737.6)pg/ml/24hrs) compared to the healthy volunteers (1502.3(1152.2)pg/ml/24hrs, $p=0.2$), but increased cortisol secretion (CI:14447.0(5709.3) v H:5915.5(1686.7)nmol/L/24hrs, $p<0.0001$). This increase in cortisol was due to non-pulsatile (CI:9253.4(3348.8) v H:960(589.0)nmol/L/24hrs, $p<0.0001$), rather than pulsatile cortisol secretion (CI:5193.1(3018.5) v H:4955.1(1753.6)nmol/L/24hrs, $p=0.43$). Seven (35%) of the 20 CI patients had cortisol pulse nadirs below the current international guideline threshold for Critical Illness Related Corticosteroid Insufficiency, but an overall secretion that would not be considered deficient.

Conclusions: This study supports the premise that current tests of HPA axis function are unhelpful in the diagnosis of adrenal insufficiency in the critically ill. The reduced ACTH and increase in non-pulsatile cortisol secretion imply that the secretion of cortisol is driven by factors outside the HPA-axis in critical illness.

Precis: We present 24-hour ACTH / cortisol profiles in patients critically ill after cardiac surgery and show both hormones are pulsatile. Using mathematical modelling to derive secretory dynamics, we suggest that current methods of diagnosing adrenal insufficiency in the critically ill are not useful.

Accepted Manuscript

Introduction

Critical illness and multi-organ failure present a major inflammatory challenge to human physiology. Patients with critical illness require specialist management in a critical care / intensive care unit - an area which has a sole function to provide advanced monitoring or support to single or multiple body systems. Patients who are critically ill after cardiac surgery represent an important cohort for study since the inflammatory stimulus is better defined than in most other critically ill patients. Its onset time also is exactly known – allowing a standardised timeline to be drawn. The systemic inflammatory response of the individual is not stylised however; whilst over 98% of patients survive cardiac surgery(1) - 75% of patients will have only a short (<2 days) stay in a critical care unit. Twenty percent will have a moderately extended stay and around 3% will have a long (>10days) stay(2). One of the key physiological systems that regulates the inflammatory response is the hypothalamic-pituitary-adrenal (HPA) axis. Both excess and deficiency(3) of components of the system have been shown to be associated with poor patient outcomes. Developing thresholds of normality for these has stalled(4), primarily as there is no accurate model for HPA-axis function in critical illness that encompasses the dynamic and pulsatile nature of the system. The term “critical illness related corticosteroid insufficiency - (CIRCI)”(4,5) has been coined to describe patients who clinicians believe do not produce sufficient corticosteroids during their critical illness.

Adrenocorticotrophic hormone (ACTH) released from the anterior pituitary (in response to portal blood corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP)) acts on the *zona fasciculata* of the adrenal gland to synthesise and release cortisol. The necessity

to synthesise cortisol *de novo* creates a feedforward delay in the release of cortisol, while secreted cortisol then has a negative feedback effect both on the pituitary and the hypothalamus to reduce release of ACTH. This positive feed-forward and negative feed-back with delay causes the secretion of both ACTH and cortisol to oscillate with a wavelength of around an hour in health(6,7). These secretory pulses of cortisol form an *ultradian* rhythm(8). In health, this ultradian rhythm underlies the *circadian* rhythm. The peak of the circadian rhythm is formed by large amplitude pulses and the nadir occurs at a time of little or no pulsatile activity(9).

Pulsatility is important for glucocorticoid signalling, and mammalian cells have developed tissue specific mechanisms to decode these oscillating signals. Multiple genes are differentially activated or inhibited depending on whether the signal is pulsatile or constant – even when the total dose is the same(10,11). It has been shown that there is an initial rise in both hormones for the first day of critical illness and that cortisol remains high, whilst ACTH subsequently falls – so called ‘dissociation’. Previous studies in critical illness have been sampled too infrequently to appreciate any pulsatility and therefore define the interaction between ACTH and cortisol(12).

This study was designed to help develop a model of HPA-axis function in critical illness by characterizing the serum hormonal patterns of ACTH and cortisol as well as understanding the secretory patterns that underlie them.

Materials and Methods

The study was reviewed and approved by the National Health Service (NHS) National Research Ethics Service (NRES), Health Research Authority (HRA) and Institutional Review

Boards of the respective centres. Incapacitated patients were recruited with deferred consent. Healthy volunteers provided written, informed consent. Female sex hormone cycles are known to affect the HPA-axis(13) and for this reason, female participants were excluded.

Subjects – Critical care patients

Twenty patients were recruited from a single cardiac critical care unit and met all of the following inclusion criteria: 1. Male, 2. Ages 18 – 80 having undergone elective or emergency cardiac surgery, 3. Post-operative day 2 or later (to exclude the immediate inflammatory effects of the surgery), 4. At the start of sampling, the patient was receiving at least 2 of the following: a) Invasive mechanical ventilation, b) Inotropes and/or vasopressors and/or c) Haemo(dia)filtration. Patients were excluded if they met any of the following exclusion criteria: 1. Current or recent (< 3 months) use of glucocorticoids, 2. Disorders of the HPA axis, 3. Thyroid disease, 4. Etomidate use at any stage of the surgical pathway.

Subjects – Healthy controls

Healthy males were recruited. Participants were eligible if: a complete medical history, physical examination, and screening tests of haematological, renal, hepatic, metabolic, and endocrine function were normal. Participants were excluded if they had recent (<10 days) trans-meridian travel, weight change (>2 kg in 6 weeks), shift work, intercurrent psychosocial stress, prescription medication use, substance abuse, neuropsychiatric illness, or acute or chronic systemic disease and also if they were exposed to exogenous glucocorticoids in the previous 3 months. Participants maintained conventional work and sleeping patterns. Healthy volunteers were admitted to the Study Unit the evening before

sampling for adaptation. Vigorous exercise, daytime sleep, snacks, caffeinated beverages, and cigarette smoking were disallowed. Meals were provided at 0800, 1230, and 1730, and room lights were turned off between 2200 and 2400 depending upon individual sleeping habits.

Collection and processing of blood samples – critically ill patients

Patients had blood sampled for 24 hours via their *in-situ* vascular catheters from 0800hrs using a needle-free, closed loop sampling system (Edwards VAMP. Edwards Life Sciences Corp, Irvine, CA. USA). Total serum cortisol was sampled at 10-minute intervals. ACTH was sampled every hour due to limitations of blood volume sampling in patients already at risk of blood loss. Cortisol samples were collected in BD vacutainer SST Advance tubes (Becton, Dickinson and Company, Oxford. UK) and were processed immediately after centrifugation. Samples for ACTH were collected in chilled 2ml EDTA tubes and stored on ice for a maximum of 60 minutes before centrifugation at 4°C and then stored at -80°C until assay. Total cortisol and ACTH were measured by solid phase, chemo-luminescent enzyme linked immunoassay (ECLIA) using the Cobas e602 modular analyzer (Roche Diagnostics Ltd, West Sussex, UK). Measuring limits for the cortisol assay were 0.5 – 1750nmol/L (intra- and inter-assay coefficients of variation (COV): 1.5 – 1.7% and 1.8 – 2.8% respectively) and for the ACTH assay were 1.0 – 2000 pg/ml (intra- and inter-assay COV: 0.6 – 2.7% and 3.5 – 5.4% respectively).

Collection and processing of blood samples – healthy volunteers

Venous blood samples were withdrawn at 10-min intervals for 24 hours. Sampling began between 0800 and 0900hrs. Blood was collected in prechilled siliconized tubes containing

EDTA (for ACTH assay) or heparin (for cortisol assay), centrifuged at 4°C and frozen at - 20°C within 30 minutes of collection. Plasma ACTH concentrations were quantified in duplicate by high-sensitivity (3 ng/l) and high-specificity monoclonal immunoradiometric assay, using reagents from Nichols Diagnostics Institute (San Juan Capistrano, CA). Cortisol was assayed by high sensitivity (25 nmol/l) solid-phase RIA (Sorin Biomedica, Milan, Italy). Intra-assay and inter-assay COVs were 5.1 and 6.4%, respectively. No samples were undetectable in either assay.

Statistical Analysis of Demographic Data

Demographic data were compared between the critically ill patients and healthy volunteers using a t-test.

Modelling Methodology

Modelling of hormone secretory dynamics, ACTH – cortisol interaction and circadian rhythm was carried for each individual and then combined as summary statistics. Modelling and statistical analysis was carried out using user-developed programs in Matlab (Mathworks, Natick, MA).

Cortisol and ACTH secretion rates are each modulated by time delayed concentrations of the other – it is this that is responsible for the pulsatility. Such feedforward and feedback are extremely difficult to estimate and implement overall. The best strategy has therefore previously been shown to estimate the kinetics and secretion rates of each hormone individually and to then estimate their dose-response interaction(14). The concentration of

a hormone at any given time is a balance of molecules being added and removed. The concentration (X) at time t is:

$$X(t) = E(t) \cdot X(0) + \int_0^t E(t-s) \cdot Z(s) ds$$

$Z(s)$ is the secretion of a given hormone into the blood at time s . $E(t-s)$ the fraction of molecules left at time t after molecules secreted at time s have exited the blood. The secretion rate is composed of two parts: a constant basal rate and a pulsatile non-basal component. To estimate the timing of the pulses: there is a pulse time set

$T_m = (T^{(1)}, T^{(2)}, \dots, T^{(m-1)}, T^{(m)})$, where the number of pulse times (m) is unknown. At each pulse time there is a secretory-burst with mass $M^{(k)}$ described as a linear function of the preceding interpulse interval ($T^{(k)} - T^{(k-1)}$), plus a random effect ($R^{(k)}$) that allows for the biological variations in burst size that are not explicitly modelled by the linear function (e.g., desensitization, receptor or enzyme saturation).

$$M^{(k)} = (\eta_0 + \eta_1 \cdot (T^{(k)} - T^{(k-1)})) + R^{(k)}$$

η_0 denotes a minimum pulse amount and η_1 is the rate of mass accumulation over the interpulse interval ($T^{(k)} - T^{(k-1)}$). The secretion rate is therefore:

$$Z(t) = \beta_0 + \sum_{T^{(k)} \leq t} (\eta_0 + \eta_1 \cdot (T^{(k)} - T^{(k-1)}) + R^{(k)}) \psi(t - T^{(k)})$$

where β_0 is the basal rate and $\psi(s)$ is a waveform of mass release (mass/unit volume/unit time) beginning at each pulse time. To accommodate circadian rhythm modulation of the secretion rate, we allow for “day-night” differences in the waveform (one for day, one for night). It was assumed that each is a (normalized) three parameter Gamma density.

Previous work has shown that a single elimination model tends to underestimate the elimination and overestimate the secretion - thus two elimination rates were used in this study: a fast and a slow rate(15). The fast rate represents the advection, diffusion and mixing effects that the molecules undergo as the just secreted molecules diffuse, flow and become well mixed. The slow rate represents elimination from the blood. A widely applied pulse detection algorithm was used to recognise hormone pulses(14,15). An example of the application of the pulse detection and estimation of the ACTH and cortisol secretion and kinetics, to an individual's profile, are contained in Fig 1. What is then observed is a time sampling of the concentration $X(t_i)$, with measurement error:

$$Y_i = X(t_i) + \varepsilon(i), \quad i=1, \dots, n,$$

where the errors were assumed to be independently and identically distributed (IID) normal with mean zero and variance σ_ε^2 . A Gaussian (log) likelihood can then be written and penalized maximum likelihood estimation is performed (the penalty term is an Akaike Information Criterion (AIC) penalization on the number (m) and locations of the pulse times): $T_m = (T^{(1)}, T^{(2)}, \dots, T^{(m-1)}, T^{(m)})$. The pulse detection procedure is part of the sequential estimation. An example is seen in the ACTH concentration profile for Critically Ill (CI) subject 1 (Fig 1a, top row).

ACTH values in the critically ill group were measured hourly and were linearly interpolated to the 10-minute values, in order to be on the same scale as the corresponding cortisol values. Summary statistics for ACTH and cortisol were calculated over the full 24 hours. These included hormone pulse frequency and pulse regularity (interpulse variability)(16); total secretion, total pulsatile secretion, total basal secretion and mass per pulse (total pulsatile hormone / number of pulses). The fast half-lives were held fixed (ACTH: 3.5

minutes; cortisol: 2.41 minutes) and the slow half-lives were estimated. The values for the slow half-lives for ACTH were: Critically ill (Mean 23.06 minutes, Standard deviation (SD) 6.81, Standard error of the mean (SEM) 1.56) and healthy volunteers (Mean 23.55 minutes, SD 7.72, SEM 1.77). The values for the slow half-lives for cortisol were: Critically ill (Mean 53.60 minutes, SD 8.51, SEM 1.95) and healthy volunteers (Mean 56.16 minutes, SD 8.85, SEM 2.03).

Once the two estimated secretion rates and pulse times for ACTH and cortisol have been determined for each subject, the ACTH drive on cortisol secretion was determined for each individual subject and then grouped. Four dose-response models of cortisol secretion (as a pulse-by-pulse function) of ACTH drive were considered. A fundamental difficulty in modelling endocrine dose-response interactions is the inherent biological variation (desensitization, receptor and enzyme saturation) and thus hysteresis occurs. Hysteresis refers to a change in cortisol responsiveness to an ACTH pulse. Three such models of ACTH – cortisol interaction incorporating hysteresis are considered (a shift in potency, sensitivity or efficacy), as well as a model of no hysteresis(17). A schematic of the models is presented in Fig 2.

Based upon the individual modelling of ACTH (e.g. as seen in Fig 1), the fitted ACTH concentrations were obtained for each subject. These values were the basis for the ACTH feedforward signal on cortisol secretion. Similarly, from the model of cortisol secretion (an example is seen in Fig 1) we obtain the estimated cortisol secretion rate for each subject. The variable time delay and biological variation (e.g., desensitization) from ACTH drive (ACTH pulse) and its resultant cortisol response was accounted for by the following:

Time delay: The ACTH feedforward signal was constructed piecewise, from one Cortisol pulse onset time to the next, thus allowing for a varying time lag between an ACTH pulse onset and a Cortisol pulse onset. For each Cortisol pulse time, the ACTH pulse nearest within the allowable time, [-60,10] min, was identified. If such an ACTH pulse existed, then it was shifted to the Cortisol onset point so that the two onset points are aligned. If no such pulse existed within the time interval, then a time lag of 40 min was applied to the ACTH concentrations, starting at the Cortisol pulse onset time. We also allowed for the possibility that the ACTH pulse may slightly (10 min) follow the Cortisol pulse, due to neural innervations.

Biological variation: Pulse-by-pulse random effects in the efficacy of the logistic dose-response function are included and we allowed hysteresis to occur, via a mid-(ACTH) pulse shift in the dose response. Specifically, the Cortisol response on the upswing of an ACTH pulse, is allowed to change on the downswing. Hence, a hysteresis-like phenomenon occurs, with the system resetting (given sufficient time) to the initial curve, ready for the next secretory pulse(18). An example of estimation for one individual is given in Fig 3.

Statistical Analysis of Four ACTH-cortisol Dose-Response Models: In Model 1 (No-Hysteresis) there are six variables as outcomes: Potency, Sensitivity, Efficacy, Baseline, as well as the error variance and the Random Effects variance. In Models 2 - 4, there are eight outcome variables, with the additional two variables being: a. the estimated ACTH Stimulatory signal Mode (the time at which there is the hysteresis shift) and b. the potency,

sensitivity and efficacy values, post hysteresis onset. Wilcoxon Ranksum test and the two-sample T test were used to compare the models.

Statistical Modelling of Circadian Rhythms: Estimation of the ACTH and cortisol circadian rhythms was achieved by modelling their secretory pulse time patterns, as described above (seen in Fig 1). Estimated secretory pulse times for both ACTH and cortisol were calculated and then a Gaussian kernel-smoothing algorithm was applied to estimate the time-varying pulse rates for each group. As a measure of a loss of circadian rhythm, the maximum minus the minimum of each curve was calculated and compared between the groups. The pulse rate for both ACTH and cortisol were estimated to obtain measures of accuracy of the estimates of circadian rhythm loss. A Gaussian kernel-smoothing algorithm was applied to estimate the time-varying ACTH and Cortisol pulse rate for each group. To examine the variation in these loss of CR calculations (as if repeated experiments were performed) a resampling procedure was constructed. A leave 2 out, including 1 out, if chosen twice (which is a 10% resampling rate) was applied. Hence, an estimate of the probability distribution for each of the pulse rate functions (ACTH and cortisol) for the CI and H groups was obtained. To estimate the Loss of Circadian Rhythm for the hormone, a random pulse rate for the CI group and from the H group are selected. The difference between the maximum and the minimum is calculated for each. The ratio of the CI difference to the H difference is calculated, and one minus this ratio is one (random) observation for loss of circadian rhythm. This was repeated 1000 times. By construction, this is precisely the sampling (i.e., probability) distribution of the CR loss.

Results

Complete 24-hour profiles for cortisol were available for 20 critically ill patients and complete ACTH profiles were available for 19/20 patients. Index operations were: 6 isolated CABG, 3 isolated valve replacements, 2 single valve replacements, 3 combined CABG and valve replacements, 1 replacement of the thoracic part of the aorta, 3 valve replacements and replacement of thoracic part of aorta, 1 CABG and replacement of thoracic part of the aorta and 2 operations that did not fit these categories. Seven (35%) of these operations were carried out as emergencies (requiring surgery before the start of the next working day). One patient died 30 minutes before the end of the 24-hour sampling period. Data for this patient were included up until the point of death. There were 19 healthy controls with both ACTH and cortisol profiles available. Therefore, in the analysis of ACTH alone, there were 19 subjects, and for cortisol alone, there were 20 subjects; in the joint analysis of ACTH and cortisol, there were 19 subjects.

Characteristics

Baseline characteristics for both groups are shown in Table 1 and post-operative characteristics are shown in Table 2. Critically ill patients were on average twenty years older and had a greater body mass index than healthy volunteers. All critically ill patients were mechanically ventilated following surgery and all except one were receiving treatment with intravenous vasopressors or inotropes.

ACTH and Cortisol profiles and secretory patterns

Twenty-four-hour profiles of serum ACTH and cortisol were pulsatile in both the critically ill and healthy volunteer groups (Examples of individuals are given in Fig 4 and by group(19)). There was visual concordance between the ACTH and cortisol in all patients except for the

patient who died towards the end of the sampling period. This patient also lost pulsatility before death. Seven of the 20 critically ill patients (35%) had cortisol pulse nadirs that were below the international guideline threshold for the diagnosis of CIRCI (10mcg/dl / 276nmol/L)(5).

Measures of secretory dynamics are shown in Table 3. The pulse rates and regularity of both ACTH and cortisol were similar between both the critically ill and the healthy volunteer groups. The total secretion of ACTH was statistically similar in the two groups, as was the amount of pulsatile ACTH secretion and the basal (non-pulsatile) ACTH secretion. The mass of ACTH released per pulse was similar between the two groups. There *were* differences in cortisol secretion - total cortisol secretion was significantly increased in the critically ill group and all of this increase appears to come from basal (non-pulsatile secretion). The amount of cortisol secretion that is pulsatile is broadly similar between the two groups; in terms of pulse rate, regularity and mass of cortisol per pulse.

ACTH-cortisol interaction

The results from all models are shown in Fig 5 and show that in the critically ill group, the adrenal glands are more sensitive to ACTH, but with reduced efficacy and similar potency (i.e. the ACTH-cortisol dose-response curve is steeper but reaches a lower maximum response). All models show a significant increase in the sensitivity of the adrenal glands as characterised by ACTH-cortisol dose-response. In the models encompassing hysteresis, the efficacy (i.e., ability to produce the maximal cortisol output) of ACTH was reduced in the critically ill group. Again, in all models, there was an increase in baseline (ie non-pulsatile)

cortisol secretion in the critically ill group, as well as less random variation in the critically ill group. There were no differences in random effects variances in any of the models.

Circadian rhythm

There was a reduction in circadian variation of both ACTH and cortisol (See Fig 6). The reduction in circadian variation of the critically ill group of ACTH was by a median 51% and cortisol by median 74% when compared to the healthy volunteers. The lower quartile, median and upper quartile, for each distribution of the difference between maximum and minimum of the time varying pulse-rates per 24 hour period was for ACTH: (CI): 2.8, 3.0, 3.3; (H): 6.0, 6.2, 6.4; and for cortisol: (CI): 1.1, 1.3, 1.6; (H): 5.0, 5.2, 5.5. The lower, median and upper quartile of the distributions of a loss of Circadian Rhythm in the Critically ill compared to the Healthy group was calculated: ACTH: **46%, 51% and 55%**, and Cortisol: **69.0%, 74% and 79%**.

Discussion

The HPA-axis is frequently tested by clinicians on critical care units using a single-point serum sample or stimulation with synthetic ACTH. The results of these tests are used to determine the need for administration of synthetic corticosteroids to patients who are deemed to be 'deficient' – so called Critical Illness Related Corticosteroid Insufficiency (CIRCI)(4,5). There is currently no model of HPA-axis function during critical illness that takes into account the dynamic, pulsatile nature of this system. This has made delineating the thresholds for deficiency and 'excess' almost impossible. There is also no clear evidence-based treatment regimen for cortisol replacement or supplementation in critical illness(20) despite the evidence that the *pattern* of corticosteroid delivery differentially regulates the

activation or inhibition of glucocorticoid responsive genes(10,11,21). In this study, we used high-frequency sampling of both ACTH and cortisol for a 24-hour period to study the profiles and elicit the dynamic interaction of both these hormones in critically ill patients after cardiac surgery and compared them to a cohort of healthy volunteers.

The most striking result was that the profiles of both ACTH and cortisol in the critically ill patients remained pulsatile with concordance between the pulses of ACTH and cortisol throughout the 24-hour period. The observed pulsatility fits with previously studied examples of chronic illness such as obstructive sleep apnoea(22), coronary artery surgery(23) and a nocturnal only study of critical illness(24).

The results illustrate why single point testing of cortisol in critically ill patients lacks specificity and is unhelpful in diagnosing CIRCI if such a syndrome exists. Seven of the 20 patients (35%) had cortisol pulse nadirs that were below the international guideline threshold for the diagnosis of CIRCI (10mcg/dl / 276nmol/L)(5), even though a subsequent pulse peak would be markedly above this threshold. This concurs with previous work that shows that a single-point test of cortisol in the 24-hour peri-operative period of cardiac surgery is not predictive of the cortisol value even 40 minutes later(25). Furthermore, previous models of HPA axis function have been derived from aggregated patient data.

Aggregated profiles of pulsatile hormones lead to smooth curves – thus there is a situation in which the aggregated model of HPA axis function currently used for diagnosis of adrenal suppression in the critically ill, represents virtually no individuals *in* that population.

Techniques aiming to circumvent the problems of point testing of serum cortisol and derive a temporally longer “overview” of cortisol concentration and production without taking

multiple frequent samples have included measuring cortisol in the saliva(26) and urine(27). Salivary cortisol has not been recommended(5) due to it failing to demonstrate any benefit in patient outcomes, but more importantly; practically collecting samples of saliva in critically ill patients is difficult, as they often do not produce sufficient volumes for assay(26). This is similar to urinary cortisol; critically ill patients have a markedly reduced glomerular filtration rate (GFR) and many will be receiving renal replacement therapy (8/20 patients in this case). Free cortisol excretion is dependent on the GFR and therefore using urine in the critically ill is not useful (28). The new development of frequent cortisol sampling by microdialysis from the subcutaneous tissue(27) has not yet been tested in critical care. It will be interesting to see this type of data in critically ill patients as the data will reflect the levels of free cortisol in the tissues rather than total cortisol in the blood.

Because pulsatility emerges as a consequence of the feedforward-feedback interaction of ACTH and cortisol(6,29), our data implies that the pituitary-adrenal connection remains intact in critical illness and that there is no 'disconnect'(30) between these two hormones. The sensitivity of the interaction is changed however; the adrenal gland is more sensitive to ACTH in critical illness. ACTH has a reduced efficacy and similar potency when compared to healthy individuals. i.e. the dose-response curve for ACTH-cortisol starts at the same point (similar potency) but is steeper (more sensitive) and reaches a lower maximum (reduced efficacy) than in healthy individuals. The increased adrenal sensitivity seen *immediately* after cardiac surgery appears to be related in part to increased expression of the ACTH receptor accessory protein, MRAP(23), but we could not test this hypothesis in this study. Other mechanisms mediating this increased adrenal sensitivity and elevated cortisol production include circulating inflammatory mediators that are prevalent in critical illness,

such as IL1, IL6 and TNF- α (30). Receptors for these on *zona fasciculata* cells are able to stimulate cortisol production in a dose-dependent manner. Activity of the splanchnic nerves can also modulate adrenal production of cortisol, and increase blood flow to the adrenal gland - which is sufficient to increase cortisol secretion(31-33). In the setting of systemic inflammatory vasodilation, it seems likely that any additional effect of the splanchnic nerves would be minimal.

All cortisol that is secreted needs to be synthesised *de novo* – its fat-soluble properties preclude its storage in vesicles. The rate limiting step of cortisol synthesis is the transport of cholesterol from the outer to the inner mitochondrial membrane by the Steroidogenic Acute Regulatory (StAR) protein. This is clearly a saturable process with a finite maximum rate. Therefore, although it appears that ACTH has a lower efficacy, this is in effect illusory, as the gland is starting at a higher secretion rate and thus any increase in secretion towards a fixed maximum would, of necessity be lower. The changed ACTH-cortisol dose-response also calls into question the use of the co-syntropin / short synacthen test to diagnose 'deficiency' in critical illness using thresholds defined in health(34).

There has been no previous study of a full 24-hour period of HPA axis hormone dynamics in critically ill patients, although short periods of pulsatility have been observed(24,35). The only similar study examined nocturnal cortisol and ACTH secretion rather than 24 hours. They also found that the number of pulses were not different between the healthy individuals and critically ill patients, but in contrast to our work they found that non-pulsatile secretion of both ACTH and cortisol was the same between the two groups and that pulsatile secretion of both hormones was reduced in the critically ill. The explanation

for the differences they found was reduced cortisol breakdown(36,37). Compared to the previous study, our group of critically ill patients was more homogenous – they were all critically ill after cardiac surgery and our sampling period was much more standardized in terms of the timescale of their critical illness. We deliberately avoided the first 48 hours after surgery to ensure that the effects we see were those of critical illness and not the immediate post-operative changes. The changes to the HPA-axis in critical illness are likely to change with time. There is a relatively short initial ACTH surge driving the cortisol secretion(23). ACTH is then suppressed by the high levels of cortisol and cortisol levels remain high. The loss of the trophic effect of ACTH in the longer term may lead to a reduction in activation of the adrenal gland to produce cortisol(24). Longer duration of critical illness is then likely to result in other changes, such as the previously described decreased cortisol metabolism (29,30). Critical illness can be a progressive condition and therefore sampling at defined time-points in defined disease processes is important to truly understand the pathophysiology of changes in HPA-axis function. One of the advantages of using cardiac surgery to investigate the HPA-axis response to systemic inflammation is that the timeline can be standardized because the timing of the inflammatory stimulus is known.

Although reduced circadian variation of HPA activity has been described in patients with critical illness(35,38), this had not been previously quantified and the differences between ACTH and cortisol variations have not been compared. Blunting of the circadian rhythm in critical care units is not only a function of illness itself, but can also occur for a range of environmental and internal disturbances. Light (particularly low intensity blue) transmitted outside the visual pathways is the major stimulus that entrains circadian rhythms(39). The light intensity on a critical care unit changes little across the 24 hours and is around 50 times

less than that on an overcast day and around 1000 times less than a sunny day(40,41). Some of the blunting may be attributed to this, but it seems more likely that the inflammatory drive to the system overcomes the decrease in adrenal activation over the nocturnal nadir(35,36).

This study has some limitations. First, ACTH could not be measured as frequently in the critically ill patients as the healthy volunteers due to the clinical team's concern over blood loss in a cohort who were already at risk of transfusion. Secondly, we were unable to exactly match the critically ill and healthy volunteers for age and body habitus, although the changes in ultradian rhythm with age(42) and body mass index(43,44) are small. The secretory dynamics of ACTH and cortisol in this study are based on mathematical models and not direct experimentation. However, the results using these empirical models broadly correlate broadly with what has been found previously in critical illness using other techniques – that although the adrenal gland is more sensitive to ACTH(23), non-ACTH mechanisms drive the increased cortisol secretion(36) with ACTH being permissive. We used separate assays in the patients and the healthy volunteers due to the sampling being carried out on 2 separate sites. The laboratories of the hospital provide immediate access to automated ACTH and cortisol assays which we utilized for the patients. Samples from volunteers in the clinical research facility were rapidly spun and frozen for subsequent assay. Although there is no direct comparison of the two assays in studies of ECLIA versus RIA for both cortisol and ACTH, the $r=0.9$ (45,46) suggests good comparability of the 2 assays. The population size was small and so establishing the effect of multiple interventions (vasoactive and other drugs, haemofilters and ventilators) used in critical care is not statistically possible. It has been shown that haemo(dia)filtration on the critical care unit is

associated with minimal cortisol loss from the plasma(47) and so for the 8/20 patients receiving this treatment modality, we can say that plasma concentrations of cortisol are not artificially suppressed. We did not include women in this study because practically, it would have been impossible to get a large enough sample size to allow for comparison of sex differences. This is due to around 75% of cardiac surgery in the UK being performed on men(1) (for the reason of higher rates of acquired heart disease in men – despite the changing profile of cardiac surgery from coronary artery bypass grafting to valve surgery). Our aim in this study was for homogeneity so as to allow us to make clear conclusions on the effects of critical illness. Now that we have this data we shall be able to include women.

There are two questions that have dominated critical care research into HPA-axis function(4,5): i) Is there a group of patients who are deficient or relatively deficient in glucocorticoids in critical illness and ii) do supplementary glucocorticoids improve outcome in critical illness? These two questions are often confused, but future work is needed to answer them independently. To begin to answer the first question, we require a valid model of HPA-axis function in critical care that should encompass the dynamic interactions in HPA function and the temporal changes seen in critical illness . Ideally this should also reflect the tissue activity of the cortisol(9), rather than surrogates in the blood. Until we produce this model, however, the safe answer to the second question is straightforward; those managing critically ill patients should give so-called ‘low-dose’ corticosteroids (<200mg/day) to all patients receiving at least modest doses of vasopressor *without* testing their adrenal function, as there is evidence that this positively impacts morbidity (but not mortality) with a low-risk of harm(20,48). This does not, of course, take into account the question raised by our paper and a variety of sources from the bench(10,21) to the bedside(4) which suggest

that pulsatility is important for function. This has yet to be tested in critical care practice. Once we have a useful model of dynamic HPA-axis function, then we can design diagnostic and treatment strategies for the individual that produce maximum clinical effectiveness with the fewest adverse events. This is already being done for patients with chronic hypoadrenalism (49-51), and these same techniques should now be brought to the critical care unit.

This study has shown that both ACTH and cortisol are pulsatile in critical illness and the pulses are concordant. The elevated serum cortisol in critical illness clearly involves other adrenal sensitizing factors in addition to ACTH. The nadir of cortisol pulses in 35% of the patients dropped below the threshold for deficiency in critical illness as defined by international guidelines, although values shortly before and shortly after would be well above this. This should stop the practice of point testing of cortisol in critical illness. The modelled alteration in the pharmacodynamics of ACTH in terms of cortisol response also calls into question the use of the co-syntropin test to diagnose deficiency, when the test thresholds are not derived from this population(34). A new model of HPA-axis function in critical care is required; encompassing the dynamic pituitary-adrenal interactions, as well as other factors such as inflammatory mediators. Once this has been generated, then we can move forward establishing if CIRCI exists and if so, develop appropriate diagnostics and therapeutic interventions based on that model.

Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Author contributions

BG and FR designed the study, analysed the data and wrote and edited the manuscript.

DMK developed the modelling methodologies, analysed the data and wrote and edited the manuscript.

JE carried out the study and collated the data.

KS performed the laboratory tests and collated the data.

CR, GDA and SLL designed the study and edited the manuscript.

References

1. The Society of Cardiothoracic Surgeons of Great Britain and Ireland. National Adult Cardiothoracic Surgical Database Report. 2017.
2. McIsaac DI, McDonald B, Wong CA, van Walraven C. Long-term survival and resource use in critically ill cardiac surgery patients: a population-based study. *Can J Anaesth.* 2018;65(9):985-995.
3. Waterhouse R. Case of suprarenal apoplexy. *Lancet.* 1911;1:577.
4. Annane D, Pastores SM, Arlt W, Balk RA, Beishuizen A, Briegel J, Carcillo J, Christ-Crain M, Cooper MS, Marik PE, Meduri GU, Olsen KM, Rochweg B, Rodgers SC, Russell JA, Van den Berghe G. Critical Illness-Related Corticosteroid Insufficiency (CIRCI): A Narrative Review from a Multispecialty Task Force of the Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM). *Crit Care Med.* 2017;45(12):2089-2098.
5. Annane D, Pastores SM, Rochweg B, Arlt W, Balk RA, Beishuizen A, Briegel J, Carcillo J, Christ-Crain M, Cooper MS, Marik PE, Umberto Meduri G, Olsen KM, Rodgers SC,

- Russell JA, Van den Berghe G. 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. *Critical Care Medicine*. 2017; 45(12): 2078-2088
6. Walker JJ, Terry JR, Lightman S. Origin of ultradian pulsatility in the hypothalamic-pituitary-adrenal axis. *Proc Biological Sci* 2010;277(1688):1627-1633.
 7. Walker JJ, Terry JR, Tsaneva-Atanasova K, Armstrong SP, McArdle CA, Lightman SL. Encoding and decoding mechanisms of pulsatile hormone secretion. *J Neuroendocrinology*. 2010;22(12):1226-1238.
 8. Veldhuis JD, Iranmanesh A, Lizarralde G, Johnson ML. Amplitude modulation of a burstlike mode of cortisol secretion subserves the circadian glucocorticoid rhythm. *Am J Phys* 1989;257(1 Pt 1):E6-14.
 9. Henley DE, Leendertz JA, Russell GM, Wood SA, Taheri S, Woltersdorf WW, Lightman SL. Development of an automated blood sampling system for use in humans. *J Med Eng Tech*; 2009;33(3):199-208.
 10. Conway-Campbell BL, Sarabdjitsingh RA, McKenna MA, Pooley JR, Kershaw YM, Meijer OC, De Kloet ER, Lightman SL. Glucocorticoid ultradian rhythmicity directs cyclical gene pulsing of the clock gene period 1 in rat hippocampus. *J Neuroendocrinology*. 2010;22(10):1093-1100.
 11. Conway-Campbell B, Birnie M, Flynn B, Kershaw Y, Lightman S. Glucocorticoid pattern-dependent gene regulation in the rat hippocampus. *Endocrine Abstracts*. 2015.

12. Vermes I, Beishuizen A, Hampsink RM, Haanen C. Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. *J Clin Endo Metab*; 1995;80(4):1238-1242.
13. Veldhuis JD, Roelfsema F, Iranmanesh A, Carroll BJ, Keenan DM, Pincus SM. Basal, pulsatile, entropic (patterned), and spiky (staccato-like) properties of ACTH secretion: impact of age, gender, and body mass index. *J Clin Endo Metab*; 2009;94(10):4045-4052.
14. Keenan DM, Chattopadhyay S, Veldhuis JD. Composite model of time-varying appearance and disappearance of neurohormone pulse signals in blood. *J Theo Bio*; 2005;236(3):242-255.
15. Keenan DM, Veldhuis JD. Pulsatility of Hypothalamo-Pituitary Hormones: A Challenge in Quantification. *Physiology*; 2016;31(1):34-50.
16. Keenan DM, Evans WS, Veldhuis JD. Control of LH secretory-burst frequency and interpulse-interval regularity in women. *Am J Phys: Endocrinology and metabolism*. 2003;285(5):E938-948.
17. Gibbison B, Keenan DM, Roelfsema F. Hysteresis models for cortisol and ACTH in the critically ill. Available at: 10.6084/m9.figshare.9992324.v1
18. Keenan DM, Roelfsema F, Veldhuis JD. Dose-response downregulation within the span of single interpulse intervals. *Am J Phys: Regulatory, integrative and comparative physiology*. 2010;299(1):R11-18.
19. Gibbison B, Keenan DM, Roelfsema F. 24 hour cortisol and ACTH profiles in patients with critical illness and control. Available at: 10.6084/m9.figshare.9933902.v1

20. Gibbison B, López-López JA, Higgins JPT, Miller T, Angelini GD, Lightman SL, Annane D. Corticosteroids in septic shock: a systematic review and network meta-analysis. *Crit Care*; 2017;21(1):78.
21. Lightman SL, Conway-Campbell BL. The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration. *Nat Rev Neuroscience*. 2010;11(10):710-718.
22. Henley DE, Russell GM, Douthwaite JA, Wood SA, Buchanan F, Gibson R, Woltersdorf WW, Catterall JR, Lightman SL. Hypothalamic-pituitary-adrenal axis activation in obstructive sleep apnea: the effect of continuous positive airway pressure therapy. *J Clin Endo Metab*. 2009;94(11):4234-4242.
23. Gibbison B, Spiga F, Walker JJ, Russell GM, Stevenson K, Kershaw Y, Zhao Z, Henley D, Angelini GD, Lightman SL. Dynamic pituitary-adrenal interactions in response to cardiac surgery. *Crit Care Med*. 2015;43(4):791-800.
24. Boonen E, Bornstein SR, Van den Berghe G. New insights into the controversy of adrenal function during critical illness. *Lancet Diabetes Endocrinol*. 2015;3(10):805-815.
25. Powell B, Nason GP, Angelini GD, Lightman SL, Gibbison B. Optimal Sampling Frequency of Serum Cortisol Concentrations After Cardiac Surgery. *Crit Care Med* 2017;45(10):e1103-e1104.
26. Galbois A, Rudler M, Massard J, Fulla Y, Bennani A, Bonnefont-Rousselot D, Thibault V, Reigner S, Bourrier A, Poynard T, Thabut D. Assessment of adrenal function in cirrhotic patients: salivary cortisol should be preferred. *J Hepatol*. 2010;52(6):839-845.

27. Kraan GP, Dullaart RP, Pratt JJ, Wolthers BG, Drayer NM, De Bruin R. The daily cortisol production reinvestigated in healthy men. The serum and urinary cortisol production rates are not significantly different. *J Clin Endocrinol Metab.* 1998;83(4):1247-1252.
28. Chan KCA, Lit LCW, Law ELK, Tai MHL, Yung CU, Chan MHM, Lam CWK. Diminished Urinary Free Cortisol Excretion in Patients with Moderate and Severe Renal Impairment. *Clin Chem* 2004;50(4):757-759.
29. Walker JJ, Spiga F, Waite E, Zhao Z, Kershaw Y, Terry JR, Lightman SL. The origin of glucocorticoid hormone oscillations. *PLoS Biology.* 2012;10(6):e1001341.
30. Chrousos GP. The Hypothalamic–Pituitary–Adrenal Axis and Immune-Mediated Inflammation. *N Eng J Med.* 1995;332(20):1351-1363.
31. Edwards AV, Jones CT. The effect of splanchnic nerve stimulation on adrenocortical activity in conscious calves. *J Phys.* 1987;382:385-396.
32. Edwards AV, Jones CT. The effect of splanchnic nerve section on the sensitivity of the adrenal cortex to adrenocorticotrophin in the calf. *J Phys.* 1987;390:23-31.
33. Engeland WC, Gann DS. Splanchnic nerve stimulation modulates steroid secretion in hypophysectomized dogs. *Neuroendocrinology.* 1989;50(2):124-131.
34. Harris MJ, Baker RT, McRoberts JW, Mohler JL. The adrenal response to trauma, operation and cosyntropin stimulation. *Surg Gyn Obs;* 1990;170(6):513-516.
35. Boonen E, Meersseman P, Vervenne H, Meyfroidt G, Guiza F, Wouters PJ, Veldhuis JD, Van den Berghe G. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *J Clin Endo Metab.* 2014;306(8):E883-892.
36. Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden YM, Spriet I, Wouters PJ, Vander Perre S, Langouche L, Vanhorebeek

- I, Walker BR, Van den Berghe G. Reduced Cortisol Metabolism during Critical Illness. *N Eng J Med*; 2013; 368:1477-1488
37. Boonen E, Van den Berghe G. Understanding the HPA response to critical illness: novel insights with clinical implications. *Int Care Med* 2015;41(1):131-133.
38. McKenna HT, Reiss IK, Martin DS. The significance of circadian rhythms and dysrhythmias in critical illness. *Journal of the Intensive Care Society*. 2017;18(2):121-129.
39. Foster RG, Hankins MW. Circadian vision. *Curr Bio*; 2007;17(17):R746-751.
40. Verceles AC, Silhan L, Terrin M, Netzer G, Shanholtz C, Scharf SM. Circadian rhythm disruption in severe sepsis: the effect of ambient light on urinary 6-sulfatoxymelatonin secretion. *Int Care Med* 2012;38(5):804-810.
41. Turner PL, Van Someren EJW, Mainster MA. The role of environmental light in sleep and health: effects of ocular aging and cataract surgery. *Sleep Medicine Revs*. 2010;14(4):269-280.
42. Veldhuis JD, Pincus SM, Keenan DM. Noninvasive in vivo investigative probes of adaptive mechanisms of neuroendocrine regulation: the paradigm of the hypothalamo-pituitary-gonadal axis in the aging male. *J Endocrinol Invest* 2003;26(7 Suppl):2-14.
43. Dodt C, Theine KJ, Uthgenannt D, Born J, Fehm HL. Basal secretory activity of the hypothalamo-pituitary-adrenocortical axis is enhanced in healthy elderly. An assessment during undisturbed night-time sleep. *Eur J Endo* 1994;131(5):443-450.
44. Roelfsema F, van Heemst D, Iranmanesh A, Takahashi P, Yang R, Veldhuis JD. Impact of age, sex and body mass index on cortisol secretion in 143 healthy adults. *Endocrine Connections*. 2017;6(7):500-509.

45. Lindsay JR, Shanmugam VK, Oldfield EH, Remaley AT, Nieman LK. A comparison of immunometric and radioimmunoassay measurement of ACTH for the differential diagnosis of Cushing's syndrome. *J Endocrinol Invest*. 2006;29(11):983-988.
46. Deutschbein T, Broecker-Preuss M, Hartmann MF, Althoff R, Wudy SA, Mann K, Petersenn S. Measurement of urinary free cortisol by current immunoassays: need for sex-dependent reference ranges to define hypercortisolism. *Horm Metab Res*. 2011;43(10):714-719.
47. Bellomo R, McGrath B, Boyce N. Effect of continuous venovenous hemofiltration with dialysis on hormone and catecholamine clearance in critically ill patients with acute renal failure. *Crit Care Med*. 1994;22(5):833-837.
48. Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y. Corticosteroids for treating sepsis. *Cochrane Database of Syst Rev*. 2015;12(12):CD002243.
49. Russell GM, Durant C, Ataya A, Papastathi C, Bhake R, Woltersdorf W, Lightman S. Subcutaneous pulsatile glucocorticoid replacement therapy. *Clin Endo* 2014;81(2):289-293.
50. Bhake RC, Leendertz JA, Linthorst ACE, Lightman SL. Automated 24-hours sampling of subcutaneous tissue free cortisol in humans. *J Med Eng Tech* 2013;37(3):180-184.
51. Bhake R, Russell GM, Kershaw Y, Stevens K, Zaccardi F, Warburton VEC, Linthorst ACE, Lightman SL. Continuous Free Cortisol Profiles in Healthy Men - Validation of Microdialysis Method. *J Clin Endocrinol Metab*. 2019.

Figure Legends

Fig 1. Graphs to show how the pulse detection algorithm was applied to individual hormone profiles (ACTH – a and b, Cortisol – c and d). The pulse detection algorithm begins with all of

the concentration profile local minima (red asterisks panel a. second row). It then progressively and selectively smooths the profile, with the degree of smoothing being inversely related to its rate of increase. Panel a, row 3 displays the smoothed profile after 3000 algorithmic steps (red asterisks are the corresponding remaining putative pulse times). Panel a, 4th row displays the potential pulse time sets (red asterisks) as the algorithm proceeds. Hence, putative pulse times are progressively removed. The number of pulse times is therefore between 21 pulses (panel a, row 3) and 10 pulses (panel a, 4th row). The estimation algorithm then proceeds by estimating the secretory and elimination parameters for each potential pulse set (each horizontal collection of pulse times), with the number of such pulses being a penalizing term in penalized maximum likelihood estimation (PMLE). **Fig 1b** shows the result of the estimation. In the top row, the observed concentration profile (solid blue) and its fitted profile (dashed red) are displayed, as well as the eleven (red asterisks) estimate pulses from the PMLE. The 2nd row is the resulting estimated time-evolving secretion rate. The 3rd row are the two estimated waveforms of mass release (night - red, day - blue). Statistics for the pattern of pulsing, based upon the estimated pulse times are calculated under the assumption that the pulse time process is a Weibull renewal process, a generalization that includes the Poisson process as a special case. The Weibull model describes the inter-pulse interval lengths, allowing highly regular, as well as highly irregular pulsing patterns. The estimates summarize the resulting observed pulse time pattern. In the 4th row of panel b is the estimated Weibull distribution for the interpulse intervals (observed interpulse = red asterisks). The analogous results for the cortisol profile for CI subject 1 are given in Figure 1c-d.

Fig 2. Schematic of the four models used for the ACTH-cortisol dose-response calculation.

Fig 3. Example of how the dose-response models for ACTH – cortisol interaction were applied to one individual. In Fig. 3a(CI Subject 1) the top row is their estimated cortisol secretion rate, with the 2nd row consisting of the fitted ACTH concentration profile (red line) and the resulting ACTH feedforward signal ($F_A(t)$) - green dashed line) for which the time-varying forward shifting has occurred, as a result of the ACTH and cortisol pulse times alignment. On the time axis, the ACTH (asterix) and cortisol (diamonds) are plotted to elucidate the procedure. In the bottom row of Fig 3a, all three of the above curves are superimposed to visualize the alignment of the ACTH feedforward signal and the cortisol secretion rate. In Fig 3b, the four estimated dose response models are displayed. For the three hysteresis models (shift in either Potency, Sensitivity or Efficacy), there is a pulse-by-pulse random shift in the dose-response before hysteresis (blue dashed curves) and the corresponding plots after hysteresis (red dashed) curves; the solid blue and red curves are the mean pre- and post-hysteresis dose-response functions. For the Non-Hysteresis (NH) model, there is clearly only one set of pulse-by-pulse curves. In Fig 3c, the fit (red dashed curve) of result of the four dose-response models to CI subject 1's cortisol secretion rate (blue solid curve) is shown. The Non-Hysteresis model does not capture the dynamic nature of cortisol, whereas the three hysteresis models do.

Fig 4. 24-hour ACTH and cortisol profiles of one critically ill patient (top) and one healthy volunteer (bottom). Time points are every 10 minutes starting from 0800 in the critically ill patient and 0900 in the healthy volunteer. Note that the ACTH scale in the critically ill patient is smaller to show the ACTH – cortisol interaction.

Fig 5. Summary diagram of the four models used to test the ACTH induced stimulation of cortisol secretion. In each subplot, the values for each subject are displayed as black asterisks and the sample means are linearly connected (Red). A \pm SEM is plotted about the mean (Red). **6a-d.** The Four Models are: No Hysteresis (**6a**); Hysteresis with a Potency shift (**6b**); Hysteresis with a Sensitivity shift (**6c**); and Hysteresis with an Efficacy shift (**6d**). Two statistical tests of a difference in their means were applied: Wilcoxon Ranksum test and the two-sample T test - when P-values are given, they are given in that order and are two-sided.

Fig 6. Graphs to show the loss of circadian rhythm in critically ill patients of ACTH and cortisol. **a.** The ACTH pulse times for each subject (horizontal rows) for the critically ill (Top) and healthy (Bottom) groups. **b.** The cortisol pulse times for each subject (horizontal rows) for the critically ill (Top) and healthy (Bottom) groups. All pulse times, in all subjects, are displayed on the baseline. **c and d.** The sampled rate functions for the critically ill (Left) and healthy volunteers (Middle) are plotted in (c) ACTH and (d) Cortisol. The panels on the right of (c) and (d) show the probability density for the loss of circadian rhythm for ACTH (c) and cortisol (d). Median, lower quartile (Q1) and upper quartile (Q3) values are stated for each.

Table 1. Demographics of Critically Ill patients and Healthy Volunteers

Demography	Critically Ill (n = 20)	Healthy (n = 19)	P-value
Age Years Mean +/-SD	63.9 +/-13.7	43.0 +/-11.6	<0.001
Body Mass Index kg/m ² Mean +/-SD	29.6 +/-5.4	25.5 +/-3.2	0.003

Table 2. Post-operative characteristics of critically ill patients.

Post-operative details of patients	
Mechanical Ventilation	20 (100)
N (%)	
Inotropes / Vasopressors	19 (95)
N (%)	
Norepinephrine	19 (95)
N (%)	
Vasopressin	15 (75)
N (%)	
Dobutamine	12 (60)
N (%)	
Adrenaline	4 (20)
N (%)	
Enoximone / Milrinone	11 (55)
N (%)	
Levosimendan	2 (10)
N (%)	
Renal Replacement Therapy	8 (40)
N (%)	
Operation to 1 st Sample interval	3 (2-13)
Days Median (range)	
ICU Length of Stay	18 (5 – 37)
Days Median (range)	
Acute Hospital Length of Stay	24 (7 – 44)
Days Median (range)	
Sequential Organ Failure (SOFA) Score	9 (7-10)
Median (IQR)	
At sampling start	
Albumin at sampling start	24.2 (5.0)
g/dl Mean (SD)	
Albumin at sampling end	22.45 (4.3)
g/dl Mean (SD)	
Cortisol Binding Globulin at sampling start	26.9 (12.4)
mcg/ml Mean (SD)	

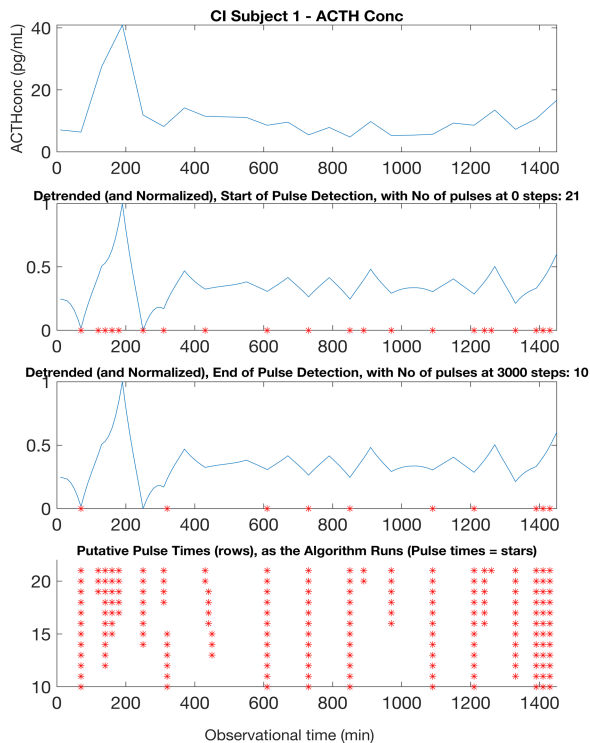
Cortisol Binding Globulin at sampling end mcg/ml Mean (SD)	28.1 (13.7)
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Table 3. Summary statistics for dynamic hormone secretion of ACTH and cortisol.

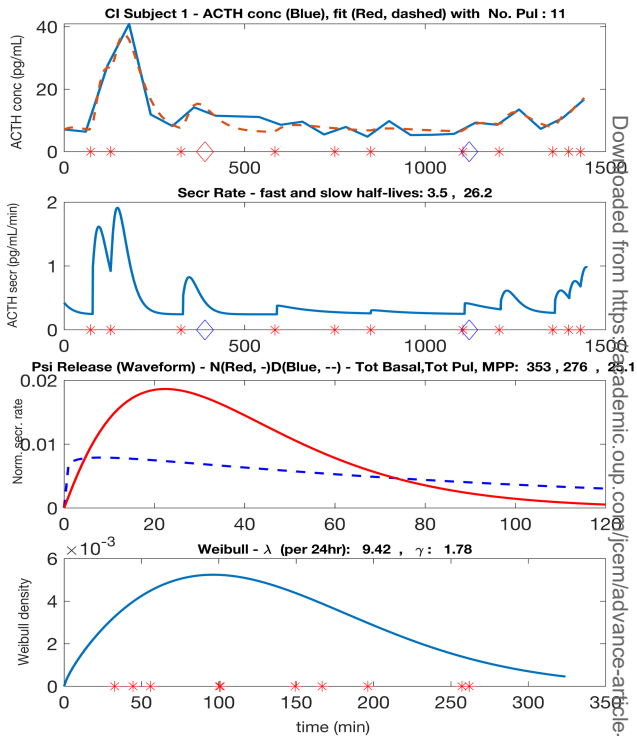
Hormone Secretion Characteristic	ACTH			Cortisol		
	Critically Ill	Healthy Volunteer	p-value	Critically Ill	Healthy Volunteer	p-value
Pulse Frequency Mean (SD)	12.4 (2.31) pulses / 24hrs	13.08 (2.05) pulses / 24hrs	0.38	14.82 (4.65) pulses / 24hrs	15.23 (2.82) pulses / 24hrs	0.16
Pulse Regularity Mean (SD)	1.3 (0.2)	1.43 (0.28)	0.27	1.80 (0.54)	1.91 (0.41)	0.12
Total Secretion Mean (SD)	1036.4 (737.6) pg/ml /24hrs	1502.3 (1152.2) pg/ml /24hrs	0.2	14447.0 (5709.3) nmol/L/24hrs	5915.5 (1686.7) nmol/L/24hrs	<0.0001
Pulsatile Secretion Mean (SD)	589.3 (479.15) pg/ml /24hrs	770.42 (579.39) pg/ml /24hrs	0.35	5193.1 (3018.5) nmol/L/24hrs	4955.1 (1753.6) nmol/L/24hrs	0.43
Basal Secretion Mean (SD)	447.2 (371.5) pg/ml /24hrs	731.85 (650.57) pg/ml /24hrs	0.08	9253.4 (3348.8) nmol/L/24hrs	960. (589.0) nmol/L/24hrs	<0.0001
Mass Per pulse Mean (SD)	45.0 (37.5) pg/ml /24hrs/pulse	60.01 (55.38) pg/ml /24hrs/pulse	0.34	316.4 (123.8) nmol/L /24hrs/pulse	300.6 (107.1) nmol/L /24hrs/pulse	0.30

ACTH and Cortisol Secretion and Parameter Estimation

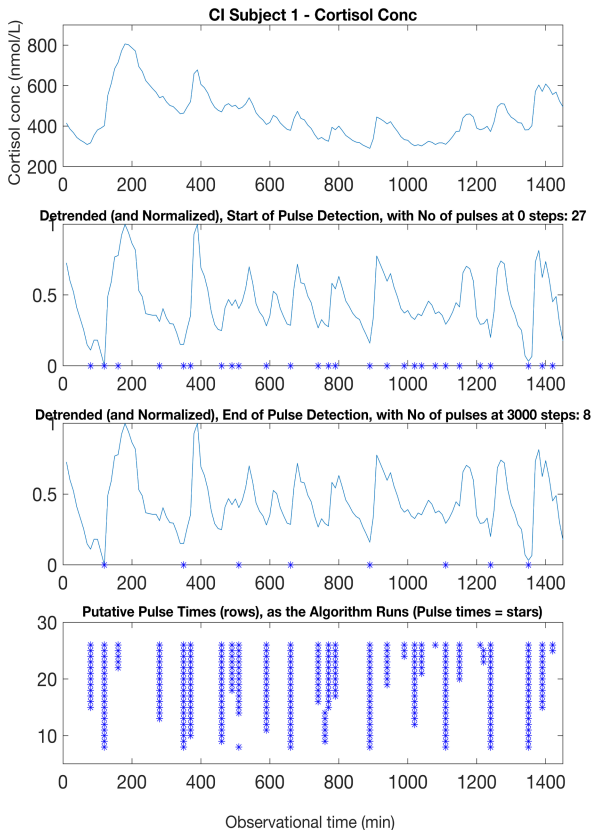
a.



b.



c.



d.

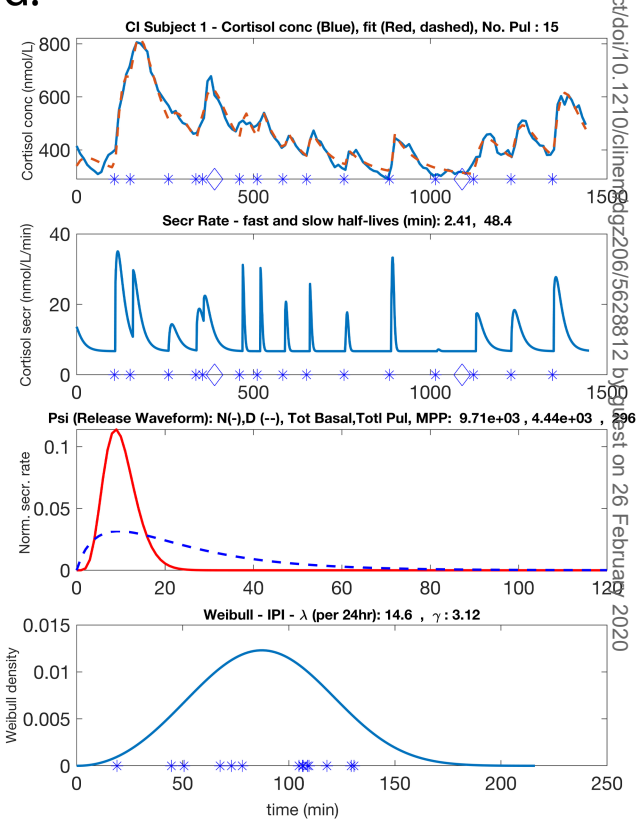
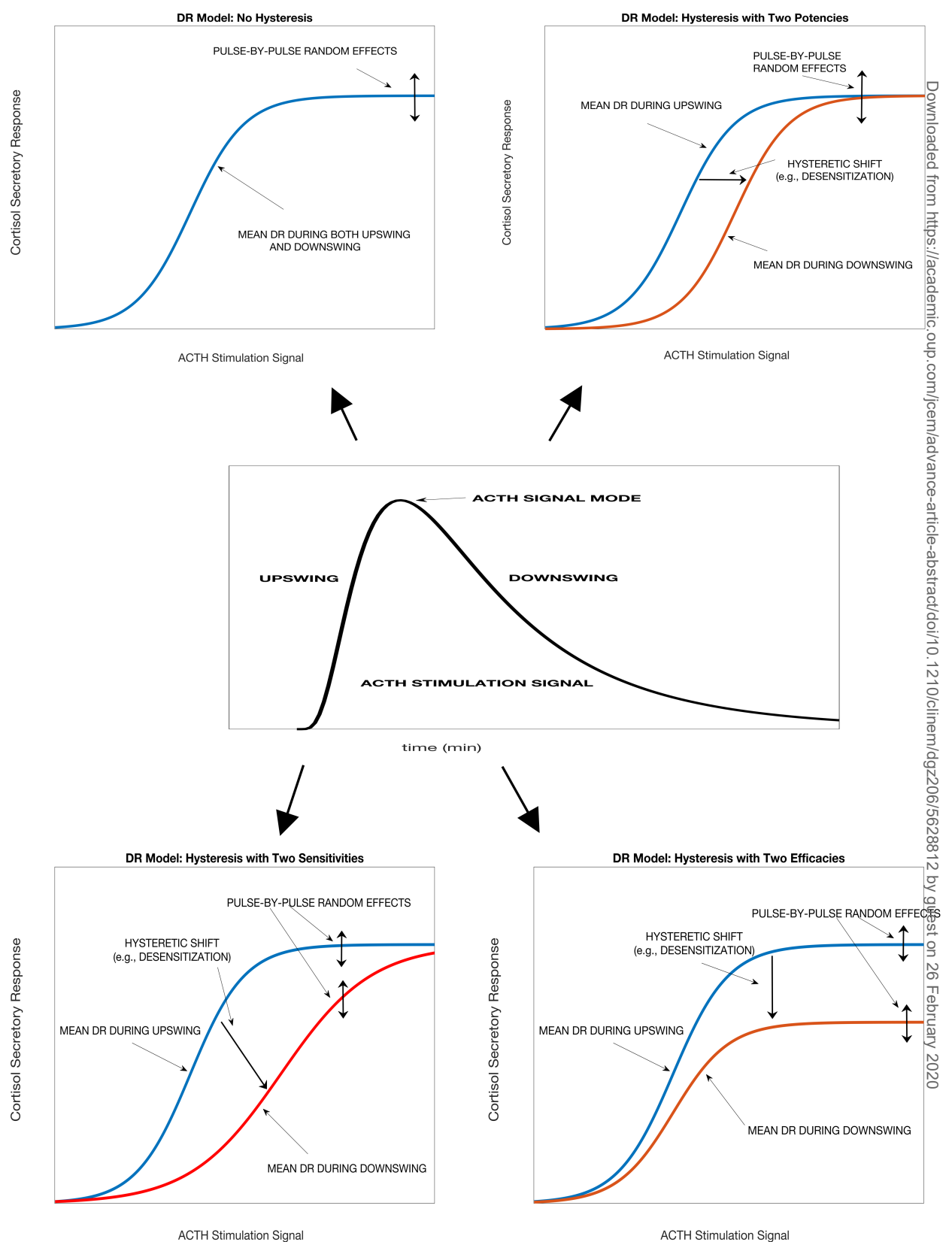
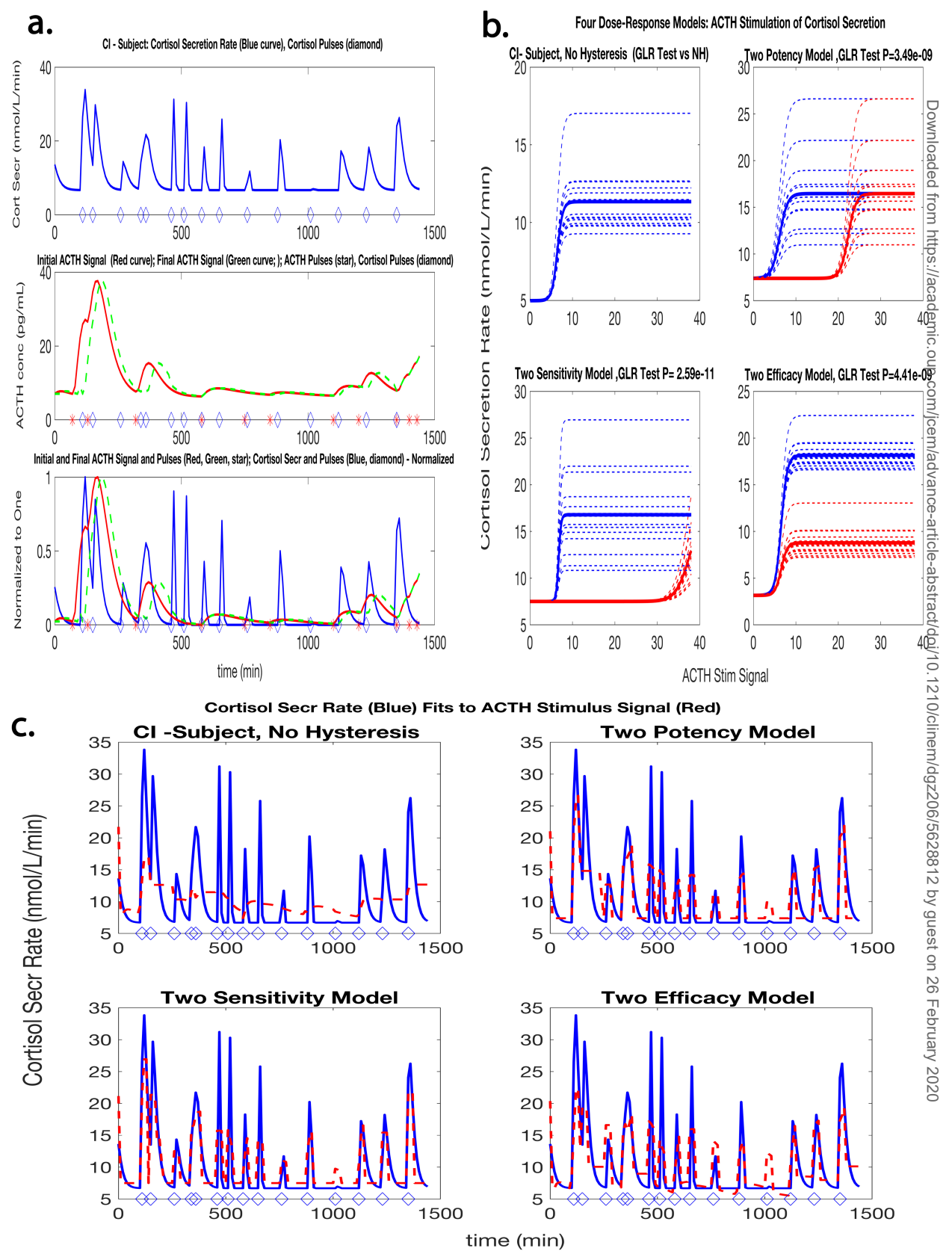


Figure 2 **Four DR Models - Cortisol Secretion In Response to ACTH Stimulation Signal**



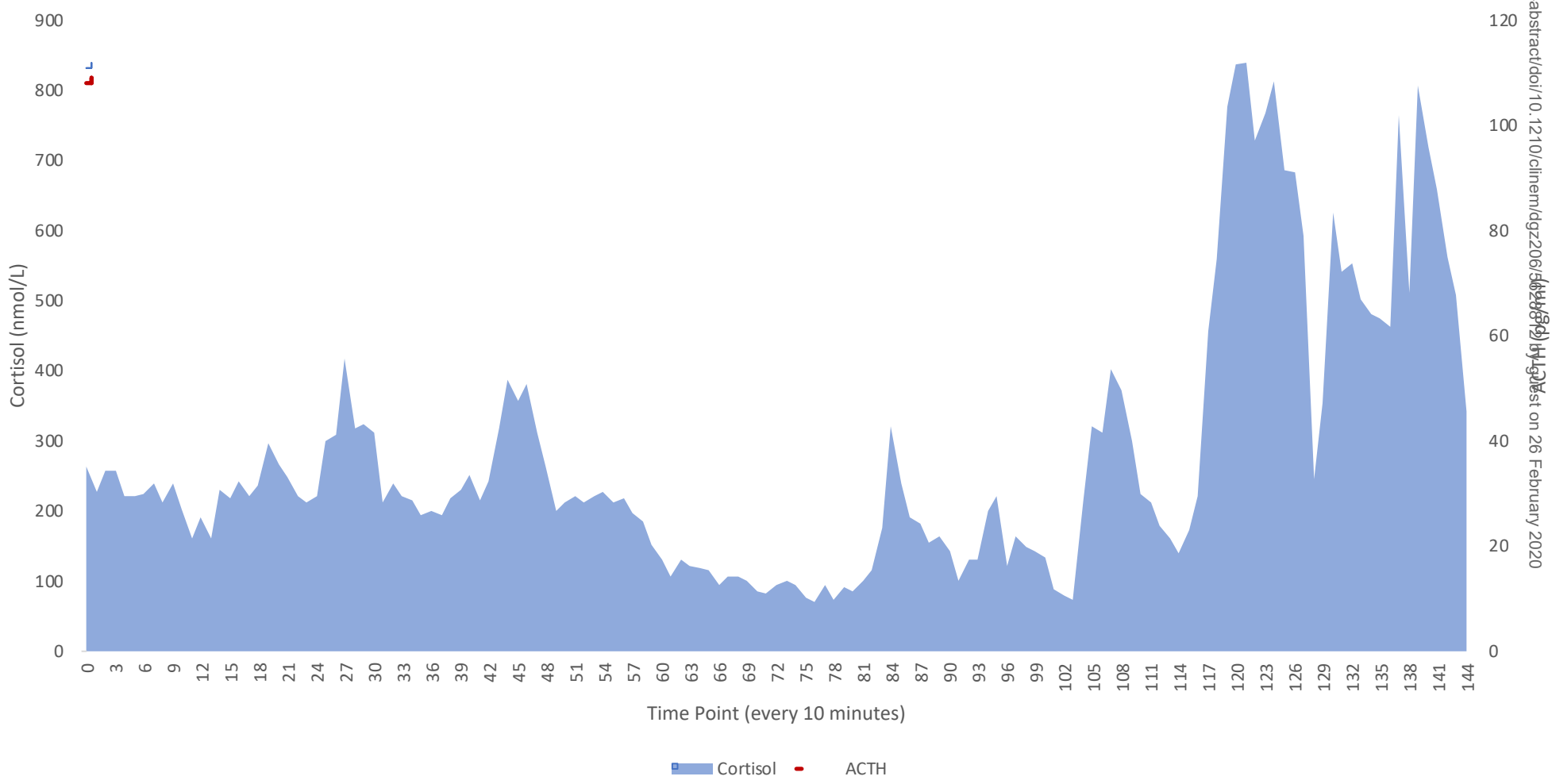
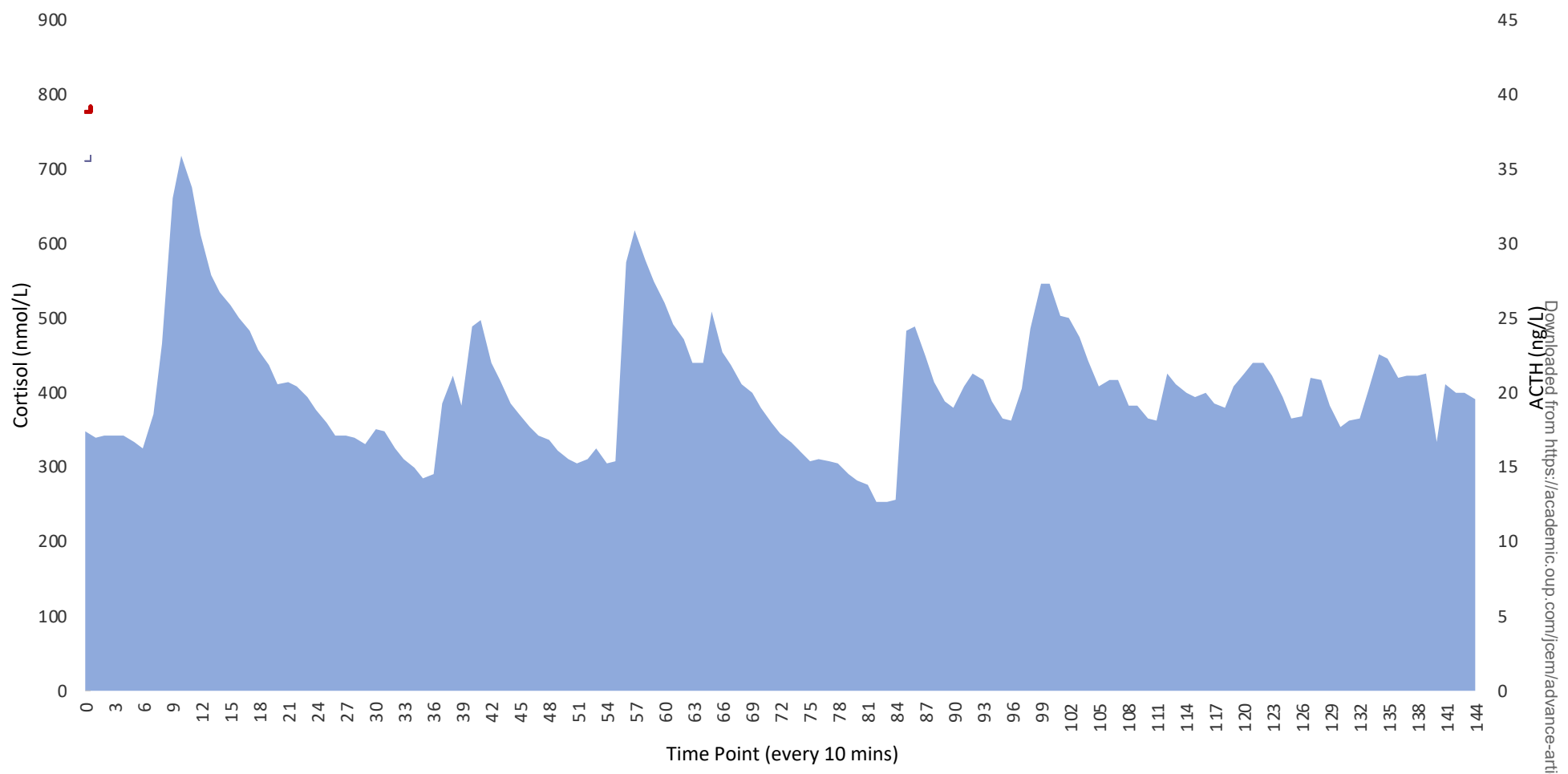
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Figure 3
CI - Subject 1 - Four DR Models - ACTH Stimulation Signal on Cortisol Secretion



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Figure 4



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Cortisol ACTH

ACTH Stimulation of Cortisol Secretion - Four DR Models

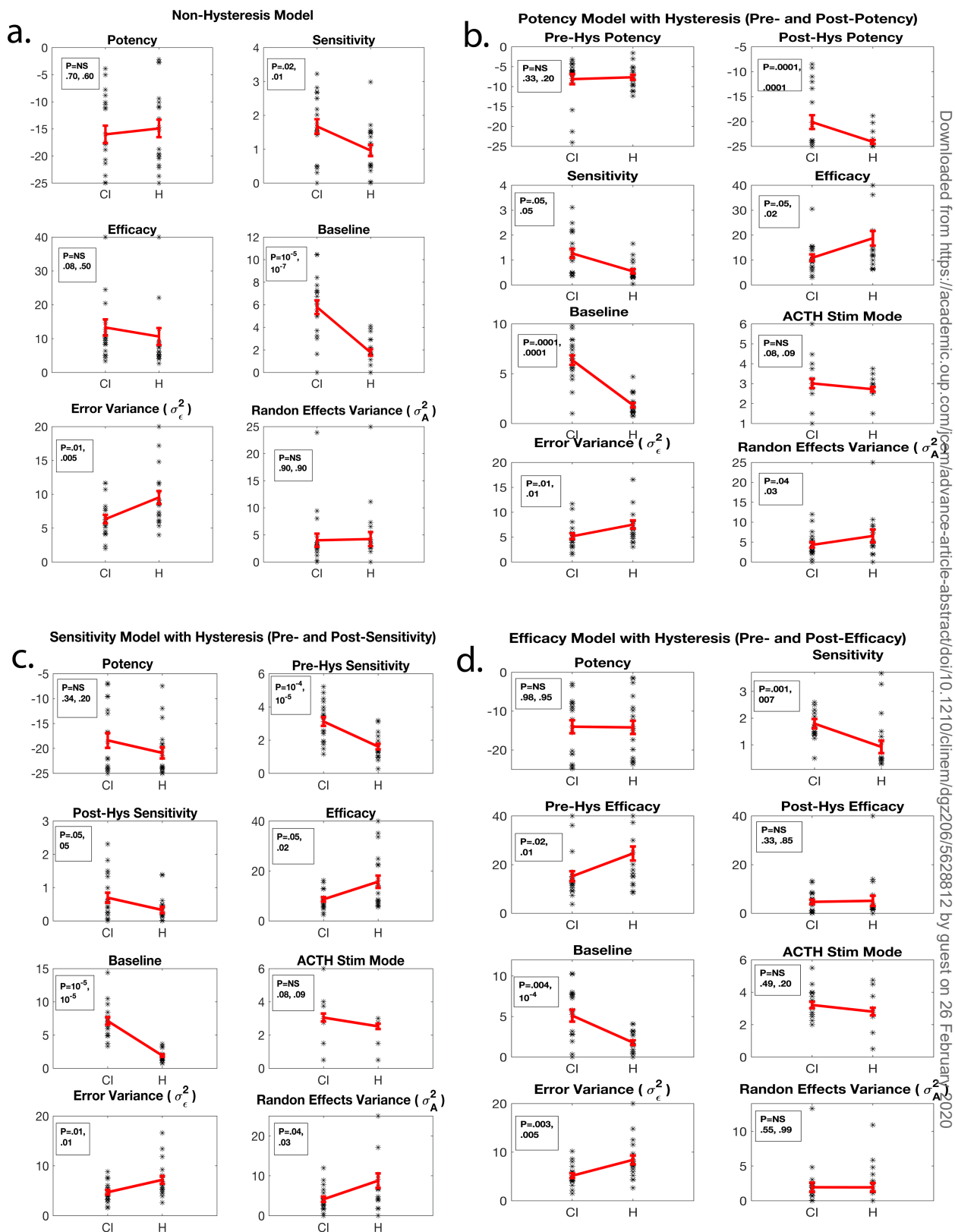
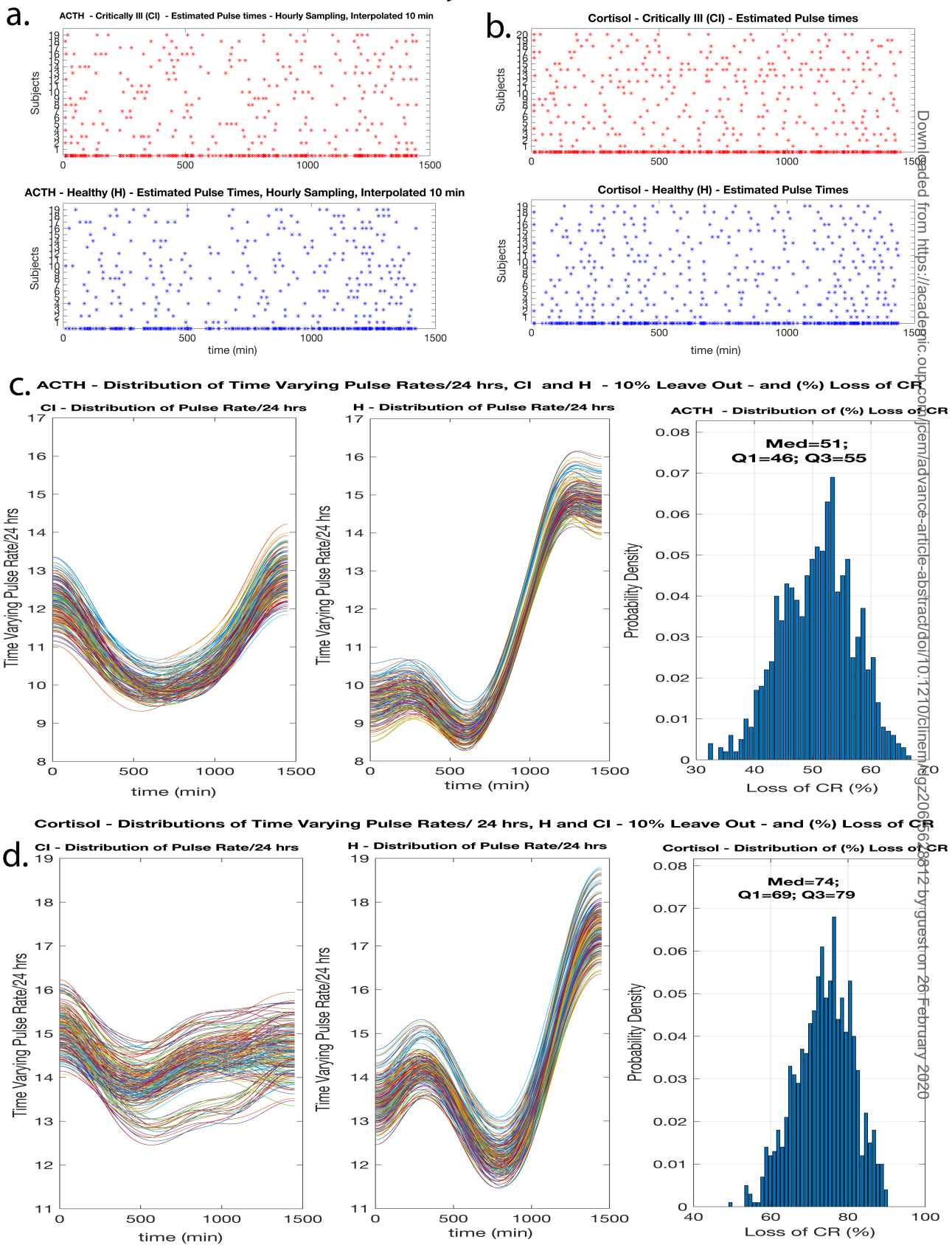


Figure 6

Loss of Circadian Rhythm - ACTH and Cortisol



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