KU Leuven
Biomedical Sciences Group
Faculty of Medicine
Department of Cellular and Molecular Medicine

HPA AXIS ALTERATIONS IN CRITICALLY ILL PATIENTS

Bram PEETERS

Jury:

Promoter:	Prof. dr. Greet Van den Berghe
Co-promoter:	Prof. dr. Lies Langouche
Chair Examining Committee:	Prof. dr. Kathleen Freson
Chair Public Defense:	Prof. dr. Joost Schymkowitz
Jury members	Prof. dr. Brigitte Decallone
	Prof. dr. Steffen Rex
	Prof. dr. Mirjam Christ-Crain
	Prof. dr. Manu Malbrain

Dissertation presented in partial fulfilment of the requirements for the degree of Doctor in Biomedical Sciences

To all critically ill and healthy individuals

who altruistically contributed

to the benefit of future patients

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Acetyl CoA	Acetyl Coenzyme A
ACTH	Adrenocorticotropic Hormone
Allo-THE	5α-tetrahydrocortisone
Allo-THF	5α-tetrahydrocortisol
ANOVA	Analysis Of Variance
APACHE	Acute Physiology and Chronic Health Assessment Evaluation
ARDS	Acute Respiratory Distress Syndrome
AUC	Area under the ROC Curve
AVP	Arginine Vasopressin
BMI	Body Mass Index
cAMP	cyclic Adenosine Monophosphate
CBG	Corticosteroid-Binding Globulin
CI	Critical Illness
CIRCI	Critical Illness-Related Corticosteroid Insufficiency
COPD	Chronic Obstructive Pulmonary Disease
CRH	Corticotropin-Releasing Hormone
CYP11A1	Cytochrome P450 family 11, subfamily A, polypeptide 1
d	Day
DAMPS	Damage-Associated Molecular Patterns
E	Cortisone
EDTA	Ethylenediaminetetraacetic Acid
eGFR	estimated Glomerular Filtration Rate

EPaNIC	Early Parenteral Nutrition Completing Enteral Nutrition in Adult Critically Ill Patients
F	Cortisol
GABA	Gamma-Aminobutyric Acid
GR	Glucocorticoid Receptor
GH	Growth Hormone
h	hour
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
HPA	Hypothalamus-Pituitary-Adrenal
HSD	Hydroxysteroid Dehydrogenase
ICU	Intensive Care Unit
IGF-1	Insulin-like Growth Factor 1
IL	Interleukin
IQR	Interquartile Range
IU	International Unit
LC-MS/MS	Liquid-Chromatography-tandem Mass-Spectrometry
LDL	Low Density Lipoprotein
MAC	Minimum Alveolar Concentration
MC2R	Melanocortin type 2 Receptor
MR	Mineralocorticoid Receptor
mRNA	messenger Ribonucleic Acid
NE	Norepinephrine
NRS	Nutritional Risk Screening
PAMPS	Pathogen-Associated Molecular Patterns
PKA	Protein Kinase A
POMC	Pro-Opiomelanocortin
PVN	Paraventricular Nucleus
PN	Parenteral Nutrition
RCT	Randomized Controlled Trial

SCN	Suprachiasmatic Nucleus
SD	Standard Deviation
SEM	Standard Error of the Mean
SIRS	Systemic Inflammatory Response Syndrome
THE	5β-tetrahydrocortisone
THF	5β-tetrahydrocortisol
TNFα	Tumor Necrosis Factor alpha
TSH	Thyroid-Stimulating Hormone
STAR	Steroidogenic Acute Regulatory Protein
SCARB1	Scavenger-Receptor class B member 1
V1b-receptor	Vasopressin 1b-receptor
Vif	Variance inflation factor
w	week

1

GENERAL INTRODUCTION

Adapted from:

- Peeters B, Boonen E, Langouche L, Van den Berghe G. The HPA axis response to critical illness: New study results with diagnostic and therapeutic implications. Mol Cell Endocrinol 2015; 408: 235-40.

- Peeters B, Langouche L, Van den Berghe G. Adrenocortical Stress Response during the Course of Critical Illness. Compr Physiol 2017; 8: 283-98.

Acute and prolonged critical illness

Critical illness represents any condition, evoked by major surgery, severe medical illnesses, or multiple trauma, that requires pharmacological and/or mechanical support of vital organ functions without which death would ensue. Sepsis, defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection, remains the major cause of death in critically ill patients. In sepsis, pathogen-associated molecular patterns (PAMPS), such as bacterial exotoxins, cause direct cellular damage and can trigger the immune response. This often leads to excessive cytokine production and tissue damage that releases damage-associated molecular patterns (DAMPS), such as activated complement, heat shock proteins, ATP, into the bloodstream, causing further organ injury.[1] Also, transcapillary flow (albumin leakage and risk of tissue edema) is increased with a persistent state of global increased permeability syndrome and ongoing fluid accumulation with new onset of organ failure.[2] Most patients admitted to the ICU only require a few days of intensive care, but about 25% of ICU patients receive vital organ support for a much longer period. This stage of prolonged critical illness is characterized by ongoing mechanical and pharmacological vital organ support with increased risk of further deterioration of organ failure and a higher risk of death. Indeed, a recent US population-based cohort study indicated an in-hospital mortality of 31% for patients with an ICU stay of at least 8 days.[3] Also, during critical illness, there is an early activation of innate immunity and suppression of adaptive immunity.[4] In the chronic phase, a failure of both innate and adaptive immune system with an immunosuppressive state leading to death has been described, but persistent activation of the innate immunity resulting in organ injury has also been suggested.[4] The exact timing of the transition from acute to chronic critical illness is however not clear, neither at the patient level, nor at the population level. A recent study defined this onset as the time at which severity of illness on admission was no longer predictive of mortality, which was after about 10 days.[5]

As such, critical illness is a condition of severe and sustained physical stress for the human body for which an adequate activation of several processes is required to provide necessary energy, to modulate the immune response, and to ensure hemodynamic homeostasis. The term 'stress response' indicates the combination of these closely interrelated physiological reactions to stress to maintain and restore homeostasis in the human body.[6] Both neuronal and endocrine systems are involved, among which the

activation of the sympathetic nervous system, the release of catecholamines from the adrenal medulla, and the activation of the hypothalamus-pituitary-adrenal (HPA) axis to increase the availability of the stress hormone cortisol.[7,8] Most hypothalamus-pituitary-peripheral-hormonal axes that play a key role in the metabolic and immunological alterations accompanying critical illness typically follow a biphasic response pattern (**Figure 1**).[9-14] For example, in the acute phase of critical illness, plasma concentrations of the anterior pituitary hormones growth hormone (GH) and thyroid-stimulating hormone (TSH) are increased, whereas plasma concentrations of their peripheral effector hormones IGF-1 and T_3 are decreased. However, when ICU-dependency continues beyond the acute time window, these pituitary hormones are typically suppressed, with a further decrease of their peripheral hormones. Whereas the acute changes can be interpreted as beneficial, bringing about the release of endogenous fatty acids and glucose into the circulation and postponing energy consuming anabolism, the uniform suppression in the prolonged phase of critical illness likely

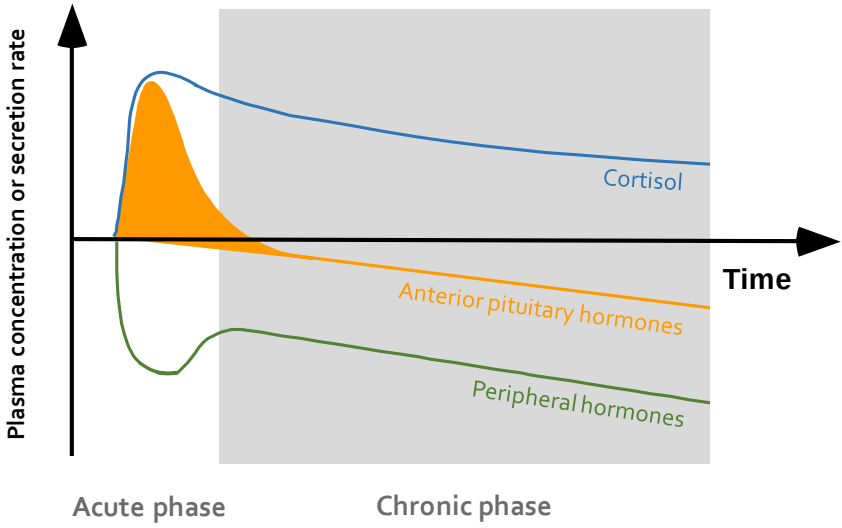


Figure 1 | Biphasic neuroendocrine responses of the anterior pituitary hormones and their peripheral hormones to acute and chronic critical illness
In the acute phase of illness the growth hormone (GH) and thyrotrophin (TSH) secretory activity is amplified (orange), and adrenocorticotropic hormone (ACTH) secretory activity is increased in some cases. Plasma concentrations of their anabolic peripheral hormones (insulin-like growth factor-I, triiodothyronine) are decreased (green), but cortisol levels are elevated (blue). In prolonged critical illness, secretion of GH, TSH and ACTH is consistently suppressed, with a further decrease of their peripheral hormones. Plasma cortisol levels remain high, but in some cases low plasma cortisol levels appear in the chronic phase of critical illness. (A*dapted from* [10])

participates in the general wasting syndrome, with persisting hypercatabolism, causing weakness and delayed or non-recovery from intensive care-dependency.[9,15]

Such bi-phasic response also applies to the HPA axis, a crucial axis in terms of acute survival. However, the anterior pituitary hormone ACTH is only very transiently elevated, and several studies have reported lower than normal plasma ACTH from quite early after admission to the ICU throughout the ICU stay. This suppression of plasma ACTH occurs while high levels of plasma cortisol are consistently observed in most ICU patients, both in the acute and the prolonged phase of critical illness (**Figure 1**). This phenomenon of low ACTH together with high cortisol is referred to as 'ACTH-cortisol dissociation'. Whether or not this dissociation points to, or can lead to, a dysfunctional HPA axis, which would warrant treatment, can only be interpreted if one correctly understands the pathophysiology of the HPA axis response during critical illness.

The adrenocortical stress response to critical illness

The normal stress response

When the human brain senses a stressful event, it signals the paraventricular nucleus (PVN) of the hypothalamus to release corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which both activate the anterior pituitary gland to release ACTH (**Figure 2**). In turn, ACTH release exerts important dose-dependent functions on the cortex of

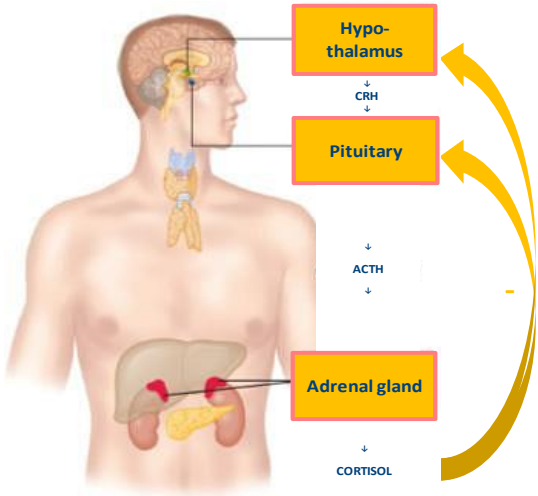


Figure 2 | The normal HPA axis regulation during health
During stress, the stress response comprises an activation of the hypothalamus-pituitary-adrenal (HPA) axis, which brings about a rise in corticotropin-releasing hormone (CRH) in the hypothalamic - hypophyseal portal system and in plasma adrenocorticotropic hormone (ACTH) and cortisol. Cortisol evokes feed-back inhibition to fine-tune its own release. (*Adapted from © 2018 Kaiser Foundation Health Plan, Inc)*

the adrenal gland to ensure immediate cortisol release into the bloodstream. Together with this activation of the HPA axis, the sympathetic nervous system is stimulated simultaneously, with a release of predominantly norepinephrine from postganglionic sympathetic nerve fibers and predominantly epinephrine from the medulla of the adrenal gland. Because cortisol secretion first requires *de novo* synthesis from cholesterol, cortisol release consequently lags behind the catecholamine secretion by several minutes during the onset of the stress response.[16] The first line response to stress is thus mediated by the effects of (nor)epinephrine, facilitating immediate physical reactions, such as an increase in blood glucose and fatty acids, an improvement of the respiratory function, and an increase in heart rate and cardiac output. However, apart from their hemodynamic effects, catecholamines can aggravate hypermetabolism and increase hyperlactatemia, and further increase oxygen demands, which can contribute to further organ damage.[17] Subsequently, the effects of increased cortisol availability become apparent, via fluid retention and increased vasopressor effects of catecholamines, via further fostering energy provision by stimulating liver gluconeogenesis, and through dampening of inflammation. In addition, studies have shown that stimulation of the sympathetic nervous system and the HPA axis synergistically interact with each other and are functionally interdependent.[18]

ACTH-cortisol dissociation during critical illness

The hypothalamic CRH release in the hypothalamic-hypophyseal portal circulation is the first step in the HPA stress response which drives the pituitary, but to our knowledge, no data on portal or systemic circulatory CRH concentrations in critically ill patients have been reported. For obvious reasons, portal veins are not accessible in critically ill patients, and CRH is rarely measured in peripheral blood, as these plasma levels are much lower than and do not correlate well with those in portal plasma and do not reflect hypothalamic activity.[19]

Also published data on circulating ACTH concentrations in the critically ill are scarce, probably explained by the cumbersome way in which blood samples should be collected (on ice) and processed (spun cold prior to assay).[20] In burn patients, plasma ACTH levels were found not to be elevated and did not show a correlation with burn size.[21] In patients undergoing minor surgical interventions, plasma ACTH increased during surgery and normalized rapidly afterwards.[22-24] Interestingly, during abdominal surgery, the rise in plasma ACTH was higher during laparotomy than during laparascopy.[25] In a study on more extensive surgery, plasma ACTH was unaltered.[26] In patients undergoing elective major surgery,

plasma ACTH, together with plasma cortisol, rose following surgery with a subsequent fall, whereas plasma cortisol remained high during the following days in ICU.[27,28] In patients suffering from severe trauma and sepsis, necessitating intensive care for 8 days and more, plasma ACTH concentrations only transiently increased, after which they fell to levels below those in healthy individuals, while plasma cortisol was elevated during the whole study period.[29] Also in septic shock patients, baseline plasma ACTH levels were low in comparison with healthy volunteers, independent of the severity of illness.[30] In a study on a mixed population of medical and surgical critically ill patients, only low plasma ACTH concentrations during the first 7 days in ICU were reported (**Figure 3, panel A**).[31]

In contrast with plasma ACTH, an increase of plasma cortisol is a hallmark of critical illness. Indeed, the more severely ill, and thus the higher the risk of dying, the higher plasma cortisol concentrations rise.[32] In burn patients, plasma cortisol concentrations were shown to be elevated in proportion to burn size.[21] In patients undergoing surgery, cortisol concentrations also reflected the degree of surgical stress.[32-35] Laparatomy caused higher plasma cortisol concentrations peroperatively than laparascopy,[22] but no differences were found between upper and lower abdominal surgical laparoscopy.[36] Interestingly, laparoscopy combined with CO_2 insufflation evoked higher plasma cortisol concentrations than laparascopy caused by mechanical elevation of the abdominal wall.[37] In addition, septic shock induced elevated cortisol levels proportionally to disease severity.[38-41] Whereas critical

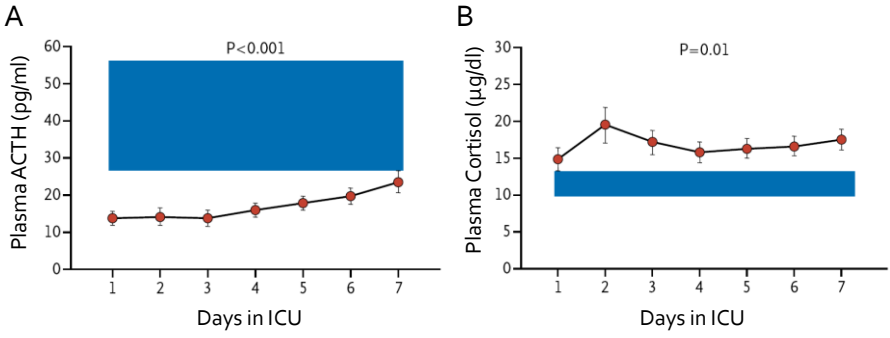


Figure 3 | ACTH-cortisol dissociation
Shown are mean values for ACTH (Panel A) and cortisol (Panel B) in patients during the first week of critical illness. The I bars indicate standard errors. The shaded area represents the interquartile range of values in controls. From day 1 to day 7, plasma cortisol concentrations in patients remained elevated (P=0.01), and patients had lower plasma ACTH concentrations than did controls (P<0.001). (*Adapted from [31]*)

illnesses evoke severe and sustained physical stress for the human body, for which one would expect that a direct and adequate activation of the stress response is required, the exact timing of the rise of plasma cortisol concentrations during the course of illness is not that clear. In patients undergoing minor and more extensive surgery, a cortisol increase was only observed during or near the end of surgery, with a rapid normalization during the following hours on the ward.[22-24,26] Patients undergoing elective major surgery displayed a large cortisol response that occurred hours after, not during, surgery and remained high during the following days in ICU.[27,28] In a mixed population of severely ill medical and surgical patients, elevated plasma cortisol concentrations were reported from the first day until day 7 in ICU, in the face of continuously low plasma ACTH concentrations, which is referred to as the 'ACTH-cortisol dissociation' during critical illness (**Figure 3, panel B**).[31]

Hence, the dynamics of the HPA axis response to severe and prolonged life-threatening stress and to less severe stress appear to differ. Based on the general concept of the stress response, high plasma cortisol levels are predominantly attributed to an increased cortisol production. However, plasma hormone concentrations are the net result of hormone secretion, distribution, binding to plasma proteins, and plasma clearance.

Cortisol production and metabolism

In a set of clinical studies performed in 158 mixed medical and surgical ICU patients, the rate of cortisol production and plasma clearance has been quantified and compared with a matched population of healthy control subjects.[31] Cortisol production rate, measured via a stable isotope technique, was found to be only slightly elevated in critically ill patients suffering from the systemic inflammatory response syndrome (SIRS) and unchanged in critically ill patients without SIRS, whereas plasma free and total cortisol concentrations were several-fold higher in all patients. The pro-inflammatory cytokines TNF-α and IL-6 correlated positively with cortisol production, suggesting that these could play a role as a driver of the moderately increased cortisol production during critical illness. Surprisingly, the cortisol production rates observed in these very ill patients on vital organ support were in the same range as those reported in old studies for patients with less severe stress, e.g. patients suffering from mild infections or during a COPD exacerbation.[42,43] Strikingly, the stable isotope study indicated that the plasma clearance of cortisol was suppressed to less than half in all patients, regardless of the inflammation status. Also the plasma clearance of 100 mg hydrocortisone, the pharmaceutical form of cortisol, administered as an intravenous bolus,

was found to be 60% lower than normal, with a half-life of a median 5-fold longer than in healthy subjects. Hence, although cortisol production rate is not much (if at all) elevated, the reduced breakdown better explains the typically elevated plasma cortisol observed in the critically ill.

Cortisol is normally mainly broken down in the liver via A-ring reductases to the metabolites 5α- and 5β-tetrahydrocortisol (**Figure 4**). In the kidney, cortisol can be inactivated to cortisone via 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), and further degraded to 5α- and 5β-tetrahydrocortisone by 5α- and 5β-reductase in the liver. Indeed, the expression and activity of the hepatic A-ring reductases and the 11β-HSD2 activity were significantly diminished in critically ill patients.[31]

As a side note, the suppression of cortisol breakdown during critical illness can be interpreted as a smart adaptive and energy-efficient mechanism to rapidly increase cortisol availability in those vital organs and tissues that express these enzymes, which could be required to deal with and overcome life-threatening illnesses or trauma.

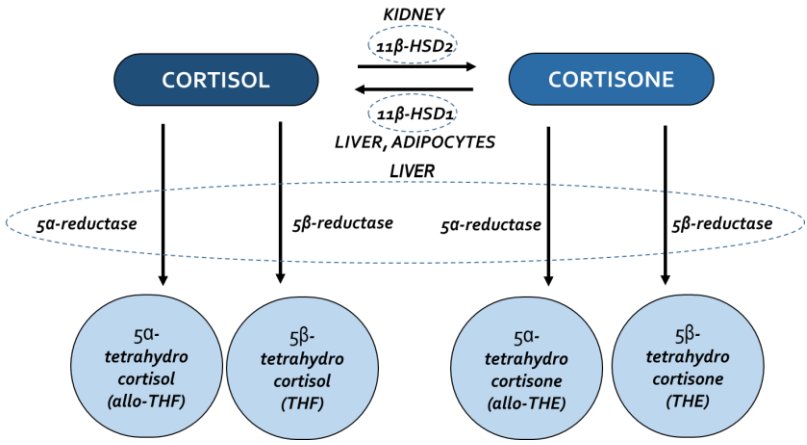


Figure 4 | Cortisol metabolism in humans
Cortisol and cortisone are mainly broken down via A-ring reductases, 5α-reductase and 5β-reductase, in the liver to generate 5α- and 5β-tetrahydrocortisol. In the kidney, cortisol is metabolized by 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2, generating cortisone, which can further be broken down to 5α- and 5β-tetrahydrocortisone by 5α- and 5β-reductase. 11β-HSD type 1 can reconvert cortisone to cortisol.

Non-ACTH driven cortisol production?

A dissociation of ACTH and cortisol levels has also been observed in other non-critically-ill stress conditions such as depression and anxiety.[44,45] Also in Alzheimer disease patients, high plasma cortisol though normal plasma ACTH levels have been shown [46]. In mice, it has been shown that adrenal responsiveness to plasma ACTH is increased with endurance training, chronic stress, or hypoxia.[47-49] Other clinical conditions ranging from metabolic disease, chronic pulmonary disease, and alcoholism, also show a dissociation between plasma ACTH and cortisol.[50-52] In abdominal obesity, and in patients with a high waist-to-hip ratio in comparison with a low waist-to-hip ratio, a hyperresponsiveness or hypersensitivity to various challenges of psychological, physiological nature result in elevated total net secretion and urinary output of cortisol.[53-55] In all these conditions, the dissociation between ACTH and cortisol has been interpreted as the consequence of an increased non-ACTH driven cortisol production. During critical illness, the observed moderately increased cortisol production[31,42,43] could indeed theoretically be brought about by alternative stimuli, such as cytokines, neuropeptides, or certain adipokines.[56] Immune cells can release cytokines that regulate cortisol secretion, but bacterial or viral pathogens can also directly interact with the adrenocortical cells via toll-like receptors.[57] Toll-like-receptor-2-deficient mice had indeed an impaired adrenal corticosterone release upon stimulation with bacterial cell wall compounds, although the initial activation of cortisol production during early sepsis depended primarily on the activation of immune cells and cytokine release.[58] Adipocytes situated in subcutaneous, visceral or peri-adrenal fat release certain adipokines, which can induce cortisol secretion but also sensitize adrenocortical cells for ACTH.[59] Tissue damage or inflammation can also induce the release of vasoactive peptides, such as endothelin, which has shown to cause a dose-dependent stimulation of cortisol production in human and rat adrenal cells and potentiated the effect of ACTH.[60]

As mentioned above, the sympathetic nervous system and the HPA axis synergistically interact with each other in the complex microenvironment of the adrenal gland.[18] The addition of chromaffin cells to intact isolated perfused pig adrenals with preserved nerve supply showed that the release of corticosteroids could be stimulated through the sympathetic nervous system.[61]

Also an increased ACTH sensitivity, i.e. an upregulation of its melanocortin 2 receptor (MC2R), could play a role.[56] However, a human study that quantified pulsatile and non-pulsatile secretion of cortisol and ACTH overnight revealed that the amount of cortisol

released in response to a given ACTH level was found to be normal, arguing against an increased ACTH sensitivity.[62] This preserved dose-response between ACTH and cortisol suggested that the term 'ACTH-cortisol dissociation' (referring to total plasma concentrations) may not be entirely correct, as an association between ACTH secretion and cortisol secretion was in fact maintained, but both were lower than in healthy subjects. On the contrary, the presence of more asynchrony and irregularity in the patterns of cortisol and ACTH secretion suggested other ACTH-independent mechanisms contributing to the cortisol availability.[62]

It might also be possible that critical illness induces an increase in other active splice-forms of ACTH, undetected by the classic immunoenzymometric ACTH assays. However, in an observational study of septic and non-septic ICU patients, where plasma cortisol was found to be increased in all patients, plasma ACTH was not increased, both measured by the highly specific immunoenzymometric assay as by a less specific single antibody competitive binding assay which would have detected other fragments or precursors of ACTH.[63] These findings disproved the hypothesis that such biologically active forms of ACTH could be responsible for increased cortisol production during critical illness.

Cortisol transport

Once secreted into the bloodstream, the relatively insoluble cortisol is transported predominantly bound to corticosteroid-binding globulin (CBG, transcortin) (80%) and to a lesser extent to albumin (10-15%). Since only free (unbound) cortisol can exert its biological and clinical effects, low CBG levels increase cortisol availability at the tissue level.[64] Also CBG affinity can be modulated by pH and temperature, and by elastases produced by neutrophils at sites of inflammation, converting the high-affinity conformation of CBG to a low-affinity conformation, as such increasing free cortisol levels.[65] Thus in patients with systemic infection, free rather than total cortisol correlate with the severity of disease and better reflect biologically active cortisol availability.[66] In the clinical setting however, total plasma cortisol is usually measured, because ultra-filtration and equilibrium dialysis are rarely available and time-consuming. Alternatively, an estimation of free cortisol can be made with use of the validated Coolens formula, based on total plasma cortisol, CBG, and albumin levels:

$$\text{Free cortisol} = \sqrt{\left(0.0167 + (G - T)\frac{1}{2(1 + N'')}\right)^2 + T * \frac{1}{(1 + N'') * K}} - 0.0167 + (G - T)\frac{1}{2(1 + N'')}$$

where G = plasma CBG concentration (in µmol/l), T = plasma total cortisol concentration (in µmol/l), K = affinity of CBG for cortisol = 3.10^7 M^{-1}, and N″ = 1.74/43 × individual albumin concentration(g/l).[67]

In patients in the early stage of septic shock and multiple trauma, plasma CBG levels have shown to be immediately and significantly lowered which reflected much higher free cortisol levels than indicated by total cortisol.[68] Plasma CBG levels were also transiently decreased following abdominal surgery, with a normalization on postoperative day 2.[69] In an observational cohort study in patients with sepsis and septic shock, total CBG levels decreased in proportion to disease severity.[70] This was explained by an increase in cleaved low-affinity CBG, which was associated with the plasma neutrophil concentration.

Elevated free plasma cortisol during severe stress is mainly determined by the combined effect of a decrease in high-affinity CBG due to elastase cleavage and reduced CBG and albumin synthesis by the liver, and increased total cortisol levels.[70] In theory, cleavage of CBG can be interpreted as a beneficial response that may target increased cortisol bioavailability to sites of interest during critical illness.[71] However, a depletion of high-affinity CBG, possibly worsened by reduced synthesis, can evoke a loss of the circulating cortisol reservoir, resulting in a failure of cortisol supply to the inflammatory sites and a loss of ability to dampen inflammation.[70]

Cortisol signaling

Local cortisol activity is also further regulated by tissue-specific alterations of glucocorticoid signaling.[72,73] At the levels of the target cells, free cortisol diffuses through the cell membrane into the cytoplasm where it can bind to the cytoplasmic glucocorticoid receptor (GR), which form dimers that translocate to the nucleus and act as a ligand-dependent transcription factor to regulate target gene expression, in order to exert its effects.[74] Alternative splicing of the GR gene can generate different isoforms of the receptor, of which GRα, the active positive isoform, and GRβ, the negative isoform, are the most

important ones.[73] Other common GR isoforms are GRγ and GR-P. Expression of the GR receptor is normally downregulated by cortisol to maintain homeostasis.[75] Cortisol can also bind to the mineralocorticoid receptor (MR) with a 10-fold higher affinity.[76] In contrast with the GR, which is widely expressed in all tissues, the MR is expressed only in certain tissues, such as the kidneys, where it mainly regulates salt and water homeostasis. Although affinity to cortisol is higher, cortisol signaling through the MR is limited by the activity of 11β-HSD2 in cells in which MR is expressed.[77]

Evidence from animal and human studies indicate that, besides alternative splicing of the GR, also GR expression, GR affinity and GR translocation are regulated and could be tissue-specific during critical illness.[78-81] GRβ expression was found to be transiently increased in white blood cells of adult septic patients.[79] White blood cell binding capacity of labeled dexamethasone was markedly reduced in ventilated critically ill patients with the lowest GR receptor levels in the more severely ill patients.[81] In white blood cells of critically ill children, suffering from trauma and sepsis, lower total and cytoplasmatic GR levels than in healthy individuals have been reported.[82] A suppression of GR expression in white blood cells has also been reported in adult septic patients.[79] In patients with sepsis, the hepatic GR expression was reduced, and further suppressed by treatment with glucocorticoids.[83]In contrast to liver GR expression, muscle GR expression was not lower in patients receiving exogenous glucocorticoids in tissue samples of patients who died in the ICU, which might imply that muscle tissue is less sensitive to down-regulating effects of glucocorticoids in critical illness.[80] *In vitro* and animal research indeed suggested increased GR expression in muscle tissue, but decreased GR expression in liver tissue during critical illness.[84,85] Also, pro-infammatory cytokines decrease the expression of the glucocorticoid receptor and increase its oxidation, which hampers both ligand and DNA binding.[86] Vitamin C has been suggested to reverse these changes and restore glucocorticoid function, a mechanism that could explain the potential reduction in mortality of patients with severe sepsis and septic shock from glucocorticoid administration together with vitamin C.[86]

A tissue-specific regulation of glucocorticoid signaling may limit undue cortisol exposure in vulnerable vital organs that would suffer from an excess of cortisol and increase it in cells that might require more cortisol action. However, further research regarding tissue-specific changes is needed to unravel whether this phenomenon is adaptive or maladaptive.

The hypothalamic-pituitary feedback mechanism

The hypothalamic-pituitary feedback regulation is central in the physiological response to maintain and restore homeostasis during stress. Cortisol exerts fast (seconds to minutes), intermediate (hours) and slow (days) feedback inhibition at the level of the hypothalamus and the pituitary to fine-tune its own release.[87] Fast feedback exerts negative feedback by inhibiting ACTH and CRH release, and does not influence gene expression or protein synthesis.[88] Intermediate feedback also does not inhibit CRH and ACTH expression and synthesis, but slow feedback involves regulation of pro-opiomelanocortin (POMC) mRNA levels and POMC synthesis in the pituitary, and lowering of CRH and AVP expression.[88,89] However, the hypothalamic-pituitary feedback regulation appears much more complex than the initially proposed simple closed loop feedback system.[90] For example, suprahypothalamic brain regions, which are also targeted by cortisol, can influence CRH neuronal function in the hypothalamus, thereby regulating the set-point of pituitary responsiveness to cortisol.[90]

The sustained high circulating cortisol levels during critical illness could potentially exert negative feedback inhibition at the pituitary and/or the hypothalamic level, as such lowering ACTH, CRH, and AVP expression, which would explain the low plasma ACTH concentrations.[89] This would be similar to the inhibition of ACTH and CRH synthesis and secretion in response to a prolonged exposure to high doses of exogenous corticosteroids, or in patients with adrenal Cushing's syndrome.[63,87,91] However, such a negative feedback inhibition exerted by high levels of cortisol, normally induces much lower plasma ACTH concentrations than those observed in critically ill patients, which suggests that increased central stress inputs, such as via stress-induced increased AVP which could potentiate CRH effects, might maintain some degree of ACTH secretion and partially overcome the feedback inhibition.[19,87,90,92,93]

Whether during prolonged critical illness ACTH secretion is also suppressed because of reduced CRH and/or AVP signaling is however currently unclear. One could speculate that the longer the feedback inhibition persists, the more ACTH synthesis and secretion would be suppressed. However, a possible progressive loss of responsiveness of the HPA axis to negative feedback regulation, probably due to degenerative changes in the hippocampus, could also play a role.[94] On the other hand, long-term administration of exogenous glucocorticoids or endogenously elevated plasma cortisol concentrations in patients with Cushing syndrome have been shown to cause tertiary, and not secondary,

adrenal insufficiency by prolonged suppression of the hypothalamic CRH and AVP neurons and/or its higher regulatory inputs.[95] Alternatively, inflammation or hypoperfusion-induced damage to cells of the hypothalamus, whereby synthesis of CRH and AVP is hampered, could be responsible for the low plasma ACTH, particularly in critically ill patients with severe infections.[96-99] However, shock or inflammation could also directly damage the anterior pituitary gland.[99] Additional well-controlled studies on central HPA axis changes over time will be highly informative to understand illness evolution.

Iatrogenic modulation of the stress response

During surgery, at the emergency ward and during stay in the ICU, patients receive a broad variety of drugs. Importantly, many of these drugs can theoretically affect the HPA axis activity, either directly at the level of the hypothalamus, pituitary, and/or adrenal gland, or indirectly via a modulation of the activity of the sympathetic nervous system.[100-102] A well-known suppressor of adrenocortical cortisol production, by inhibiting 11-beta-hydroxylase, is etomidate.[103] Prolonged etomidate infusion has been shown to be associated with an increased mortality and was therefore abandoned as a sedative from all ICUs.[104] A single induction dose of etomidate, however, was not related with an increase in mortality, but still lowered plasma cortisol concentrations.[105] Also opioids, frequently used as strong analgetics that act on the opioid receptor to produce morphine-like effects, have shown to result in suppressed plasma ACTH and/or cortisol concentrations when administered to healthy individuals, to patients suffering from chronic pain, and to surgical patients.[26,106-112] Furthermore, many other frequently used drugs such as anesthetics and sedatives may have HPA suppressive properties as suggested by animal experiments,[113-115] small interventional studies in surgical and ICU patients,[26,110,112,116] and by observational studies in surgical patients.[117,118] Based on this available evidence, the pharmacological effects of drugs used during surgery or during ICU-stay may explain the acute 'ACTH-cortisol dissociation', as observed already upon the first day in ICU. Further research is needed to determine these drugs and their effect on the HPA axis.

Loss of circadian and ultradian rhythm during critical illness

During health, ACTH and cortisol follow a circadian rhythm, with the highest levels of ACTH and cortisol secretion observed in the morning in anticipation of wakening, and the lowest levels during sleep.[119] The tightly coupled release of ACTH and cortisol also follows an

ultradian rhythm, with rapid secretory pulses superimposed on a continuous non-pulsatile release. Mainly the pulse amplitude, not the pulse frequency, determines the circadian rhythm. Evidence grows stronger that, instead of a continuous exposure, pulsatile release is necessary for normal transcriptional and behavioral responses, and plays a role in health and disease.[120]

Circadian rhythms in physiological processes are ubiquitous in living organisms and rely on a complex system of self-sustained clocks with approximately 24h periods.[121] To maintain daily homeostasis, the PVN receives information from the suprachiasmatic nucleus (SCN), which is needed to bring about the circadian pattern of HPA axis activity.[122] Moreover, the SCN directly signals the adrenal cortex by a multi-synaptic neural pathway.[123] The SCN was typically regarded as the only self-sustained clock to act as a master pacemaker for the entire organism, influenced by the light-dark cycle, physical activity, and food intake and fasting.[124] Remarkably, many peripheral tissues, including endocrine glands such as the adrenal gland, are capable of generating self-sustained oscillations independently of the master SCN clock.[125] Indeed, there is evidence that an intrinsic adrenocortical circadian oscillator drives the adrenal response to ACTH, defining a time window in which the cortisol response to ACTH is the highest.[126] This sensitivity is also increased by the SCN, especially during the rising part of the diurnal rhythm, mediated through autonomic pathways.[127] In addition, the sensitivity of the pituitary to negative feedback from cortisol appears to be modulated in a diurnal fashion, with a higher effect during the nadir of the diurnal rhythm.[128]

Although plasma ACTH levels are low in all ICU patients, ACTH secretion is not completely suppressed. The dynamics and interaction of cortisol and ACTH during critical illness have been assesed with use of repeated sampling time series of plasma levels in a mixed set of 40 surgical and medical ICU patients as compared with 8 healthy matched volunteers.[129] Hormonal secretory profiles were created by deconvolution analysis, which took into account the substantially prolonged cortisol half-life, and which allowed to quantify pulsatile and non-pulsatile secretion rates of cortisol and ACTH.[62] This study indicated that nocturnal ACTH as well as cortisol pulsatile secretion rates were reduced in patients, attributed to reduction of pulse masses rather than a reduction of number of pulses. No diurnal rhythm was present for ACTH, nor for cortisol, and plasma (total and free) cortisol concentrations were constantly high and ACTH levels constantly low.

Failure of the adrenocortical stress response

Critical illness-associated acquired adrenal insufficiency

When cortisol availability is insufficient, this has immediate potentially lethal consequences, as demonstrated by the phenotype of patients with Addison's crisis and Addison's disease.[130] Also in adrenalectomized mice, it was shown that mortality strongly increased when sepsis was induced by bacterial endotoxin administration.[131]

ACTH is responsible for both the short- and long-term regulation of cortisol synthesis from the adrenal gland. In the normal stress response, when ACTH binds to its receptor on the membrane of the adrenocortical cells, it activates its receptor. This activates adenylyl cyclase, increases cAMP, and stimulates protein kinase A (PKA) (**Figure 5**).[132,133] PKA activates cholesterol esterase through phosphorylation, which leads to the release of cholesterol from the lipid droplets (intracellular vesicles) into the cytoplasm of the adrenocortical cell.[134] Furthermore, ACTH rapidly increases the expression of the steroidogenic acute regulatory protein (STAR), which is responsible for the transport of cholesterol from the cytoplasm to the inner membrane of the mitochondria where steroidogenesis takes place.[132] STAR is indispensable for cortisol production.[135] Next, cholesterol is converted into different steroid hormones by their respective catalyzing enzymes, in which the final step of the synthesis of cortisol is the hydroxylation of 11-deoxycortisol by 11β-hydroxylase.[136] This process, which in total takes only a few minutes, does not depend on new mRNA synthesis, but on the activation of several proteins, primarily caused by phosphorylation through PKA.[136] The long-term impact of sustained ACTH activity on its receptor involves increased transcription of genes important for cholesterol uptake, cholesterol synthesis, and steroidogenesis as such enhancing the synthetic capacity of the cells.[132,136-139] In addition, increased availability of ACTH affects adrenal gland structure and growth, by first inducing hypertrophy and hyperplasia later on, and by increasing blood flow to the adrenal glands through stimulation of vascular endothelial growth.[140,141] Finally, ACTH has a direct stimulatory effect on the expression of its own receptor (MC2R) which amplifies the responsiveness to ACTH.[142] The extensive acute and chronic impact of ACTH on the adrenal cortex ensures normal adrenal gland structure and functioning. As such, ICU acquired adrenal failure could be the consequence of continuously low plasma ACTH, which negatively affects the adrenal cortex. Indeed, continuously low plasma ACTH negatively affects the adrenal cortex, as evidenced by POMC knockout mice, which suffer from adrenal atrophy and

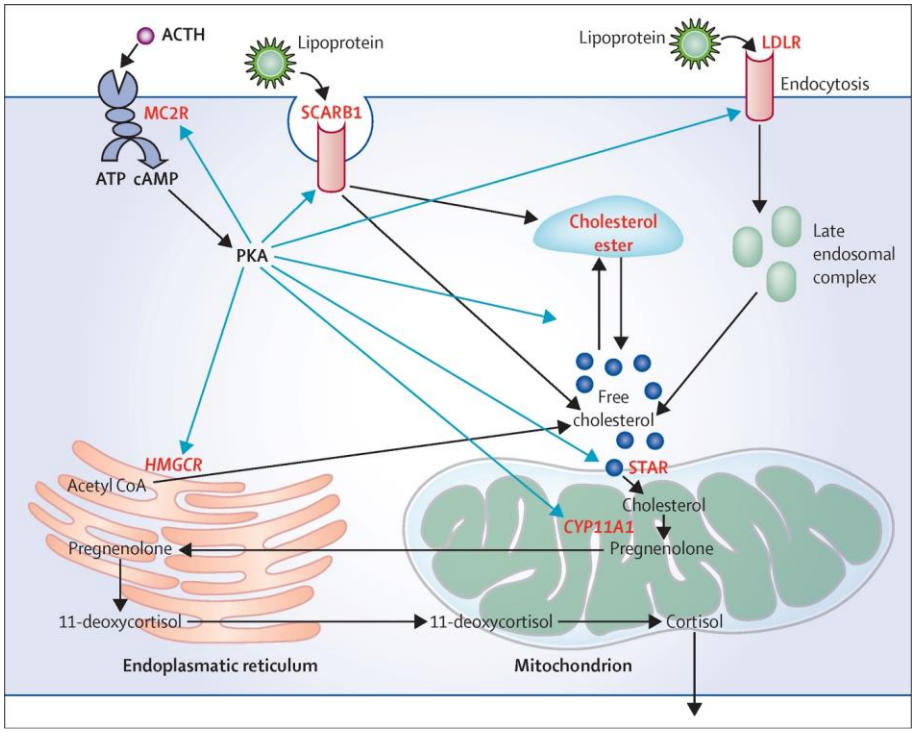


Figure 5 | Short- and long-term impact of ACTH on cortisol synthesis
Adrenocorticotropic hormone (ACTH) binds to its receptor, the melanocortin 2 receptor (MC2R), on the membrane of the adrenocortical cells, which increases cyclic AMP (cAMP) and stimulates protein kinase A (PKA). PKA causes the release of cholesterol from the lipid droplets into the cytoplasm and de novo production from acetyl coenzyme A (acetyl CoA). ACTH increases the expression of the steroidogenic acute regulatory protein (STAR) to transport cholesterol from the cytoplasm to the inner membrane of the mitochondria where steroidogenesis takes place. Cholesterol is converted into different steroid hormones. The long-term impact of ACTH involves increased transcription of genes important for cholesterol uptake (scavenger-receptor class B, member 1 (SCARB1), LDL receptor (LDLR)) and cholesterol synthesis (3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)), and for steroidogenesis (STAR and CYP11A1). ACTH has a direct stimulatory effect on the expression of its own receptor (MC2R). Blue lines represent ACTH effects. (A*dapted from* [133])

hypofunction.[143,144] Also in human patients with POMC-deficiency, a loss of adrenocortical zonational structure, lipid depletion, reduced ACTH signaling and adrenal atrophy is observed.[145] In adrenal glands, harvested postmortem from patients who had been critically ill for several weeks, the adrenal cortex revealed a distorted architecture, lipid droplet depletion, and suppressed ACTH-regulated gene expression as compared with patients dying after short illness or individuals dying suddenly out of hospital (**Figure 6**).[146] Normal pulsatile

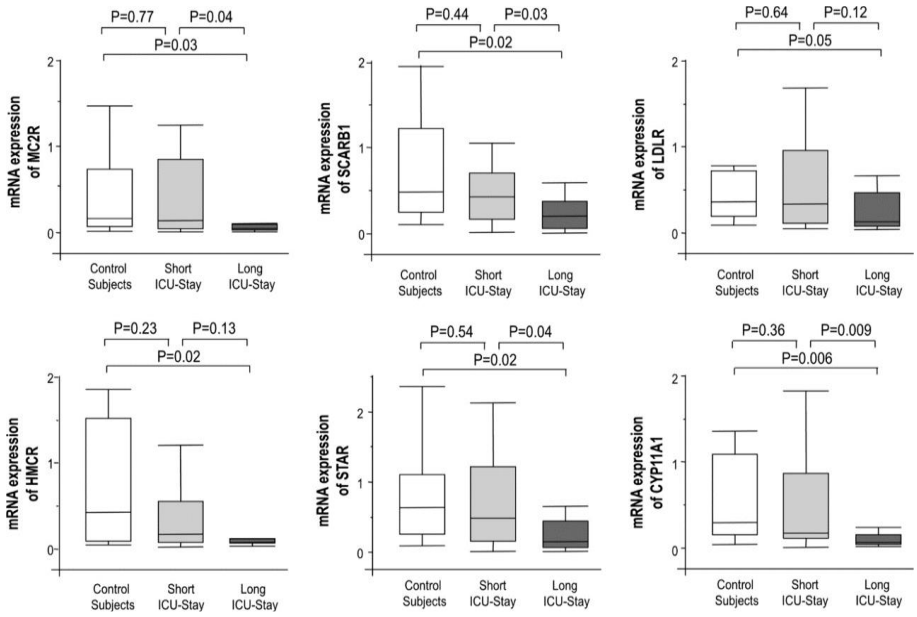


Figure 6 | mRNA expression of ACTH-regulated proteins in adrenal glands
The adrenal glands were harvested from individuals dying suddenly out of hospital (control subjects), from patients dying after short critical illness and from patients after prolonged critical illness. The mRNA data are expressed, normalized to RNA18S as a fold difference from the mean of the controls. Boxes represent medians and interquartile ranges and whiskers represent firstquartile-1.5*IQR and thirdquartile+1.5*IQR. (*Adapted from* [146])

release of ACTH is necessary for transcriptional and behavioral responses.[120] Given the fact that during critical illness pulse masses of ACTH were reduced, this - with time - could also lead to loss of trophic effects of ACTH on the adrenal gland.[120] Of note, these observations argue against an increased ACTH sensitivity during critical illness. However, it remains unknown whether prolonged suppression of plasma ACTH in critically ill patients associates with critical illness-associated acquired loss of adrenal function and low plasma cortisol.

Insufficient cortisol availability during critical illness could also be the result of failure at any level of the HPA axis, from low CBG or altered CBG binding capacity with a loss of the circulating cortisol reservoir, to an inadequate cortisol production.[147] Ischemia or hemorrhage within the adrenal cortex during severe stress or sepsis can cause changes that impair cortisol production. Furthermore, tumor necrosis factor (TNF), produced by microphages and adrenocortical cells, not only stimulates steroidogenesis,[148,149] but also inhibitory effects have been described.[150,151] Also, a decreased blood supply to the pituitary

can evoke ischemia, followed by accumulation of nitric oxide and impaired ACTH secretion.[152] Decreased cortisol production during acute illness may theoretically also be due to substrate deficiency, since HDL cholesterol has been shown to be substantially reduced during sepsis.[153] Other possible causes of ICU acquired adrenal failure, are the administration of drugs that interfere with steroidogenesis, such as etomidate and the antifungal agent ketoconazole, and chronic exogenous corticosteroid therapy.[154]

It has been suggested that adrenal failure in ICU patients ensues from glucocorticoid resistance and insufficiently elevated circulating cortisol to overcome such resistance.[147] Glucocorticoid resistance in peripheral cells could be caused by an increase of the expression of GRβ, and/or by downregulation of GRα, which could be mediated by micro RNA124.[82,155] Furthermore, reduced translocation to the nucleus or the presence of less functional GR polymorphisms may also play a role. Rodents with a general dimerization-deficient GR are indeed highly susceptible to adverse outcome when sepsis is induced.[156] However, it remains unclear whether the changes documented in peripheral blood cells are adaptive, to safeguard the function of immune cells, or, instead, maladaptive and a sign of generalized insufficient GR signaling.

In the past, experts have suggested the presence of a phenomenon that comprises a "relative exhaustion" or "insufficiently activated" adrenal cortex or critical illness-related corticosteroid insufficiency (CIRCI), insufficient to cope with the level of stress of septic shock in particular.[157] In patients suffering from such presumed "relative" adrenal failure, plasma (free) cortisol concentrations are still much higher than normal, but it is assumed that this is not enough to cope with the level of stress and inflammation, and therefore to negatively affect outcome.[40] However, the term "relative" adrenal insufficiency is currently quite controversial and many experts now challenge its existence.[158]

Diagnosis of adrenal failure during critical illness

Diagnosis of adrenal failure is complex and prevalence among ICU patients varies widely from 0% to 77% depending on the definition and criteria used.[159] Diagnosis of adrenal failure starts with a clinical suspicion such as hypotension that is resistant to vasopressors, unexplained coma, hyponatremia and hyperkalemia. Outside the ICU, this clinical suspicion can be confirmed by the presence of a low total morning plasma cortisol (<3 μg/dl or <80nmol/L), although this can be highly variable.[160,161] Therefore, plasma cortisol

concentrations <18 µg/dL or <500 nmol/L upon stimulation with 250 µg of cosyntropin (synthetic ACTH(1-24), Synacthen®), are more indicative of primary adrenal failure.[19]

Using the same cut-off levels for cortisol as used in healthy individuals has potential pitfalls, since basal cortisol levels are much higher in ICU patients. Furthermore, low CBG and albumin levels might further increase free cortisol levels, which makes total cortisol levels less relevant for this diagnostic question.[162] Indeed, a study in 66 critically ill patients reported that hypoproteinemia results in low total plasma cortisol levels with a low total cortisol response to cosyntropin, indicating adrenal insufficiency, while free plasma cortisol levels were consistently elevated and several times higher than in healthy volunteers.[163] Therefore, the free cortisol response to cosyntropin in critically ill patients might be a more valuable clinical determination than the total cortisol response, to avoid treatment of patients with a normal adrenal function.[164] Some authors even doubt that an increase in total cortisol would be essential to survive acute stress, given that the free fraction is so much higher.[158] Studies of critically ill patients, investigating the association between plasma cortisol and mortality, failed to show a minimum level of plasma cortisol concentration below which mortality clearly increased.[165-167] Hence, there is currently no consensus on a cut-off for plasma cortisol to diagnose adrenal failure in ICU patients and even less to indicate the need for treatment with hydrocortisone.

Salivary cortisol levels might be a surrogate for free plasma cortisol in the diagnosis of adrenal failure in critically ill patients, but has not been validated extensively. Salivary cortisol is in close equilibrium with free cortisol and might offer an accurate measure of the biologically active cortisol availability.[168,169] However, local conversion to cortisone through 11β-HSD2 presence in the salivary gland, and reduced salivary flow due to stress, hypovolemia, and opioids effects might limit the use of this technique.[170-172] Also potential blood contamination, by presence of mucositis and/or pathogenic microorganisms, constitutes a major challenge during sampling of pure saliva in critically ill patients.[171,172] Nevertheless, several studies in both adult and pediatric critically ill patients found excellent correlations of salivary and free plasma cortisol, strengthening its potential clinical use.[63,168,173,174] In the diagnosis of adrenal failure, in accordance with morning plasma cortisol, morning salivary cortisol levels vary widely and are not advised to be used.[175] But ACTH-stimulated free plasma and salivary cortisol concentrations increased in parallel in both adult and pediatric critically ill patients.[168,176] However, also opposing results between free and salivary cortisol were measured in patients with severe sepsis.[176,177] Interestingly, in a study of

28 acutely ill patients with a clinical suspicion of adrenal insufficiency, 13 patients had a similar response to cosyntropin in peak serum total and salivary cortisol, whereas 15 patients displayed a subnormal serum total cortisol response, but a normal salivary cortisol response.[178] Salivary cortisol measurements can thus potentially identify patients with a normal adrenal function but an abnormal total cortisol response. As such, salivary cortisol might be a clinically useful and easily obtainable parameter to exclude adrenal failure in ICU patients and thus avoid unnecessary treatment. This possibility should be further investigated.

Experts have advised to diagnose "relative" adrenal failure in ICU patients by the incremental cortisol response to cosyntropin, irrespective of the baseline plasma cortisol. A cortisol increase of less than 9 µg/dL or 240 nmol/L after stimulation with 250 µg cosyntropin, irrespective of the baseline plasma cortisol, or a high baseline plasma cortisol levels >34 µg/dL or 907 nmol/L, have been proposed, as these were most discriminative for increased risk of death.[40] A low cortisol response to cosyntropin was also associated with a higher baseline cortisol and ACTH and with more severe disease and presence of sepsis and septic shock.[179,180] However, in critically ill patients, a low rise in plasma cortisol in response to cosyntropin was associated both with a low cortisol production rate (but still equal to healthy individuals), and, more importantly, with low clearance of plasma cortisol.[31] This suggested that a suppression of cortisol breakdown may explain a reduced cortisol response to cosyntropin, and actually may reflect the degree of negative feedback inhibition exerted by supra-normal cortisol availability. This mechanism is also observed in patients treated with exogenous glucocorticoids, with a lower response to cosyntropin.[181] Therefore, in the presence of increased plasma cortisol and suppressed cortisol metabolism, a reduced cortisol response to cosyntropin may not necessarily point to an insufficient cortisol availability.

Patients suffering from septic shock[40] and long-stay ICU patients[182,183] are considered to be particularly at risk of developing "relative" adrenal failure. Guidelines advised to diagnose "relative" adrenal failure either by an incremental cortisol response to 250 µg of cosyntropin that is below 9 µg/dl or by a random plasma total cortisol below 10 µg/dl, in which case treatment with stress doses of hydrocortisone is advised for sepsis/septic shock patients on vasopressors.[184,185] However, clinicians do not use these diagnostic criteria in their routine practice, and the latest Surviving Sepsis Campaign guidelines suggest not using the ACTH stimulation test to select patients with septic shock that may be treated with hydrocortisone.[186] However, the concept of "relative" adrenal failure as a clinical entity that

should be treated remains controversial. Also, it has not been investigated whether initiation of glucocorticoid treatment in the ICU is supported by abnormalities in adrenocortical function parameters. Clearly, more research on this topic is needed.

Treatment

Evidently, ICU patients suffering from adrenal failure should receive coverage to cope with the stress.[187] Currently, it is recommended to treat adrenal failure during critical illness with an immediate IV injection of 100 mg hydrocortisone and 200 mg of hydrocortisone/24 hours (via continuous iv therapy or 6 hourly injection).[188] As such, this dose is the equivalent of a several-fold increased daily cortisol production, which has been reported to be about 5–8 mg/m²/d, which is equivalent to an oral replacement with 15–25 mg/d.[188]

Patients with presumed "relative" adrenal failure during critical illness with signs of shock should not be treated with hydrocortisone, if adequate fluid resuscitation and vasopressor therapy are able to restore hemodynamic stability during shock.[186] However, there is a weak recommendation, with low quality of evidence, that if this is not achievable, this condition can be treated with hydrocortisone at a dose of 200 mg per day. Indeed, a rise in blood pressure following treatment with hydrocortisone has been used as a proof for underlying adrenal failure.[162] However, this might be related to a pharmacological effect of these doses of hydrocortisone on the vasculature rather than indicating a successful treatment of any form of suspected adrenal failure.[158,189] Empiric treatment with hydrocortisone, which results in hemodynamic improvement in some patients, does not assume a previous diagnosis of "relative" adrenal failure. In 2002, a large French randomized controlled trial (RCT) investigated the impact of 200 mg hydrocortisone (in combination with 50 µg fludrocortisone) in patients with septic shock.[190] This pioneer study showed reduction in mortality with this treatment only in patients who did not have an incremental cortisol response to cosyntropin above 9 µg/dl.[190] In contrast, in a subsequent large European RCT, the CORTICUS trial, the use of low-dose hydrocortisone had no significant effect on the rate of death in patients with septic shock at 28 days, regardless of the patients' adrenal responsiveness to corticotropin.[189] The proportion of patients in whom reversal of shock was achieved, was similar in the two groups, though this goal was achieved earlier in patients who received hydrocortisone. However, the study showed an increased incidence of superinfection, including new episodes of sepsis or septic shock, in the hydrocortisone group.

Patients whose septic shock is treated with hydrocortisone commonly have blood glucose levels higher than 180 mg/dL, which have been associated with increased risk of death.[191] However, in the COIITSS trial in 2010, intensive insulin therapy compared with conventional insulin therapy did not improve in-hospital mortality among patients who were treated with hydrocortisone for septic shock.[192] The addition of oral fludrocortisone did not result in a statistically significant improvement in in-hospital mortality. The HYPRESS trial in 2016 included patients with severe sepsis who were not in septic shock, and showed that the use of hydrocortisone compared with placebo did not reduce the risk of septic shock within 14 days.[193] A Chinese RCT assessed the importance of early initiation of 200 mg hydrocortisone in adults with septic shock, and showed that early initiation did not decrease the risk of mortality and the length of stay in the ICU or hospital.[194] Interestingly, in a retrospective clinical study, Marik and colleagues compared the outcome and clinical course of septic patients treated with intravenous vitamin C, hydrocortisone, and thiamine with a control group, and found an association between the early use of intravenous vitamin C, together with corticosteroids and thiamine with prevention of organ dysfunction and with reduction in mortality.[86] Additional studies are required to confirm these preliminary findings. Recently, in 2018, the largest RCT investigating 200mg hydrocortisone treatment for septic shock, the ADRENAL trial including 3800 patients, showed no mortality benefit at 28 or 90 days.[195] In this trial, secondary outcomes demonstrated that patients in the hydrocortisone group had a more rapid resolution of shock, lower incidence of blood transfusion, a shorter time to ICU discharge, and earlier cessation of mechanical ventilation. In the most recent smaller RCT, the APPROCHSS trial including 1241 patients, a reduced 90-day mortality with hydrocortisone plus fludrocortisone among patients with septic shock was demonstrated.[196] Strangely, this survival benefit is explained by the authors by the addition of 50 µg fludrocortisone, whereas doses that exceed 40 mg of hydrocortisone already provide maximal activation of mineralocorticoid receptors.[188] Surprisingly, in this trial, in patients who underwent a cosyntropin test, the survival benefit was only present in the subgroup of patients who had a response to corticotropin, and thus the controversy remains actual. Subgroups of ICU patients, more specifically patients with acute respiratory distress syndrome (ARDS) and patients with severe community acquired pneumonia appear to benefit from treatment with corticosteroids.[197-199] A speculative explanation for the conflicting results of these studies might be that, given the reduced breakdown and prolonged cortisol half-life during critical illness,[31] doses of 200 mg hydrocortisone could be

too high, and induce side effects such as myopathy, muscle wasting, whereby extending the intensive care dependency.[200,201] It is reasonable to assume that lower doses of hydrocortisone, for any indication during critical illness, might actually be sufficient.[202] It was demonstrated that cortisol production during critical illness is only moderately increased, more or less doubled but only in patients with excessive inflammation, whereas in other critically ill patients, cortisol production is not different from that in healthy subjects.[31] Hence, during critical illness, cortisol production rates range from about 30 to 60 mg per day. It is thus possible that 60 mg hydrocortisone per day could suffice as a substitution dose during critical illness, but further research is needed to determine the optimal therapeutic dose and potential benefits.

Conclusion

During critical illness, normal to slightly increased cortisol production and a substantially reduced cortisol breakdown appear to be the main drivers of hypercortisolemia during critical illness (**Figure 7**). Besides total plasma cortisol levels, the dynamics of increased biologically active free plasma cortisol, and tissue-specific alterations of glucocorticoid signaling, further characterize these changes. While plasma cortisol levels are increased, plasma ACTH levels, however, are decreased, which implies that critical illness is not hallmarked by a full central activation of the HPA axis, but by an 'ACTH cortisol dissociation', with loss of the diurnal rhythm of ACTH and cortisol. These findings have revived the ongoing debate about which level of cortisol availability is sufficient in the struggle for survival of the critically ill, about the concept of "relative" adrenal failure, and about how to correctly interpret diagnostic laboratory tests. The ongoing controversy clearly indicates the need for further research on this important clinical problem.

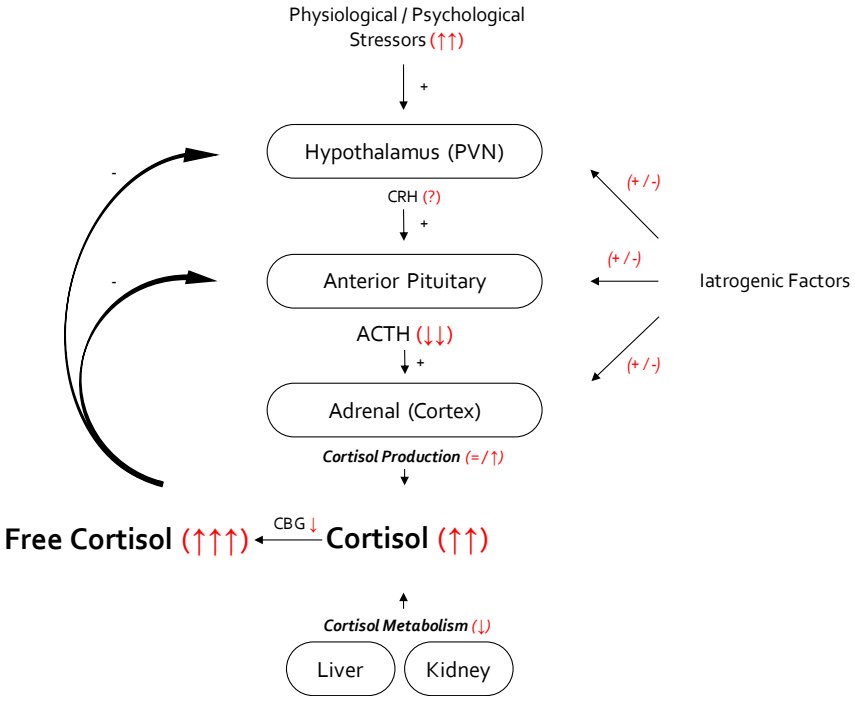


Figure 7 | Overview of the regulation of hypercortisolism during critical illness.
↑, elevated plasma concentrations; ↓, decreased plasma concentrations; ?, no univocal data available; +, stimulates; -, inhibits; PVN, paraventricular nucleus; ACTH, adrenocorticotropic hormone; CBG, corticosteroid-binding globulin.

References

1. Gentile LF, Moldawer LL. DAMPs, PAMPs, and the origins of SIRS in bacterial sepsis. *Shock* 2013; **39**: 113-4.
2. Malbrain M, Van Regenmortel N, Saugel B, et al. Principles of fluid management and stewardship in septic shock: it is time to consider the four D's and the four phases of fluid therapy. *Ann Intensive Care* 2018; **8**: 66.
3. Kahn JM, Le T, Angus DC, et al. The epidemiology of chronic critical illness in the United States*. *Crit Care Med* 2015; **43**: 282-7.
4. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol* 2013; **13**: 862-74.
5. Iwashyna TJ, Hodgson CL, Pilcher D, et al. Timing of onset and burden of persistent critical illness in Australia and New Zealand: a retrospective, population-based, observational study. *Lancet Respir Med* 2016; **4**: 566-73.
6. Selye H. A syndrome produced by diverse nocuous agents. *Nature* 1936; **138**: 32.
7. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol* 2009; **5**: 374-81.
8. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci* 2006; **8**: 383-95.
9. Vanhorebeek I, Langouche L, Van den Berghe G. Endocrine aspects of acute and prolonged critical illness. *Nat Clin Pract Endocrinol Metab* 2006; **2**: 20-31.
10. Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. *J Clin Endocrinol Metab* 1998; **83**: 1827-34.
11. Van den Berghe G, de Zegher F, Veldhuis JD, et al. The somatotropic axis in critical illness: effect of continuous growth hormone (GH)-releasing hormone and GH-releasing peptide-2 infusion. *J Clin Endocrinol Metab* 1997; **82**: 590-9.
12. Van den Berghe G, de Zegher F, Baxter RC, et al. Neuroendocrinology of prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone secretagogues. *J Clin Endocrinol Metab* 1998; **83**: 309-19.
13. Van den Berghe G, Wouters P, Weekers F, et al. Reactivation of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotropin-releasing hormone in patients with protracted critical illness. *J Clin Endocrinol Metab* 1999; **84**: 1311-23.
14. Van den Berghe G, de Zegher F, Veldhuis JD, et al. Thyrotrophin and prolactin release in prolonged critical illness: dynamics of spontaneous secretion and effects of growth hormone-secretagogues. *Clin Endocrinol (Oxf)* 1997; **47**: 599-612.
15. Preiser JC, van Zanten AR, Berger MM, et al. Metabolic and nutritional support of critically ill patients: consensus and controversies. *Crit Care* 2015; **19**: 35.
16. de Kloet ER, Karst H, Joels M. Corticosteroid hormones in the central stress response: quick-and-slow. *Front Neuroendocrinol* 2008; **29**: 268-72.
17. Hartmann C, Radermacher P, Wepler M, Nussbaum B. Non-Hemodynamic Effects of Catecholamines. *Shock* 2017; **48**: 390-400.
18. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *EndocrRev* 1998; **19**: 101-43.
19. Raff H, Sharma ST, Nieman LK. Physiological basis for the etiology, diagnosis, and treatment of adrenal disorders: Cushing's syndrome, adrenal insufficiency, and congenital adrenal hyperplasia. *Compr Physiol* 2014; **4**: 739-69.
20. Livesey JH, Dolamore B. Stability of plasma adrenocorticotrophic hormone (ACTH): influence of hemolysis, rapid chilling, time, and the addition of a maleimide. *Clin Biochem* 2010; **43**: 1478-80.
21. Vaughan GM, Becker RA, Allen JP, Goodwin CW, Jr., Pruitt BA, Jr., Mason AD, Jr. Cortisol and corticotrophin in burned patients. *J Trauma* 1982; **22**: 263-73.
22. Marana E, Annetta MG, Meo F, et al. Sevoflurane improves the neuroendocrine stress response during laparoscopic pelvic surgery. *Can J Anaesth* 2003; **50**: 348-54.
23. Marana E, Colicci S, Meo F, Marana R, Proietti R. Neuroendocrine stress response in gynecological laparoscopy: TIVA with propofol versus sevoflurane anesthesia. *J Clin Anesth* 2010; **22**: 250-5.

24. Udelsman R, Norton JA, Jelenich SE, et al. Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. *J Clin Endocrinol Metab* 1987; **64**: 986-94.
25. Marana E, Scambia G, Maussier ML, et al. Neuroendocrine stress response in patients undergoing benign ovarian cyst surgery by laparoscopy, minilaparotomy, and laparotomy. *J Am Assoc Gynecol Laparosc* 2003; **10**: 159-65.
26. Watanabe K, Kashiwagi K, Kamiyama T, et al. High-dose remifentanil suppresses stress response associated with pneumoperitoneum during laparoscopic colectomy. *J Anesth* 2014; **28**: 334-40.
27. Roth-Isigkeit AK, Schmucker P. Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids* 1997; **62**: 695-9.
28. Gibbison B, Spiga F, Walker JJ, et al. Dynamic pituitary-adrenal interactions in response to cardiac surgery. *Crit Care Med* 2015; **43**: 791-800.
29. 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 Endocrinol Metab* 1995; **80**: 1238-42.
30. Annane D, Maxime V, Ibrahim F, Alvarez JC, Abe E, Boudou P. Diagnosis of adrenal insufficiency in severe sepsis and septic shock. *AmJRespirCrit Care Med* 2006; **174**: 1319-26.
31. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477-88.
32. Widmer IE, Puder JJ, Konig C, et al. Cortisol response in relation to the severity of stress and illness. *J Clin Endocrinol Metab* 2005; **90**: 4579-86.
33. Mohler JL, Michael KA, Freedman AM, Griffen WO, Jr., McRoberts JW. The serum and urinary cortisol response to operative trauma. *Surg Gynecol Obstet* 1985; **161**: 445-9.
34. Chernow B, Alexander HR, Smallridge RC, et al. Hormonal responses to graded surgical stress. *Arch Intern Med* 1987; **147**: 1273-8.
35. McIntosh TK, Lothrop DA, Lee A, Jackson BT, Nabseth D, Egdahl RH. Circadian rhythm of cortisol is altered in postsurgical patients. *J Clin Endocrinol Metab* 1981; **53**: 117-22.
36. Hirvonen EA, Nuutinen LS, Vuolteenaho O. Hormonal responses and cardiac filling pressures in head-up or head-down position and pneumoperitoneum in patients undergoing operative laparoscopy. *Br J Anaesth* 1997; **78**: 128-33.
37. Ishizuka B, Kuribayashi Y, Kobayashi Y, et al. Stress responses during laparoscopy with CO_2 insufflation and with mechanical elevation of the abdominal wall. *J Am Assoc Gynecol Laparosc* 2000; **7**: 363-71.
38. Dalegrave D, Silva RL, Becker M, Gehrke LV, Friedman G. Relative adrenal insufficiency as a predictor of disease severity and mortality in severe septic shock. *Rev Bras Ter Intensiva* 2012; **24**: 362-8.
39. Marik PE, Zaloga GP. Adrenal insufficiency in the critically ill: a new look at an old problem. *Chest* 2002; **122**: 1784-96.
40. Annane D, Sebille V, Troche G, Raphael JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000; **283**: 1038-45.
41. Rothwell PM, Udwadia ZF, Lawler PG. Cortisol response to corticotropin and survival in septic shock. *Lancet* 1991; **337**: 582-3.
42. Cornil A, Copinschi G, Leclercq R, Franckson JR. Cortisol secretion during acute bacterial infections in man. *Acta Endocrinol (Copenh)* 1968; **58**: 1-5.
43. Cornil A, Glinoer D, Leclerco R, Copinschi G. Adrenocortical and somatotrophic secretions in acute and chronic respiratory insufficiency. *Am Rev Respir Dis* 1975; **112**: 77-81.
44. Carroll BJ, Cassidy F, Naftolowitz D, et al. Pathophysiology of hypercortisolism in depression. *Acta Psychiatr Scand Suppl* 2007; **115**: 90-103.
45. Rubin RT, Miller TH, Rhodes ME, Czambel RK. Adrenal cortical responses to low- and high-dose ACTH(1-24) administration in major depressives vs. matched controls. *Psychiatry Res* 2006; **143**: 43-50.
46. Pascualy M, Petrie EC, Brodkin K, Peskind ER, Wilkinson CW, Raskind MA. Hypothalamic pituitary adrenocortical and sympathetic nervous system responses to the cold pressor test in Alzheimer's disease. *Biol Psychiatry* 2000; **48**: 247-54.

47. Droste SK, Schweizer MC, Ulbricht S, Reul JM. Long-term voluntary exercise and the mouse hypothalamic-pituitary-adrenocortical axis: impact of concurrent treatment with the antidepressant drug tianeptine. *J Neuroendocrinol* 2006; **18**: 915-25.
48. Droste SK, Chandramohan Y, Hill LE, Linthorst AC, Reul JM. Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. *Neuroendocrinology* 2007; **86**: 26-37.
49. Raff H, Lee JJ, Widmaier EP, Oaks MK, Engeland WC. Basal and adrenocorticotropin-stimulated corticosterone in the neonatal rat exposed to hypoxia from birth: modulation by chemical sympathectomy. *Endocrinology* 2004; **145**: 79-86.
50. Vierhapper H, Nowotny P, Waldhausl W. Production rates of cortisol in obesity. *Obes Res* 2004; **12**: 1421-5.
51. Rask E, Olsson T, Soderberg S, et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metab* 2001; **86**: 1418-21.
52. Gianoulakis C, Dai X, Thavundayil J, Brown T. Levels and circadian rhythmicity of plasma ACTH, cortisol, and beta-endorphin as a function of family history of alcoholism. *Psychopharmacology (Berl)* 2005; **181**: 437-44.
53. Bjorntorp P, Rosmond R. The metabolic syndrome--a neuroendocrine disorder? *Br J Nutr* 2000; **83 Suppl 1**: S49-57.
54. Epel EE, Moyer AE, Martin CD, et al. Stress-induced cortisol, mood, and fat distribution in men. *Obes Res* 1999; **7**: 9-15.
55. Rosmond R, Dallman MF, Bjorntorp P. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab* 1998; **83**: 1853-9.
56. Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends EndocrinolMetab* 2008; **19**: 175-80.
57. Bornstein SR, Zacharowski P, Schumann RR, et al. Impaired adrenal stress response in Toll-like receptor 2-deficient mice. *ProcNatlAcadSciUSA* 2004; **101**: 16695-700.
58. Kanczkowski W, Alexaki VI, Tran N, et al. Hypothalamo-pituitary and immune-dependent adrenal regulation during systemic inflammation. *ProcNatlAcadSciUSA* 2013; **110**: 14801-6.
59. Krug AW, Vleugels K, Schinner S, et al. Human adipocytes induce an ERK1/2 MAP kinases-mediated upregulation of steroidogenic acute regulatory protein (StAR) and an angiotensin II-sensitization in human adrenocortical cells. *IntJObes(Lond)* 2007; **31**: 1605-16.
60. Hinson JP, Vinson GP, Kapas S, Teja R. The role of endothelin in the control of adrenocortical function: stimulation of endothelin release by ACTH and the effects of endothelin-1 and endothelin-3 on steroidogenesis in rat and human adrenocortical cells. *J Endocrinol* 1991; **128**: 275-80.
61. Bornstein SR, Ehrhart-Bornstein M, Scherbaum WA, Pfeiffer EF, Holst JJ. Effects of splanchnic nerve stimulation on the adrenal cortex may be mediated by chromaffin cells in a paracrine manner. *Endocrinology* 1990; **127**: 900-6.
62. Veldhuis JD, Keenan DM, Pincus SM. Motivations and methods for analyzing pulsatile hormone secretion. *Endocr Rev* 2008; **29**: 823-64.
63. Raff H, Biru N, Reisinger N, Kramer DJ. Dissociation of ACTH and cortisol in septic and non-septic ICU patients. *Endocrine* 2017; **55**: 307-10.
64. Perogamvros I, Aarons L, Miller AG, Trainer PJ, Ray DW. Corticosteroid-binding globulin regulates cortisol pharmacokinetics. *Clin Endocrinol (Oxf)* 2011; **74**: 30-6.
65. Henley DE, Lightman SL. New insights into corticosteroid-binding globulin and glucocorticoid delivery. *Neuroscience* 2011; **180**: 1-8.
66. Ho JT, Al-Musalhi H, Chapman MJ, et al. Septic shock and sepsis: a comparison of total and free plasma cortisol levels. *J Clin Endocrinol Metab* 2006; **91**: 105-14.
67. Coolens JL, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem* 1987; **26**: 197-202.
68. Beishuizen A, Thijs LG, Vermes I. Patterns of corticosteroid-binding globulin and the free cortisol index during septic shock and multitrauma. *Intensive Care Med* 2001; **27**: 1584-91.
69. Dimopoulou I, Tzanela M, Vassiliadi D, et al. Pituitary-adrenal responses following major abdominal surgery. *Hormones (Athens)* 2008; **7**: 237-42.

70. Nenke MA, Rankin W, Chapman MJ, et al. Depletion of high-affinity corticosteroid-binding globulin corresponds to illness severity in sepsis and septic shock; clinical implications. *Clin Endocrinol (Oxf)* 2015; **82**: 801-7.
71. Nguyen PT, Lewis JG, Sneyd J, Lee RS, Torpy DJ, Shorten PR. Development of a formula for estimating plasma free cortisol concentration from a measured total cortisol concentration when elastase-cleaved and intact corticosteroid binding globulin coexist. *JSteroid BiochemMolBiol* 2014; **141**: 16-25.
72. Chapman K, Holmes M, Seckl J. 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev* 2013; **93**: 1139-206.
73. Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. *Mol Cell Endocrinol* 2009; **300**: 7-16.
74. Vandevyver S, Dejager L, Libert C. Comprehensive overview of the structure and regulation of the glucocorticoid receptor. *Endocr Rev* 2014; **35**: 671-93.
75. Desmet SJ, De Bosscher K. Glucocorticoid receptors: finding the middle ground. *J Clin Invest* 2017; **127**: 1136-45.
76. Reichardt HM, Kaestner KH, Tuckermann J, et al. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 1998; **93**: 531-41.
77. Fiebeler A, Muller DN, Shagdarsuren E, Luft FC. Aldosterone, mineralocorticoid receptors, and vascular inflammation. *Curr Opin Nephrol Hypertens* 2007; **16**: 134-42.
78. Bergquist M, Nurkkala M, Rylander C, Kristiansson E, Hedenstierna G, Lindholm C. Expression of the glucocorticoid receptor is decreased in experimental Staphylococcus aureus sepsis. *JInfect* 2013; **67**: 574-83.
79. Guerrero J, Gatica HA, Rodriguez M, Estay R, Goecke IA. Septic serum induces glucocorticoid resistance and modifies the expression of glucocorticoid isoforms receptors: a prospective cohort study and in vitro experimental assay. *Crit Care* 2013; **17**: R107.
80. Peeters RP, Hagendorf A, Vanhorebeek I, et al. Tissue mRNA expression of the glucocorticoid receptor and its splice variants in fatal critical illness. *ClinEndocrinol(Oxf)* 2009; **71**: 145-53.
81. Siebig S, Meinel A, Rogler G, et al. Decreased cytosolic glucocorticoid receptor levels in critically ill patients. *AnaesthIntensive Care* 2010; **38**: 133-40.
82. Indyk JA, Candido-Vitto C, Wolf IM, et al. Reduced glucocorticoid receptor protein expression in children with critical illness. *HormResPaediatr* 2013; **79**: 169-78.
83. Jenniskens M, Weckx R, Dufour T, et al. The Hepatic Glucocorticoid Receptor Is Crucial for Cortisol Homeostasis and Sepsis Survival in Humans and Male Mice. *Endocrinology* 2018; **159**: 2790-802.
84. Sun X, Mammen JM, Tian X. Sepsis induces the transcription of the glucocorticoid receptor in skeletal muscle cells. *Clin Sci (Lond)* 2003; **105**: 383-91.
85. Ali M, Allen HR, Vedeckis WV, Lang CH. Depletion of rat liver glucocorticoid receptor hormone-binding and its mRNA in sepsis. *Life Sci* 1991; **48**: 603-11.
86. Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. *Chest* 2017; **151**: 1229-38.
87. Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev* 1984; **5**: 1-24.
88. Russell GM, Henley DE, Leendertz J, et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci* 2010; **30**: 6106-15.
89. Jameson J, De Groot L. Endocrinology: Adult and Pediatric, 7th Edition. Philadelphia: Elsevier; 2016: 142-3.
90. Watts AG. Glucocorticoid regulation of peptide genes in neuroendocrine CRH neurons: a complexity beyond negative feedback. *Front Neuroendocrinol* 2005; **26**: 109-30.
91. Wagner-Bartak NA, Baiomy A, Habra MA, et al. Cushing Syndrome: Diagnostic Workup and Imaging Features, With Clinical and Pathologic Correlation. *AJR Am J Roentgenol* 2017; **209**: 19-32.
92. Hauger RL, Aguilera G. Regulation of pituitary corticotropin releasing hormone (CRH) receptors by CRH: interaction with vasopressin. *Endocrinology* 1993; **133**: 1708-14.
93. Watanabe T, Oki Y, Orth DN. Kinetic actions and interactions of arginine vasopressin, angiotensin-II, and oxytocin on adrenocorticotropin secretion by rat anterior pituitary cells in the microperifusion system. *Endocrinology* 1989; **125**: 1921-31.

94. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev* 1986; **7**: 284-301.
95. Gomez MT, Magiakou MA, Mastorakos G, Chrousos GP. The pituitary corticotroph is not the rate limiting step in the postoperative recovery of the hypothalamic-pituitary-adrenal axis in patients with Cushing syndrome. *J Clin Endocrinol Metab* 1993; **77**: 173-7.
96. Polito A, Sonneville R, Guidoux C, et al. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoSOne* 2011; **6**: e25905.
97. Bornstein SR. Predisposing factors for adrenal insufficiency. *N Engl J Med* 2009; **360**: 2328-39.
98. Deutschman CS, Raj NR, McGuire EO, Kelz MB. Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med* 2013; **41**: e368-e75.
99. Annane D. The Role of ACTH and Corticosteroids for Sepsis and Septic Shock: An Update. *Front Endocrinol (Lausanne)* 2016; **7**: 70.
100. Lindgren C, Dahlqvist P, Lindvall P, Nilsson L, Koskinen LO, Naredi S. Cortisol levels are influenced by sedation in the acute phase after subarachnoid haemorrhage. *Acta Anaesthesiol Scand* 2013; **57**: 452-60.
101. Plunkett JJ, Reeves JD, Ngo L, et al. Urine and plasma catecholamine and cortisol concentrations after myocardial revascularization. Modulation by continuous sedation. Multicenter Study of Perioperative Ischemia (McSPI) Research Group, and the Ischemia Research and Education Foundation (IREF). *Anesthesiology* 1997; **86**: 785-96.
102. Brorsson C, Dahlqvist P, Nilsson L, Thunberg J, Sylvan A, Naredi S. Adrenal response after trauma is affected by time after trauma and sedative/analgesic drugs. *Injury* 2014; **45**: 1149-55.
103. Hildreth AN, Mejia VA, Maxwell RA, Smith PW, Dart BW, Barker DE. Adrenal suppression following a single dose of etomidate for rapid sequence induction: a prospective randomized study. *J Trauma* 2008; **65**: 573-9.
104. Watt I, Ledingham IM. Mortality amongst multiple trauma patients admitted to an intensive therapy unit. *Anaesthesia* 1984; **39**: 973-81.
105. Bruder EA, Ball IM, Ridi S, Pickett W, Hohl C. Single induction dose of etomidate versus other induction agents for endotracheal intubation in critically ill patients. *Cochrane Database Syst Rev* 2015; **1**: CD010225.
106. Taylor T, Dluhy RG, Williams GH. beta-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J Clin Endocrinol Metab* 1983; **57**: 592-6.
107. Rittmaster RS, Cutler GB, Jr., Sobel DO, et al. Morphine inhibits the pituitary-adrenal response to ovine corticotropin-releasing hormone in normal subjects. *J Clin Endocrinol Metab* 1985; **60**: 891-5.
108. Aloisi AM, Aurilio C, Bachiocco V, et al. Endocrine consequences of opioid therapy. *Psychoneuroendocrinology* 2009; **34 Suppl 1**: S162-8.
109. Abs R, Verhelst J, Maeyaert J, et al. Endocrine consequences of long-term intrathecal administration of opioids. *J Clin Endocrinol Metab* 2000; **85**: 2215-22.
110. Shinoda T, Murakami W, Takamichi Y, Iizuka H, Tanaka M, Kuwasako Y. Effect of remifentanil infusion rate on stress response in orthopedic surgery using a tourniquet application. *BMC Anesthesiol* 2013; **13**: 14.
111. Taniguchi H, Sasaki T, Fujita H, et al. The effect of intraoperative use of high-dose remifentanil on postoperative insulin resistance and muscle protein catabolism: a randomized controlled study. *Int J Med Sci* 2013; **10**: 1099-107.
112. Raff H, Flemma RJ, Findling JW. Fast cortisol-induced inhibition of the adrenocorticotropin response to surgery in humans. *J Clin Endocrinol Metab* 1988; **67**: 1146-8.
113. Dispersyn G, Sage D, Challet E, Pain L, Touitou Y. Plasma corticosterone in rats is specifically increased at recovery from propofol anesthesia without concomitant rise of plasma ACTH. *Chronobiol Int* 2009; **26**: 697-708.
114. Kostopanagiotou G, Kalimeris K, Christodoulaki K, et al. The differential impact of volatile and intravenous anaesthetics on stress response in the swine. *Hormones (Athens)* 2010; **9**: 67-75.
115. Pascoe JE, Williams KL, Mukhopadhyay P, Rice KC, Woods JH, Ko MC. Effects of mu, kappa, and delta opioid receptor agonists on the function of hypothalamic-pituitary-adrenal axis in monkeys. *Psychoneuroendocrinology* 2008; **33**: 478-86.
116. Aitkenhead AR, Pepperman ML, Willatts SM, et al. Comparison of propofol and midazolam for sedation in critically ill patients. *Lancet* 1989; **2**: 704-9.

117. Skoglund K, Enblad P, Hillered L, Marklund N. The neurological wake-up test increases stress hormone levels in patients with severe traumatic brain injury. *Crit Care Med* 2012; **40**: 216-22.
118. Marty J, Gauzit R, Lefevre P, et al. Effects of diazepam and midazolam on baroreflex control of heart rate and on sympathetic activity in humans. *Anesth Analg* 1986; **65**: 113-9.
119. Hastings M, O'Neill JS, Maywood ES. Circadian clocks: regulators of endocrine and metabolic rhythms. *J Endocrinol* 2007; **195**: 187-98.
120. Stavreva DA, Wiench M, John S, et al. Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. *Nat Cell Biol* 2009; **11**: 1093-102.
121. Oster H, Damerow S, Kiessling S, et al. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab* 2006; **4**: 163-73.
122. Walker JJ, Terry JR, Lightman SL. Origin of ultradian pulsatility in the hypothalamic-pituitary-adrenal axis. *Proc Biol Sci* 2010; **277**: 1627-33.
123. Buijs RM, Wortel J, Van Heerikhuize JJ, et al. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *Eur J Neurosci* 1999; **11**: 1535-44.
124. Albrecht U. Timing to perfection: the biology of central and peripheral circadian clocks. *Neuron* 2012; **74**: 246-60.
125. Garaulet M, Madrid JA. Chronobiological aspects of nutrition, metabolic syndrome and obesity. *Adv Drug Deliv Rev* 2010; **62**: 967-78.
126. Son GH, Chung S, Kim K. The adrenal peripheral clock: glucocorticoid and the circadian timing system. *Front Neuroendocrinol* 2011; **32**: 451-65.
127. Sage D, Maurel D, Bosler O. Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress. *Am J Physiol Endocrinol Metab* 2001; **280**: E260-9.
128. Girotti M, Weinberg MS, Spencer RL. Diurnal expression of functional and clock-related genes throughout the rat HPA axis: system-wide shifts in response to a restricted feeding schedule. *Am J Physiol Endocrinol Metab* 2009; **296**: E888-97.
129. Boonen E, Meersseman P, Vervenne H, et al. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab* 2014; **306**: E883-92.
130. Ten S, New M, Maclaren N. Clinical review 130: Addison's disease 2001. *J Clin Endocrinol Metab* 2001; **86**: 2909-22.
131. Bertini R, Bianchi M, Ghezzi P. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *J Exp Med* 1988; **167**: 1708-12.
132. Simpson ER, Waterman MR. Regulation by ACTH of steroid hormone biosynthesis in the adrenal cortex. *CanJBiochemCell Biol* 1983; **61**: 692-707.
133. Boonen E, Bornstein SR, Van den Berghe G. New insights into the controversy of adrenal function during critical illness. *Lancet Diabetes Endocrinol* 2015; **3**: 805-15.
134. Toth IE, Szabo D, Bruckner GG. Lipoproteins, lipid droplets, lysosomes, and adrenocortical steroid hormone synthesis: morphological studies. *Microsc Res Tech* 1997; **36**: 480-92.
135. Lin D, Sugawara T, Strauss JF, 3rd, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science* 1995; **267**: 1828-31.
136. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *EndocrRev* 1996; **17**: 221-44.
137. Liu J, Heikkila P, Meng QH, Kahri AI, Tikkanen MJ, Voutilainen R. Expression of low and high density lipoprotein receptor genes in human adrenals. *EurJEndocrinol* 2000; **142**: 677-82.
138. Lehoux JG, Lefebvre A, Belisle S, Bellabarba D. Hormonal regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in the rat adrenal gland. *JSteroid Biochem* 1989; **34**: 379-84.
139. Lehoux JG, Fleury A, Ducharme L. The acute and chronic effects of adrenocorticotropin on the levels of messenger ribonucleic acid and protein of steroidogenic enzymes in rat adrenal in vivo. *Endocrinology* 1998; **139**: 3913-22.
140. Ferreira JG, Cruz CD, Neves D, Pignatelli D. Increased extracellular signal regulated kinases phosphorylation in the adrenal gland in response to chronic ACTH treatment. *J Endocrinol* 2007; **192**: 647-58.
141. Shifren JL, Mesiano S, Taylor RN, Ferrara N, Jaffe RB. Corticotropin regulates vascular endothelial growth factor expression in human fetal adrenal cortical cells. *J Clin Endocrinol Metab* 1998; **83**: 1342-7.

142. Lebrethon MC, Naville D, Begeot M, Saez JM. Regulation of corticotropin receptor number and messenger RNA in cultured human adrenocortical cells by corticotropin and angiotensin II. *JClinInvest* 1994; **93**: 1828-33.
143. Coll AP, Challis BG, Yeo GS, et al. The effects of proopiomelanocortin deficiency on murine adrenal development and responsiveness to adrenocorticotropin. *Endocrinology* 2004; **145**: 4721-7.
144. Karpac J, Czyzewska K, Kern A, Brush RS, Anderson RE, Hochgeschwender U. Failure of adrenal corticosterone production in POMC-deficient mice results from lack of integrated effects of POMC peptides on multiple factors. *Am J Physiol Endocrinol Metab* 2008; **295**: E446-55.
145. Krude H, Gruters A. Implications of proopiomelanocortin (POMC) mutations in humans: the POMC deficiency syndrome. *Trends Endocrinol Metab* 2000; **11**: 15-22.
146. Boonen E, Langouche L, Janssens T, et al. Impact of Duration of Critical Illness on the Adrenal Glands of Human Intensive Care Patients. *J Clin Endocrinol Metab* 2014; **99**: 4214-22.
147. Marik PE. Critical illness-related corticosteroid insufficiency. *Chest* 2009; **135**: 181-93.
148. Darling G, Goldstein DS, Stull R, Gorschboth CM, Norton JA. Tumor necrosis factor: immune endocrine interaction. *Surgery* 1989; **106**: 1155-60.
149. Mikhaylova IV, Kuulasmaa T, Jaaskelainen J, Voutilainen R. Tumor necrosis factor-alpha regulates steroidogenesis, apoptosis, and cell viability in the human adrenocortical cell line NCI-H295R. *Endocrinology* 2007; **148**: 386-92.
150. Jaattela M, Ilvesmaki V, Voutilainen R, Stenman UH, Saksela E. Tumor necrosis factor as a potent inhibitor of adrenocorticotropin-induced cortisol production and steroidogenic P450 enzyme gene expression in cultured human fetal adrenal cells. *Endocrinology* 1991; **128**: 623-9.
151. Jaattela M, Carpen O, Stenman UH, Saksela E. Regulation of ACTH-induced steroidogenesis in human fetal adrenals by rTNF-alpha. *Mol Cell Endocrinol* 1990; **68**: R31-6.
152. Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. *Lancet* 2014; **383**: 2152-67.
153. van der Voort PH, Gerritsen RT, Bakker AJ, Boerma EC, Kuiper MA, de Heide L. HDL-cholesterol level and cortisol response to synacthen in critically ill patients. *Intensive Care Med* 2003; **29**: 2199-203.
154. Cooper MS, Stewart PM. Corticosteroid insufficiency in acutely ill patients. *N Engl J Med* 2003; **348**: 727-34.
155. Ledderose C, Mohnle P, Limbeck E, et al. Corticosteroid resistance in sepsis is influenced by microRNA-124--induced downregulation of glucocorticoid receptor-alpha. *Crit Care Med* 2012; **40**: 2745-53.
156. Kleiman A, Hubner S, Rodriguez Parkitna JM, et al. Glucocorticoid receptor dimerization is required for survival in septic shock via suppression of interleukin-1 in macrophages. *FASEB J* 2012; **26**: 722-9.
157. SELYE H. The general adaptation syndrome and the diseases of adaptation. *J Clin Endocrinol Metab* 1946; **6**: 117-230.
158. Loriaux DL, Fleseriu M. Relative adrenal insufficiency. *Curr Opin Endocrinol Diabetes Obes* 2009; **16**: 392-400.
159. 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**: 1937-49.
160. Hagg E, Asplund K, Lithner F. Value of basal plasma cortisol assays in the assessment of pituitary-adrenal insufficiency. *Clin Endocrinol (Oxf)* 1987; **26**: 221-6.
161. Dickstein G, Shechner C, Nicholson WE, et al. Adrenocorticotropin stimulation test: effects of basal cortisol level, time of day, and suggested new sensitive low dose test. *J Clin Endocrinol Metab* 1991; **72**: 773-8.
162. Hamrahian AH, Fleseriu M, Committee AAS. Evaluation and Management of Adrenal Insufficiency in Critically Ill Patientsdisease State Review. *Endocr Pract* 2017; **23**: 716-25.
163. Hamrahian AH, Oseni TS, Arafah BM. Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 2004; **350**: 1629-38.
164. Loriaux L. Glucocorticoid therapy in the intensive care unit. *N Engl J Med* 2004; **350**: 1601-2.
165. Span LF, Hermus AR, Bartelink AK, et al. Adrenocortical function: an indicator of severity of disease and survival in chronic critically ill patients. *Intensive Care Med* 1992; **18**: 93-6.

166. Jurney TH, Cockrell JL, Jr., Lindberg JS, Lamiell JM, Wade CE. Spectrum of serum cortisol response to ACTH in ICU patients. Correlation with degree of illness and mortality. *Chest* 1987; **92**: 292-5.
167. Jarek MJ, Legare EJ, McDermott MT, Merenich JA, Kollef MH. Endocrine profiles for outcome prediction from the intensive care unit. *Crit Care Med* 1993; **21**: 543-50.
168. Arafah BM, Nishiyama FJ, Tlaygeh H, Hejal R. Measurement of salivary cortisol concentration in the assessment of adrenal function in critically ill subjects: a surrogate marker of the circulating free cortisol. *J Clin Endocrinol Metab* 2007; **92**: 2965-71.
169. Vining RF, McGinley RA. The measurement of hormones in saliva: possibilities and pitfalls. *J Steroid Biochem* 1987; **27**: 81-94.
170. Perogamvros I, Keevil BG, Ray DW, Trainer PJ. Salivary cortisone is a potential biomarker for serum free cortisol. *J Clin Endocrinol Metab* 2010; **95**: 4951-8.
171. Dennesen P, van der Ven A, Vlasveld M, et al. Inadequate salivary flow and poor oral mucosal status in intubated intensive care unit patients. *Crit Care Med* 2003; **31**: 781-6.
172. Bartanusz V, Corneille MG, Sordo S, et al. Diurnal salivary cortisol measurement in the neurosurgical-surgical intensive care unit in critically ill acute trauma patients. *J Clin Neurosci* 2014; **21**: 2150-4.
173. Gunnala V, Guo R, Minutti C, et al. Measurement of salivary cortisol level for the diagnosis of critical illness-related corticosteroid insufficiency in children. *Pediatr Crit Care Med* 2015; **16**: e101-6.
174. Estrada YMR, Orlander PR. Salivary cortisol can replace free serum cortisol measurements in patients with septic shock. *Chest* 2011; **140**: 1216-22.
175. Marcus-Perlman Y, Tordjman K, Greenman Y, et al. Low-dose ACTH (1 microg) salivary test: a potential alternative to the classical blood test. *Clin Endocrinol (Oxf)* 2006; **64**: 215-8.
176. Balbao VM, Costa MM, Castro M, Carlotti AP. Evaluation of adrenal function in critically ill children. *Clin Endocrinol (Oxf)* 2014; **81**: 559-65.
177. Elbuken G, Karaca Z, Tanriverdi F, et al. Comparison of total, salivary and calculated free cortisol levels in patients with severe sepsis. *J Intensive Care* 2016; **4**: 3.
178. Raff H, Brock S, Findling JW. Cosyntropin-stimulated salivary cortisol in hospitalized patients with hypoproteinemia. *Endocrine* 2008; **34**: 68-74.
179. de Jong MF, Molenaar N, Beishuizen A, Groeneveld AB. Diminished adrenal sensitivity to endogenous and exogenous adrenocorticotropic hormone in critical illness: a prospective cohort study. *Crit Care* 2015; **19**: 1.
180. Festti J, Grion CM, Festti L, et al. Adrenocorticotropic hormone but not high-density lipoprotein cholesterol or salivary cortisol was a predictor of adrenal insufficiency in patients with septic shock. *Shock* 2014; **42**: 16-21.
181. Sacre K, Dehoux M, Chauveheid MP, et al. Pituitary-adrenal function after prolonged glucocorticoid therapy for systemic inflammatory disorders: an observational study. *J Clin Endocrinol Metab* 2013; **98**: 3199-205.
182. Barquist E, Kirton O. Adrenal insufficiency in the surgical intensive care unit patient. *JTrauma* 1997; **42**: 27-31.
183. Van den Berghe G. On the Neuroendocrinopathy of Critical Illness. Perspectives for Feeding and Novel Treatments. *Am J Respir Crit Care Med* 2016; **194**: 1337-48.
184. 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**: 1751-63.
185. 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. *Crit Care Med* 2017; **45**: 2078-88.
186. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med* 2017; **43**: 304-77.
187. Debaveye Y, Vandenbrande J, Van den Berghe G. Endocrine emergencies. In: Tubaro M, Danchin N, Filippatos G, Goldstein P, Vranckx P, Zahger D, eds. The ESC Textbook of Intensive and Acute Cardiac Care. New York: Oxford University Press; 2011: 709-17.

188. Bornstein SR, Allolio B, Arlt W, et al. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2016; **101**: 364-89.
189. Sprung CL, Annane D, Keh D, et al. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111-24.
190. Annane D, Sebille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002; **288**: 862-71.
191. Finney SJ, Zekveld C, Elia A, Evans TW. Glucose control and mortality in critically ill patients. *JAMA* 2003; **290**: 2041-7.
192. Investigators CS, Annane D, Cariou A, et al. Corticosteroid treatment and intensive insulin therapy for septic shock in adults: a randomized controlled trial. *JAMA* 2010; **303**: 341-8.
193. Keh D, Trips E, Marx G, et al. Effect of Hydrocortisone on Development of Shock Among Patients With Severe Sepsis: The HYPRESS Randomized Clinical Trial. *JAMA* 2016; **316**: 1775-85.
194. Lv QQ, Gu XH, Chen QH, Yu JQ, Zheng RQ. Early initiation of low-dose hydrocortisone treatment for septic shock in adults: A randomized clinical trial. *Am J Emerg Med* 2017; **35**: 1810-4.
195. Venkatesh B, Finfer S, Cohen J, et al. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. *N Engl J Med* 2018; **378**: 797-808.
196. Annane D, Renault A, Brun-Buisson C, et al. Hydrocortisone plus Fludrocortisone for Adults with Septic Shock. *N Engl J Med* 2018; **378**: 809-18.
197. Meduri GU, Bridges L, Shih MC, Marik PE, Siemieniuk RA, Kocak M. Prolonged glucocorticoid treatment is associated with improved ARDS outcomes: analysis of individual patients' data from four randomized trials and trial-level meta-analysis of the updated literature. *Intensive Care Med* 2016; **42**: 829-40.
198. Tagami T, Matsui H, Horiguchi H, Fushimi K, Yasunaga H. Low-dose corticosteroid use and mortality in severe community-acquired pneumonia patients. *Eur Respir J* 2015; **45**: 463-72.
199. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 1511-8.
200. Hermans G, Wilmer A, Meersseman W, et al. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. *Am J Respir Crit Care Med* 2007; **175**: 480-9.
201. Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G. Clinical review: Critical illness polyneuropathy and myopathy. *Crit Care* 2008; **12**: 238.
202. Patel GP, Balk RA. Systemic steroids in severe sepsis and septic shock. *Am J Respir Crit Care Med* 2012; **185**: 133-9.

2

AIM AND STUDY OBJECTIVES

The **general aim** of this doctoral thesis was to gain more insight into the regulation of the HPA axis response during the course of critical illness, in order to understand the so-called "ACTH-cortisol dissociation" and the pathophysiology of "critical illness related adrenal insufficiency". Many drugs may have HPA suppressive properties as suggested by small studies in surgical patients, ICU patients, and animal experiments. Therefore, the **first** hypothesis of my PhD postulated that the **pharmacological effects of frequently used drugs** used during surgery or the acute phase of critical illness may **explain the acute "ACTH-cortisol dissociation"** as observed already upon admission to the ICU. Identifying iatrogenic HPA axis suppressive drugs could then potentially guide treatment in the ICU. **Second**, as adrenal glands, harvested postmortem from patients who had been critically ill for several weeks, revealed a distorted architecture, lipid droplet depletion, and suppressed ACTH-regulated gene expression as compared with patients dying after short illness or individuals dying suddenly out of hospital, we hypothesized that prolonged **deprivation of trophic ACTH-effects on the adrenal cortex** may contribute to **adrenal insufficiency**, whereas reactivation of the pituitary with **increased plasma ACTH** occurs in those patients who **recover**. It is also unknown to what extent adrenocortical function parameters relate to sepsis/septic shock, to the clinical need for glucocorticoid treatment, and to survival. **Finally**, low plasma ACTH during critical illness has been explained either by shock/inflammation-induced cell damage to hypothalamus and/or pituitary or by feedback-inhibition exerted by free-cortisol, possibly predisposing to central adrenal insufficiency. We hypothesized that sustained **elevated cortisol levels**, via reduced breakdown, may **suppress CRH and ACTH synthesis and release** via feedback-inhibition in the prolonged phase of critical illness. Testing these hypotheses will add new insights in the controversial topic of adrenal insufficiency in the ICU. The results of these studies could open perspectives towards effective preventive strategies, identifying the right timing, therapy, and patient population, to protect ICU patients against the risk of developing adrenal insufficiency.

To test the proposed hypotheses, we formulated 3 main **study objectives**.

1. **The first objective** was to investigate the **pharmacological effects** of frequently used drugs **on the acute "ACTH-cortisol dissociation"** in ICU patients in an **observational association study**.

2. **The second objective** was to document the **changes over time** - from the 7th day in ICU up to recovery or death - in plasma **ACTH** and (**free)cortisol, urinary cortisol metabolites,** and in plasma **cortisol responses to cosyntropin,** for long-stay (≥4 weeks in ICU) patients as compared with shorter stayers and for patients with and without sepsis/septic shock, in relation to subsequent clinical need for glucocorticoid treatment and to survival, in a **longitudinal prospective observational study**.

3. **The third objective** was to compare the **ACTH** and (**free)cortisol responses** to **corticorelin**, a synthetic human CRH analogue, in the acute, subacute and prolonged phases of critical illness with those of healthy subjects, in a **randomized, double-blind, placebo-controlled crossover cohort study.**

3

DRUG-INDUCED HPA AXIS ALTERATIONS DURING ACUTE CRITICAL ILLNESS

Adapted from:

- **Peeters B,** Guiza F, Boonen E, Meersseman P, Langouche L, Van den Berghe G. Drug-induced HPA axis alterations during acute critical illness: a multivariable association study. Clin Endocrinol (Oxf) 2017; 86: 26-36.

Abstract

Background Critical illness is hallmarked by low plasma ACTH in the face of high plasma cortisol. We hypothesized that frequently used drugs could play a role by affecting the hypothalamic-pituitary-adrenal axis. We therefore performed an observational association study in 156 medical-surgical critically ill patients.

Methods Plasma concentrations of ACTH and total/free cortisol were quantified upon ICU admission and throughout the first 3 ICU-days. The independent associations between drugs administered 24h prior to ICU-admission and plasma ACTH and cortisol concentrations upon ICU-admission were quantified with use of multivariable linear regression analyses.

Results Upon ICU-admission, compared with healthy subjects, patients revealed low mean±SEM plasma ACTH concentrations (2.7±0.6 pmol/l vs. 9.0±1.6 pmol/l, P<0.0001) in the face of unaltered total plasma cortisol (336.7±30.4 nmol/l vs. 300.8±16.6 nmol/l, P=0.3) and elevated free plasma cortisol concentrations (41.4±5.5 nmol/l vs. 5.5±0.8 nmol/l, P=0.04). Plasma ACTH concentrations remained low (P<0.001) until day 3 whereas plasma (free)cortisol concentrations steeply increased and remained high (P<0.001). No independent correlations with plasma ACTH were found. In contrast, the total admission plasma cortisol concentration was independently and negatively associated with the cumulative opioid (P=0.001) and propofol (P=0.02) dose, the use of etomidate (P=0.03), and positively with the cumulative dobutamine dose (P=0.0007).

Conclusions Besides the known suppressive effect of etomidate, also opioids and propofol may suppress and dobutamine increase plasma cortisol in a dose-dependent manner. The observed independent associations suggest drug effects not mediated centrally via ACTH, but rather peripherally by a direct or indirect action on the adrenal cortex.

Introduction

Critical illnesses necessitating intensive care are considered to represent conditions of severe physical stress. The traditional concept of the stress response comprises hypothalamic release of corticotropin-releasing hormone (CRH) that activates pituitary adrenocorticotropic hormone (ACTH), which drives adrenocortical cortisol synthesis and secretion. Cortisol controls the activation status of the hypothalamic-pituitary-adrenal (HPA) axis via negative feedback inhibition at the level of the hypothalamus and the pituitary. The stress response further comprises an activation of the sympathetic nervous system and catecholamine release by the chromaffin cells of the adrenal medulla.

Although elevated plasma ACTH concentrations are considered to be the main driver of increased cortisol availability in response to stress, this does not appear to be applicable for the critically ill. Critical illness is hallmarked by an 'ACTH-cortisol dissociation', i.e. low plasma ACTH already during the first days of intensive care in the face of high plasma cortisol.[1,2] It was recently shown that suppressed cortisol breakdown, together with a mildly increased cortisol production, predominantly determines the level of hypercortisolemia in Intensive Care Unit (ICU) patients.[2] In this context, low plasma ACTH concentrations could be explained by negative feedback inhibition exerted by high amounts of circulating cortisol that is not metabolized.

Prior to admission to ICU, surgical as well as medical patients often received drugs that can theoretically affect the HPA axis, either directly at the hypothalamus-pituitary or adrenocortical level or indirectly via a modulation of the sympathetic nervous system[3-5] and some are continued during the acute phase of critical illness. Previous studies have shown that anesthetic drugs, more specifically the hypnotic etomidate, can suppress adrenocortical synthesis of cortisol.[6,7] However, many other drugs may have HPA suppressive properties as suggested by small interventional studies in surgical[8-10] and ICU patients[11], by observational studies of surgical patients[12,13], and by animal experiments[14-16]. Based on the available evidence, we hypothesized that the pharmacological effects of drugs used during surgery or the acute phase of critical illness prior to ICU admission may explain the acute 'ACTH-cortisol dissociation', as observed already upon admission to the ICU. To test this hypothesis, we used a multivariable linear regression analysis to investigate any independent associations

between these drugs and the concentrations of ACTH and cortisol in plasma collected upon ICU admission from a mixed set of surgical and medical ICU patients.[17]

Methods

Patients and documentation of the administered drugs

This study used plasma samples, previously collected - in the context of another study - from 174 adult ICU patients who did not have predisposing risks for HPA axis dysfunction, which includes chronic treatment with glucocorticoids, steroids or anti-steroid chemotherapy within the last 3 months, or other drugs predisposing to adrenal insufficiency (phenytoin, rifampicin, glitazones, imipramin, phenothiazine, phenobarbital, drug abuse).This study showed that the use of parenteral nutrition (PN) did not explain the 'ACTH-cortisol dissociation' present from the first day in ICU onward.[17] Written informed consent was obtained from all patients or their next-of-kin. The study protocol and consent forms were approved by the Institutional Ethical Review Board (ML4190). For the current study, the electronic medical records of these 174 patients were re-analyzed and all drugs and cumulative drug doses administered 24h prior to ICU admission in the operating room, emergency room, post-anesthesia care unit, and/or on the ward were documented. This data search revealed that 18 patients had received corticosteroids within 24h prior to ICU admission and were therefore excluded for further analysis. Characteristics of the 156 remaining patients are described in **Table 1**.

All documented intravenous, subcutaneous or inhaled drugs were grouped into 5 relevant drug categories, of which a potential impact on the HPA axis has been suggested in the literature. Drugs that were given to fewer than 5 patients were not taken into account to exclude findings by chance (**Table 2**). Equipotent drug doses were calculated for opioids and for volatile anesthetics, taking into account the relative potency of each individual drug (**Table 2**). For dobutamine, cumulative doses were expressed as folds of 4200 µg, corresponding to an infusion rate of 1 µg kg^{-1} min^{-1} for a 70 kg individual during 1 hour.

As healthy references, morning ACTH and cortisol plasma concentrations were available from 20 overnight-fasted healthy volunteers with comparable demographics as the patient population (**Table 1**).[17]

Quantification of plasma ACTH and (free) cortisol concentrations

Admission (IQR 14:14PM – 19:29PM) and daily morning (6:00AM) blood samples were collected in pre-chilled ethylenediaminetetraacetic acid (EDTA) tubes and immediately

Table 1: Characteristics of the patients and healthy volunteers

	Patients (n=156)	Healthy volunteers (n=20)	P-value
Demography and anthropometry			
Male sex - no. (%)	103 (66)	11 (55)	0.3
Age - yr (mean ± SEM)	66 ± 1.1	58 ± 1.1	<0.0001
BMI - kg/m² (mean ± SEM)	26.5 ± 0.4	24.3 ± 0.7	0.007
Admission characteristics			
Diabetes mellitus - no. (%)	33 (21)		
Malignancy - no. (%)	34 (22)		
Pre-admission dialysis – no. (%)	2 (1)		
Sepsis - no. (%)	53 (34)		
APACHE II score (mean ± SEM)	24 ± 0.8		
NRS score > 4 – no. (%)	32 (21)		
eGFR - mL min-1 1.73 m-2 (mean ± SEM)	77 (2.5)		
Plasma total bilirubin - mg/dL (median (IQR))	0.8 (0.5-1.2)		
Emergency admission - no. (%)	76 (49)		
Randomization EPaNIC trial: Early - no. (%)	83 (53)		
Surgery <24h pre-admission ICU - no. (%)	113 (72)		
Diagnostic admission categories			
Cardiac surgery - no. (%)	86 (55)		
Complicated surgery / Trauma - no. (%)	44 (28)		
Medical - no. (%)	26 (17)		
Clinical outcomes			
Duration of ICU stay – median (interquartile range)	9 (4-11)		
ICU nonsurvivor - no. (%)	6 (4%)		

The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects severity of illness, with higher values indicating more severe illness, and can range from 0 to 71. Scores on nutritional risk screening (NRS) range from 0 to 7, with higher scores indicating a higher risk of malnutrition. eGFR stands for estimated glomerular filtration rate. The duration of surgery was defined as the time from skin incision until the end of skin closure, if surgery took place 24h prior to admission to the ICU.

placed on ice, centrifuged at 4°C and then stored at -80°C until assay. Total plasma cortisol concentrations (Immunotech, Prague, Czech Republic) and plasma cortisol-binding-globulin (CBG) concentrations (Riazen, Louvain-La-Neuve, Belgium) were quantified with use of

Table 2. Drug categories

Drug category	Generic drug name	Patients received - no. (%)
Anesthetics	Propofol	96 (62)
	Etomidate	62 (40)
	Volatile anesthetics (sevoflurane, desflurane)	102 (65)
Analgetics	Opioids (morphine, alfentanil, fentanyl, sufentanil, tramadol, piritramide)	134 (86)
	Acetaminophen	17 (11)
Sedatives	Midazolam	88 (56)
Vasopressors / Inotropics	Norepinephrine	116 (74)
	Dobutamine	50 (32)
	Enoximone	18 (12)
	Vasopressin / Desmopressin	11 (7)
Anticoagulants	Enoxaparin	8 (5)
	Heparin	63 (40)

Equipotent drug doses were calculated for volatile anesthetics (Minimum Alveolar Concentration (MAC) in O2 at 37°C PB760 (%) for sevoflurane = 1.8, for desflurane = 6.6) and for opioids (relative to morphine (=1): alfentanil = 30, fentanyl = 120, remifentanil = 120, sufentanil = 1200, tramadol = 0.1, piritramide = 0.75).

radioimmunoassay. Plasma ACTH concentrations were measured with a double-monoclonal immunoradiometric assay (Brahms Diagnostics, Berlin, Germany). Plasma albumin was quantified by the bromocresol green method with a Colorimetric assay (BioAssay Systems, Hayvard, CA). Plasma free cortisol was calculated with use of the previously validated Coolens' formula adapted for individual albumin and CBG concentrations.[18]

Statistical analyses

Wilcoxon rank sum test was used to compare data that did not have a normal distribution and unpaired Student's t-tests was used for comparison of normally distributed data. The Wilcoxon matched pairs signed rank test for repeated measurements was used to compare plasma concentrations within patients on the admission day with those on the consecutive days.

The primary study aim was to assess, in a multivariable linear regression analysis, the presence of an independent association between the cumulative doses of the drugs given during the 24h prior to ICU admission and the plasma ACTH or total cortisol concentrations upon ICU admission and to determine the effect size hereof. The multivariable linear

regression model was adjusted for the following baseline risk factors: gender, BMI, presence of diabetes, presence of malignancy, presence of pre-admission dialysis, presence of sepsis upon admission (according to the criteria of the American College of Chest Physicians-Society of Critical Care Medicine),[19] APACHE II score on admission, nutritional risk score (NRS) score, eGFR (estimated glomerular filtration rate), plasma total bilirubin, emergency or elective admission, randomization to early PN or late PN, diagnostic category, free plasma cortisol concentration (for investigating the association with plasma ACTH), and plasma ACTH concentration (for investigating the association with total plasma cortisol). The presence of multicollinearity among the regressor variables and baseline risk factors was assessed using the tolerance, variance inflation factor (Vif), condition indices, and variance proportions.[20] The analyses were repeated after excluding such variables from the model. To assess the presence of a (log)linear relationship between the continuous independent regressor variables and the dependent variables (plasma ACTH or plasma total cortisol concentration), the cumulative doses of each drug were automatically binned by the statistical software, to visualize the pattern of the association with the outcome of interest. Drugs that were either not given or given in a same fixed dose were dichotomized, drugs displaying a J-shaped relationship were categorized in 3 groups, all other drugs were added as continuous variables.

As a secondary aim, the time courses of plasma ACTH and total and free cortisol concentrations during the first 3 consecutive days in ICU were plotted for those drugs that were identified, in the multivariable linear regression analysis, as independently associated with admission plasma ACTH or cortisol concentrations. For this, the cumulative doses of the drugs given 24h prior to ICU admission were divided in the identified categories as explained above, or, for those drugs with a (log)linear association with the outcomes, in two groups, at or below versus above the median cumulative dose. The presence of any potential subsequent rebound effect on plasma cortisol or ACTH concentrations was investigated in these time series with use of repeated measures ANOVA. For those drugs that were identified as independently associated with admission plasma ACTH or cortisol concentrations, patient files were screened to assess whether or not and in which dose range this drug administration was continued.

Statistical analyses were performed with use of JMP® version 11.0.0 (SAS Institute, Inc, Cary, NC) and SPSS software, version 22 (IBM, Armonk, NY). Two-sided P-values of 0.05

or less were considered to indicate statistical significance. No corrections for multiple comparisons were done.

Results

Plasma concentrations of ACTH, total cortisol, and free cortisol from ICU admission up to day 3 in ICU

All 156 patients remained in the ICU for at least 3 days. Upon admission to the ICU, patients had much lower mean±SEM plasma ACTH concentrations than healthy subjects with comparable demographics (2.7±0.6 pmol/l vs. 9.0±1.6 pmol/l, P<0.0001) and plasma ACTH concentrations remained below normal throughout the 3 first days in ICU (P<0.001) (**Figure 1, panel A**). Upon ICU admission, mean±SEM total plasma cortisol concentrations in patients were not different from those in healthy subjects (336.7±30.4 nmol/l vs. 300.8±16.6 nmol/l, P=0.3) but plasma mean±SEM free cortisol concentrations were 7-fold elevated (41.4±5.5 nmol/l vs. 5.5±0.8 nmol/l, P=0.04) (**Figure 1, panel B-C**). The latter can be explained by a decrease in mean±SEM plasma CBG and albumin levels compared to healthy controls (621.4±11.5 nmol/l vs. 801.7±36.4 nmol/l (P=0.001) and 35.9±0.8 g/l vs. 47.3±1.3 g/l (P<0.0001), respectively) from ICU admission onwards. From the morning after ICU admission, total and free cortisol plasma concentrations were significantly higher than those upon ICU admission (total cortisol: 502.3±22.1 nmol/l vs. 336.7±30.4 nmol/l, P<0.0001; free cortisol: 58.0±5.5 nmol/l vs. 41.4±5.5 nmol/l, P<0.0001) and remained high until day 3 in ICU (P<0.001).

Independent association of the cumulative drug doses with ICU admission plasma ACTH and cortisol concentrations adjusted for baseline risk factors

Based on the visualisation of the association between plasma ACTH concentrations and the cumulative doses of each drug, the cumulative doses of propofol, midazolam, opioids, volatile anesthetics, dobutamine and heparin were entered into the multivariable linear regression model as continuous variables (**Figure S1 in Supplementary Appendix**). The cumulative doses of etomidate, paracetamol, enoximone, enoxaparin, desmopressin and vasopressin, were dichotomized as "given" versus "not given" for the entering into the multivariable model. The cumulative dose of norepinephrine displayed a J-shaped relationship with admission plasma ACTH concentration and was therefore added to the model categorized into 3 groups.

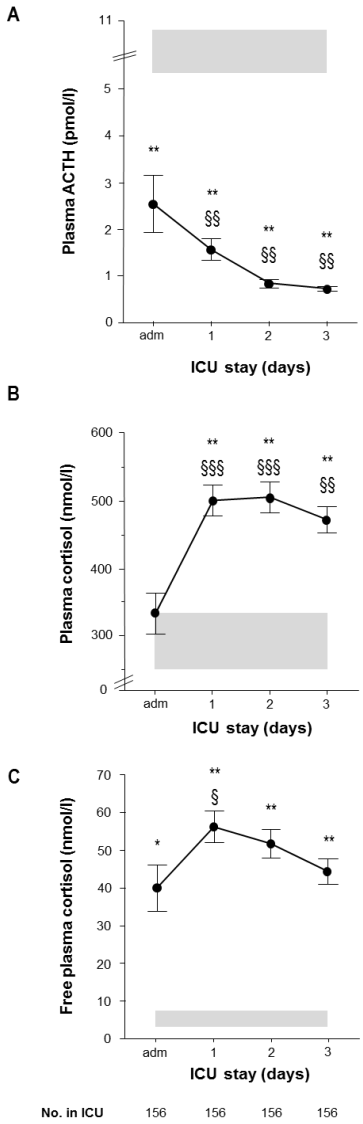


Figure 1 | Plasma ACTH, cortisol and free cortisol time course from ICU admission throughout the first 3 days of critical illness

Mean values and standard errors for plasma ACTH (Panel A), total cortisol (Panel B), and free cortisol (Panel C) in patients from admission onwards until day 3 of ICU stay. The shaded area represents the interquartile range of morning values in healthy control subjects. * P≤0.05, ** P<0.001, for the comparison with controls. § P≤0.05, §§ P<0.01, §§§ P<0.0001, for the comparison of paired values of the consecutive days with the admission sample. For each day, the number of patients still in ICU is displayed below the figure. ICU denotes intensive care unit, adm denotes admission.

In the multivariable linear regression analysis, adjusted for baseline risk factors and plasma free cortisol concentrations, none of the drugs administered within 24h prior to ICU admission were significantly associated with plasma ACTH concentrations upon admission (total model R^2=0.12, P=0.94) (**Table 3**).

For the multivariable linear regression analysis, with admission plasma total cortisol concentrations as the dependent variable, the cumulative doses of propofol, opioids, volatile anesthetics, dobutamine and heparin were added as continuous variables to the multivariable linear regression model (**Figure S2 in Supplementary Appendix**). The cumulative doses of etomidate, paracetamol, enoximone, enoxaparin, desmopressin and vasopressin were again added dichotomized, as either "given" versus "not given". The cumulative doses of midazolam and norepinephrine displayed a J-shaped relationship with admission plasma cortisol concentrations and were therefore added to the model categorized into 3 groups.

The multivariable linear regression analysis, adjusted for baseline risk factors and admission plasma ACTH concentrations, revealed that the cumulative doses of several drugs, administered within 24h prior to ICU admission, were independent determinants for total plasma cortisol concentrations upon ICU admission (total model R^2=0.56, P<0.0001) (**Table 4**). Indeed, total plasma cortisol concentration upon ICU admission was independently and negatively associated with the cumulative opioid dose [a decrease of 8.6 (95% CI -13.6 to -3.6) nmol/l in total plasma cortisol for every 10 mg morphine-equivalent given; P=0.001], the cumulative propofol dose [a decrease of 7.2 (95% CI -.13.4 to -1.0) nmol/l in total plasma cortisol for every 100 mg of propofol given; P=0.02] and the use of etomidate [a decrease of 65.6 (95% CI .125.6 to -5.7) nmol/l in total plasma cortisol when given; P=0.03], and positively with the cumulative dobutamine dose [an increase of 18.7 (95% CI 8.2 to 29.2) nmol/l plasma cortisol for every 4200 µg given (equal to 1 µg kg^{-1} min^{-1} for a 70 kg individual for one hour); P=0.0007]. Similar independent associations between plasma free cortisol and the cumulative doses of opioids and dobutamine were found (data not shown).

Using an additional forward-backward stepwise regression sensitivity analysis, with a probability to enter the model (F-to-enter) of 0.05 and a probability to leave the model (F-to-remove) of 0.05, the independent associations of etomidate, opioids, propofol and dobutamine with total plasma cortisol concentrations were confirmed (data not shown). The assessment of multicollinearity had identified potential collinearity for enoxaparin and age.

Table 3. Multivariable linear regression analyses determining significant and independent associations between drug doses of all included drugs and plasma ACTH concentrations upon admission, adjusted for baseline risk factors

Variables	*Estimated difference (95% CI in plasma ACTH concentratio (pmol/l)*	*P-value*
Gender (male vs. female)	-1,34 (-2,74 - 0,06)	0.06
BMI dichotomized (25>BMI≤40 vs. other)	-0,26 (-1,64 - 1,12)	0.7
Diabetes (present vs. not present)	1,56 (-0,19 - 3,31)	0.08
Malignancy (present vs. not present)	-0,22 (-1,97 - 1,54)	0.8
Pre-admission dialysis (present vs. not present)	0,32 (-5,69 - 6,33)	0.9
Sepsis upon admission (present vs. not present)	0,31 (-1,46 - 2,09)	0.7
APACHE II score on admission (per unit added)	-0,07 (-0,27 - 0,13)	0.5
NRS dichotomized (NRS ≥5 vs. <5)	-0,94 (-2,82 - 0,94)	0.3
eGFR (per mL min-1 1.73 m-2)	-0,01 (-0,06 - 0,04)	0.7
Plasma total bilirubin (per mg/dL)	-0,22 (-0,98 - 0,54)	0.6
Elective vs. emergency admission	-1,64 (-4,32 - 1,05)	0.2
Randomization to early PN vs. late PN	-0,64 (-1,93 - 0,64)	0.3
Diagnostic Category - as compared with Medical		
Cardiac surgery	0,70 (-3,33 - 4,72)	0.7
Complicated surgery/Trauma)	-1,15 (-3,96 - 1,65)	0.4
Free Cortisol (per nmol/l)	0,20 (-0,36 - 0,76)	0.5
Propofol (per 100 mg given)	0,00 (-0,18 - 0,17)	0.9
Etomidate (given vs. not given)	-0,21 (-1,88 - 1,46)	0.8
Midazolam (per 1 mg given)	-0,04 (-0,15 - 0,08)	0.5
Opioids (per 10 mg morphine-equivalent given)	-0,26 (-1,70 - 1,18)	0.7
Acetaminophen (given vs. not given)	-0,43 (-2,83 - 1,97)	0.7
Volatile Anesthetics (per % min equipotent dose given)	0,00 (-0,01 - 0,02)	0.4
Norepinephrine - as compared with not given		
When given >0. <2277 µg	-1,64 (-4,59 - 1,31)	0.9
When given >=2277 µg	-0,05 (-2,50 - 2,39)	0.3
Dobutamine (per 4200 µg given)	0,14 (-0,16 - 0,44)	0.4
Enoximone (given vs. not given)	-0,88 (-2,99 - 1,23)	0.4
Enoxaparin (given vs. not given)	0,17 (-2,96 - 3,31)	0.9
Heparin (per 1 IU given)	0,00 (0,00 - 0,00)	0.4
Desmopressin (given vs. not given)	-0,43 (-4,18 - 3,32)	0.8
Vasopressin (given vs. not given)	0,65 (-2,08 - 3,38)	0.6

The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects severity of illness, with higher values indicating more severe illness, and can range 0 to 71. Scores on nutritional risk screening (NRS) range from 0 to 7, with higher scores indicating a higher risk of malnutrition.

Table 4. Multivariable linear regression analyses determining significant and independent associations between drug doses of all included drugs and plasma total cortisol concentrations upon admission, adjusted for baseline risk factors

Variables	*Estimated difference (95% CI) in plasma cortisol concentration (nmol/l)*	*P-value*
Gender (male vs. female)	-44,7 (-96,3 - 6,8)	0.09
BMI dichotomized (25>BMI≤40 vs. other)	-42,6 (-91,5 - 6,2)	0.09
Diabetes (present vs. not present)	19,3 (-44,4 - 83,0)	0.6
Malignancy (present vs. not present)	39,0 (-24,8 - 102,8)	0.2
Pre-admission dialysis (present vs. not present)	-13,0 (-230,0 - 204,1)	0.9
Sepsis upon admission (present vs. not present)	56,5 (-7,0 - 120,0)	0.08
APACHE II score on admission (per unit added)	-3,2 (-10,3 - 4,0)	0.4
NRS dichotomized (NRS ≥5 vs. <5)	-19,6 (-87,4 - 48,1)	0.6
eGFR (per mL min-1 1.73 m-2)	0,7 (-0,9 - 2,4)	0.4
Plasma total bilirubin (per mg/dL)	-20,6 (-47,7 - 6,5)	0.1
Elective vs. emergency admission	-132,1 (-227,6 - -36,5)	0.008
Randomization to early PN vs. late PN	8,9 (-37,7 - 55,4)	0.7
Diagnostic Category - as compared with Medical		
Cardiac surgery	104,0 (-60,7 - 268,7)	0.2
Complicated surgery/Trauma)	-13,8 (-122,4 - 94,8)	0.8
ACTH (per pmol/l)	2,4 (-3,9 - 8,8)	0.5
Propofol (per 100 mg given)	**-7,2 (-13,4 - -1,0)**	**0.02**
Etomidate (given vs. not given)	**-65,6 (-125,6 - -5,7)**	**0.03**
Midazolam - as compared with not given		
When given >0, <13 mg	5,7 (-82,5 - 93,8)	0.9
When given >=13 mg	-34,5 (-123,2 - 54,2)	0.4
Opioids (per 10 mg morphine-equivalent given)	**-8,6 (-13,6 - -3,6)**	**0.001**
Acetaminophen (given vs. not given)	-64,4 (-149,0 - 20,2)	0.1
Volatile Anesthetics (per % min equipotent dose given)	-0,1 (-0,6 - 0,3)	0.5
Norepinephrine - as compared with not given		
When given >0, <2277 µg	35,9 (-70,9 - 142,8)	0.5
When given >=2277 µg	-57,6 (-145,5 - 30,2)	0.2
Dobutamine (per 4200 µg given)	**18,7 (8,2 - 29,2)**	**0.0007**
Enoximone (given vs. not given)	-25,1 (-101,4 - 51,3)	0.5
Enoxaparin (given vs. not given)	-25,6 (-138,6 - 87,3)	0.7
Heparin (per 1 IU given)	0,0 (0,0 - 0,0)	0.06
Desmopressin (given vs. not given)	-95,5 (-230,2 - 39,1)	0.2
Vasopressin (given vs. not given)	-80,6 (-178,9 17,6)	0.1

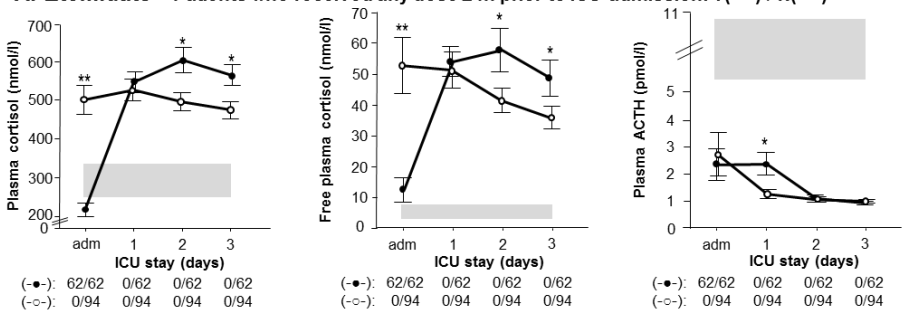
The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects severity of illness, with higher values indicating more severe illness, and can range 0 to 71. Scores on nutritional risk screening (NRS) range from 0 to 7, with higher scores indicating a higher risk of malnutrition.

Repeating the multivariable linear regression analyses after taking enoxaparin and age out of the model did not affect any of the results. In the subset of surgical ICU patients, adjusted for the same baseline risk factors and admission plasma ACTH concentrations, the independent associations of etomidate, opioids, propofol and dobutamine with total plasma cortisol concentrations were confirmed (data not shown).

Univariate time course of total/free cortisol plasma concentrations for those drugs that were identified by multivariable analysis as independently associated with cortisol

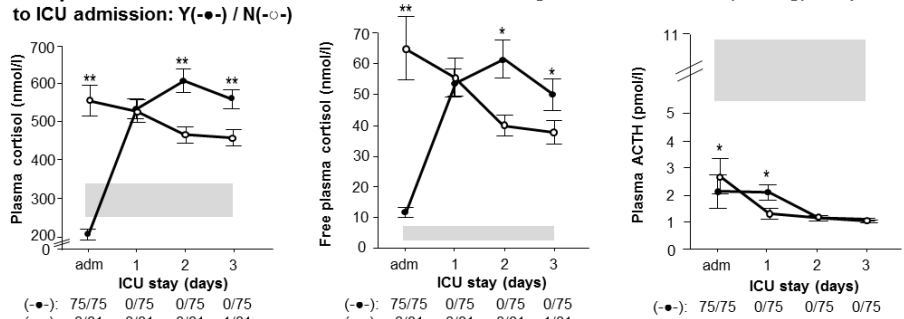
Of all patients, 142 received at least 1 of the 4 drugs that were associated with admission total cortisol levels. 21 patients received all 4 drugs. Patients who had received etomidate in the 24h prior to ICU admission, revealed significantly lower total and free plasma cortisol concentrations upon ICU admission than did patients who had not received etomidate, but equally low plasma ACTH concentrations. On the following 3 days in the ICU, the plasma cortisol levels of patients who had received etomidate prior to ICU admission showed a rebound rise reaching levels that were higher than those of patients not having received etomidate, while in this latter group plasma ACTH levels declined more quickly (**Figure 2A**). On the following 3 days in ICU, patients of both groups did not receive etomidate. A similar rebound rise in plasma cortisol levels was apparent for patients who had received a cumulative opioid dose higher than the median (180 mg morphine equivalent dose) in the 24h prior to ICU admission as compared with patients who had received less opioids (**Figure 2B**). On the following 3 days in ICU, the majority of patients of both groups (83% of patients on day 1, 83% on day 2, and 63% on day 3) still received opioids, in a dose that was much lower than the cumulative median dose upon admission of 180 mg (median dose of 26.1 mg on day 1, 17.9 mg on day 2, and 6.5 mg on day 3). In patients receiving more than the median opioid dose, plasma ACTH concentrations were lower upon ICU admission and further decreased more slowly in comparison with patients receiving opioids in a lower dose. Patients who had received a cumulative dose of propofol higher than the median of 227.5 mg in the 24h prior to ICU admission displayed lower ICU admission total and free plasma cortisol concentrations than patients who had received propofol at a lower dose, after which the two groups became comparable (**Figure 2C**). Propofol administration increased during the first day in ICU, after which it declined (81% of patients on day 1, 47% on day 2, and 27% on day 3) with a median

A. Etomidate - Patients who received any dose 24h prior to ICU admission: Y(-●-) / N(-○-)



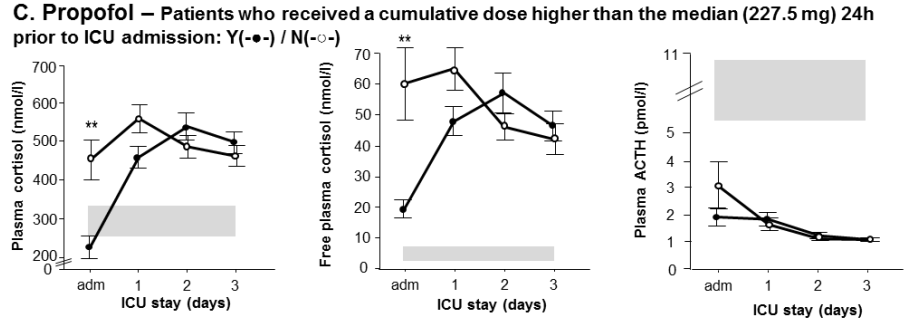
(-●-): 62/62 0/62 0/62 0/62
(-○-): 0/94 0/94 0/94 0/94

B. Opioids - Patients who received a cumulative dose higher than the median (180 mg) 24h prior to ICU admission: Y(-●-) / N(-○-)



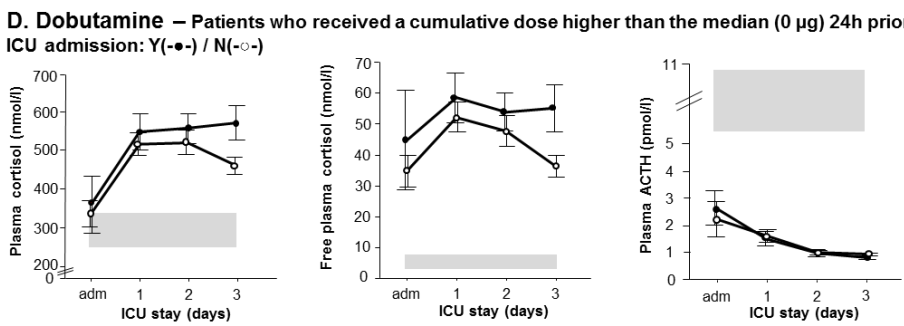
(-●-): 75/75 0/75 0/75 0/75
(-○-): 0/81 0/81 0/81 1/81

C. Propofol – Patients who received a cumulative dose higher than the median (227.5 mg) 24h prior to ICU admission: Y(-●-) / N(-○-)



(-●-): 78/78 71/78 39/78 20/78
(-○-): 0/78 50/78 25/78 18/78

D. Dobutamine – Patients who received a cumulative dose higher than the median (0 µg) 24h prior to ICU admission: Y(-●-) / N(-○-)



(-●-): 50/50 37/50 31/50 21/50
(-○-): 0/106 8/106 5/106 3/106

Figure 2 | Univariate time course of total/free cortisol and ACTH plasma concentrations for those drugs that were identified by multivariable analysis as independently associated with cortisol

Mean values and standard errors for plasma total and free cortisol in patient groups from admission onwards until day 3 of ICU stay, divided by the received/not received etomidate (panel A), median cumulative opioid dose (panel B), median cumulative propofol dose (panel C), and median cumulative dobutamine dose (panel D), during the 24h prior to ICU admission. The presence of a subsequent rebound effect on plasma total/free cortisol concentrations was investigated. * P≤0.05, ** P<0.001, comparing mean values of both groups. The shaded area represents the interquartile range of morning values in healthy control subjects. For each consecutive day, the number of patients who received the drug (panel A), or who received more than the cumulative median dose upon admission (panel B, C, D), is displayed below the figure. ICU denotes intensive care unit, adm denotes admission.

dose of 1248 mg on day 1, 0 mg on day 2, and 0 mg on day 3). No rebound rise in plasma cortisol levels was apparent. Plasma ACTH concentrations were equally low in both groups. In contrast, the stimulatory effect of dobutamine, shown by the results of the multivariable analysis, could not be illustrated in the univariate plots, and also no effect on plasma ACTH concentrations was observed (**Figure 2D**). On the following 3 days in ICU, dobutamine administration declined (29% of patients on day 1, 23% on day 2, and 15% on day 3), with a median dose of 0 mg on day 1, 2, and 3.

Discussion

In this mixed population of critically ill patients, low plasma ACTH concentrations were documented upon admission to the ICU in the face of normal total and elevated free cortisol plasma concentrations. A further lowering of plasma ACTH and a steep rise in plasma total/free cortisol were shown from the morning after admission to the ICU onwards. With multivariable analysis, adjusting for other known determinants of the stress response, it was shown that none of the drugs administered 24h prior to ICU admission independently affected plasma ACTH on ICU admission. However, for opioids, etomidate, and propofol administered 24h prior to ICU admission a suppressive effect on admission plasma cortisol was suggested, whereas for dobutamine this effect on admission plasma cortisol appeared to be stimulatory. These associations were independent of the medical or surgical diagnostic category, severity of illness, sepsis, or other patient characteristics.

Except for etomidate, it is generally assumed that sedative and analgesic drugs suppress the stress response via a central inhibition of the HPA axis and of the sympathetic nervous system resulting in a decreased release of CRH and ACTH from the hypothalamus and pituitary.[5] However, in this study, none of the drugs administered 24h before ICU-admission were found to be independently associated with the plasma ACTH concentration upon ICU admission. We can only speculate on why such a correlation was not present in the critically ill. First, plasma ACTH was only measured at one single time point, whereas ACTH release follows a dynamic pulsatile pattern. Second, higher plasma ACTH values may have occurred prior to the ICU admission and could thus have been missed. Indeed, previous studies have documented a rise in plasma ACTH and cortisol during and shortly after surgery, followed by a rapid fall in plasma ACTH to baseline levels while plasma cortisol remained high.[21,22] Third, other mechanisms responsible for a low plasma ACTH may play a dominant role. These comprise negative feedback inhibition exerted by the elevated plasma free cortisol, or by inflammation and ischemia at the level of the pituitary or the hypothalamus.[23,24] Conceivably, inhibition of ACTH release through such mechanisms may have dominated in the critically ill which may have hidden any additional central pharmacological suppression on ACTH release. Fourth, it has been shown that the stimulation of the HPA axis and of the sympathetic nervous system synergistically interact with each other in the complex microenvironment of the adrenal gland and that they are

functionally interdependent.[25] As sedative and analgesic drugs have been shown to evoke a sustained suppression of sympathetic activity[4,9,10,13], this may abolish such an effect.

In contrast with ACTH, plasma cortisol was significantly and independently associated with the cumulative doses of etomidate, propofol, opioids and dobutamine. A suppressive effect of opioids, etomidate and propofol and a stimulatory effect of dobutamine on plasma cortisol was suggested. The effect size of opioids was large and dose-dependent. Acute opioid administration to healthy individuals, prolonged opioid administration to patients suffering from chronic pain and intraoperative intravenous opioid administration in surgical patients have shown to result in suppressed plasma ACTH and/or cortisol concentrations.[8-10,26-30] Also during active heroin addiction, an opioid most commonly used as a recreational drug, or early in methadone-maintenance treatment, an opiate with slow absorption and a long half-life, the HPA axis response to the removal of glucocorticoid negative feedback by metyrapone administration was blunted.[31] However, during long-term stabilization in methadone maintenance, with cessation of illicit drug use, responsivity to metyrapone administration normalizes, and even abstinence from opioids in dependent individuals is associated with HPA axis activation.[32] An effect on the hypothalamus and the pituitary are assumed to mediate such an effect of opioids on the HPA axis27 most likely via the κ opioid receptor.[16] However, also the adrenal gland expresses specific opioid binding sites, which, after binding with opioids, could mediate a direct inhibition of cortisol secretion.[33] This could explain why only cortisol and not ACTH appeared affected by opioids in the here studied critically ill patients.

The results of this study also confirmed a suppressive effect of etomidate on plasma cortisol, even after one single induction dose. Etomidate is a known suppressor of adrenocortical cortisol production by inhibiting 11-beta-hydroxylase, a key enzyme for cortisol synthesis.[34] Prolonged etomidate infusion during critical illness has been shown to be associated with an increased mortality and was therefore abandoned as sedative for patients in ICUs.[35] However, a recent Cochrane review concluded that a single induction dose of etomidate was not related with an increase in mortality in critical ill patients, although random plasma cortisol concentrations were lowered.[6]

The current study also identified propofol as a possible suppressor of plasma cortisol. A previous study concluded that continuous intravenous infusion of propofol for up to 24h in critically ill patients did not impair adrenal steroidogenesis.[11] However, in patients admitted to the ICU after cardiopulmonary bypass surgery, continuous infusion of propofol

has been associated with an attenuated rise of plasma cortisol, when compared with an anesthetic regimen based on sufentanil and midazolam.[4] It is well known that propofol rapidly binds to GABA(gamma-aminobutyric acid)-A-receptors, which are ubiquitous in the central nervous system. However, no effect on plasma ACTH could be demonstrated in the current study, which is not in favour of a central effect of propofol. However, GABA-A-receptor sites have been reported in rat adrenal chromaffin cells[36] which modulate catecholamine secretion[37] whereby indirectly cortisol secretion could be affected in this complex microenvironment. The GABA-A-receptor has also been described in bovine glomerulosa cells of the adrenal cortex, which mediate an inhibition of aldosterone secretion.[38] Therefore, rather than via a central inhibitory effect, propofol might also directly inhibit cortisol secretion by binding to the GABA-A-receptor in the adrenal gland. This hypothesis is supported by the rise in corticosterone secretion which has been shown in rats immediately after discontinuation of propofol, in the absence of an effect on ACTH secretion.[14]

The results of the multivariable analysis suggested that dobutamine prior to ICU admission may increase plasma cortisol levels without an effect on plasma ACTH. As in univariate analysis, this effect was negligible, these data suggest that any stimulatory effect of dobutamine is likely small and context dependent. Catecholamines are known to stimulate the HPA axis by an activation of α1-adrenergic receptors and to inhibit the HPA axis by an activation of α2-adrenergic receptors in the central nervous system.[39] Dobutamine predominantly activates β1-adrenergic receptors, but also β2- and α1-adrenergic receptors are activated at doses used clinically.[40] Although dobutamine has been shown to increase plasma ACTH levels in a study of freely moving non-anesthetized rats, no results on corticosterone were given.[41] As the current evidence suggests that catecholamines activate the HPA axis via receptors in the central nervous system, the absence of an association with plasma ACTH in the current study does not support such an effect. Again, the complex interaction between the adrenal medulla and cortex suggests that a direct effect of catecholamines on the adrenal cortex is possible. In our study, the stimulatory effect on plasma cortisol levels could not be shown in a univariate analysis, suggesting that these effects are a result of the interaction of dobutamine with the HPA axis, only in the presence of the other administered drugs.

It cannot be concluded from this association study whether a suppressive effect of opioids, propofol and etomidate on plasma cortisol upon ICU admission is beneficial or

harmful. Lower plasma cortisol levels could either indicate that the drugs reduced the stress of trauma, major surgery or serious illnesses and hereby its detrimental consequences. However, the steep rise in plasma cortisol observed on the morning following ICU admission, when the transient drug effect waned off, could be an indication that patients need higher cortisol availability during critical illness. Unfortunately, the number of patients in this study was too small to investigate whether or not the iatrogenic suppression of cortisol upon ICU admission was associated with adverse outcome. However, the main clinical interest of this study is to inform physicians on the potential iatrogenic suppressive effects of commonly used drugs. Specifically, the data suggest that prior to considering treatment with hydrocortisone based on a low plasma cortisol, avoidable iatrogenic suppressive drugs should be discontinued and the effects on plasma cortisol documented.

This study has some limitations to highlight. First, the observed independent associations suggest drug effects not mediated centrally via ACTH, but rather peripherally by (in)direct actions on the adrenal cortex. However, the use of a single sample for quantification of plasma cortisol and ACTH concentrations may have precluded the detection of subtle effects on the dynamics of ACTH and cortisol secretion. Second, an association study does not provide proof of causality. Although the multivariable analyses were adjusted for known risk factors, unknown confounders may have played a role. Also, the study was not statistically powered to study outcome of any iatrogenic effect on cortisol availability. Furthermore, in this association study we did not account for differences in drug metabolism and thus only assessed drug doses rather than drug exposures.

In conclusion, besides the known suppressive effect of etomidate, also opioids and propofol may suppress plasma cortisol, and dobutamine may increase plasma cortisol, in a dose-dependent manner in critically ill patients. Whether or not drug-induced alteration of cortisol availability during acute critical illness is beneficial or harmful requires further investigation.

Acknowledgements

The authors thank the patients and healthy volunteers for participating, the clinical staff for excellent protocol compliance and the research assistants for sample handling and data entry. This work was supported by the Research Foundation-Flanders (FWO) [Grant numbers 11W9315N to Bram Peeters, G.0417.12 to Greet Van den Berghe]; by the Methusalem Program of the Flemish Government [METH/08/07 and METH/14/06 to Greet Van den Berghe via KU Leuven]; by European Research Council Advanced Grant [AdvG-2012-321670 to GVdB] from the European Union 7th framework programme.

Personal contribution

Bram Peeters participated in designing the study, conducting the study, gathering and analyzing the data, and writing the manuscript.

Conflict of interest

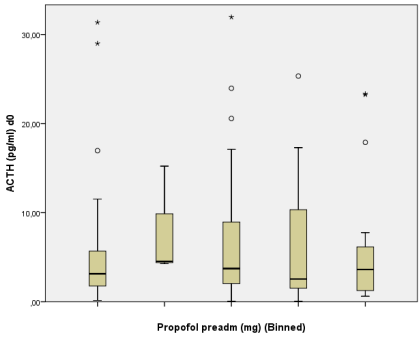
The authors declare that they have no conflict of interest.

References

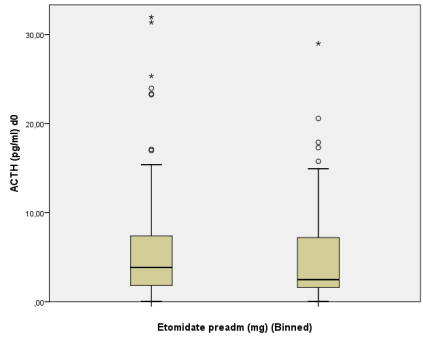
1. Vermes I, Beishuizen A, Hampsink RM, Haanen C. Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. *J Clin Endocrinol Metab* 1995; **80**: 1238-42.
2. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477-88.
3. Lindgren C, Dahlqvist P, Lindvall P, Nilsson L, Koskinen LO, Naredi S. Cortisol levels are influenced by sedation in the acute phase after subarachnoid haemorrhage. *Acta Anaesthesiol Scand* 2013; **57**: 452-60.
4. Plunkett JJ, Reeves JD, Ngo L, et al. Urine and plasma catecholamine and cortisol concentrations after myocardial revascularization. Modulation by continuous sedation. Multicenter Study of Perioperative Ischemia (McSPI) Research Group, and the Ischemia Research and Education Foundation (IREF). *Anesthesiology* 1997; **86**: 785-96.
5. Brorsson C, Dahlqvist P, Nilsson L, Thunberg J, Sylvan A, Naredi S. Adrenal response after trauma is affected by time after trauma and sedative/analgesic drugs. *Injury* 2014; **45**: 1149-55.
6. Bruder EA, Ball IM, Ridi S, Pickett W, Hohl C. Single induction dose of etomidate versus other induction agents for endotracheal intubation in critically ill patients. *Cochrane Database Syst Rev* 2015; **1**: CD010225.
7. Molenaar N, Bijkerk RM, Beishuizen A, et al. Steroidogenesis in the adrenal dysfunction of critical illness: impact of etomidate. *Crit Care* 2012; **16**: R121.
8. Raff H, Flemma RJ, Findling JW. Fast cortisol-induced inhibition of the adrenocorticotropin response to surgery in humans. *J Clin Endocrinol Metab* 1988; **67**: 1146-8.
9. Shinoda T, Murakami W, Takamichi Y, Iizuka H, Tanaka M, Kuwasako Y. Effect of remifentanil infusion rate on stress response in orthopedic surgery using a tourniquet application. *BMC Anesthesiol* 2013; **13**: 14.
10. Watanabe K, Kashiwagi K, Kamiyama T, et al. High-dose remifentanil suppresses stress response associated with pneumoperitoneum during laparoscopic colectomy. *J Anesth* 2014; **28**: 334-40.
11. Aitkenhead AR, Pepperman ML, Willatts SM, et al. Comparison of propofol and midazolam for sedation in critically ill patients. *Lancet* 1989; **2**: 704-9.
12. Skoglund K, Enblad P, Hillered L, Marklund N. The neurological wake-up test increases stress hormone levels in patients with severe traumatic brain injury. *Crit Care Med* 2012; **40**: 216-22.
13. Marty J, Gauzit R, Lefevre P, et al. Effects of diazepam and midazolam on baroreflex control of heart rate and on sympathetic activity in humans. *Anesth Analg* 1986; **65**: 113-9.
14. Dispersyn G, Sage D, Challet E, Pain L, Touitou Y. Plasma corticosterone in rats is specifically increased at recovery from propofol anesthesia without concomitant rise of plasma ACTH. *Chronobiol Int* 2009; **26**: 697-708.
15. Kostopanagiotou G, Kalimeris K, Christodoulaki K, et al. The differential impact of volatile and intravenous anaesthetics on stress response in the swine. *Hormones (Athens)* 2010; **9**: 67-75.
16. Pascoe JE, Williams KL, Mukhopadhyay P, Rice KC, Woods JH, Ko MC. Effects of mu, kappa, and delta opioid receptor agonists on the function of hypothalamic-pituitary-adrenal axis in monkeys. *Psychoneuroendocrinology* 2008; **33**: 478-86.
17. Meersseman P, Boonen E, Peeters B, et al. Effect of Early Parenteral Nutrition on the Hpa Axis and on Treatment with Corticosteroids in Icu Patients. *The Journal of clinical endocrinology and metabolism* 2015: jc20151846.
18. Boonen E, Meersseman P, Vervenne H, et al. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab* 2014; **306**: E883-92.
19. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644-55.
20. Midi H, Sarkar SK, Rana S. Collinearity diagnostics of binary logistic regression model. *Journal of Interdisciplinary Mathematics* 2010; **13**: 253-67.
21. Roth-Isigkeit AK, Schmucker P. Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids* 1997; **62**: 695-9.
22. Gibbison B, Angelini GD, Lightman SL. Dynamic output and control of the hypothalamic-pituitary-adrenal axis in critical illness and major surgery. *BrJAnaesth* 2013; **111**: 347-60.

23. Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends EndocrinolMetab* 2008; **19**: 175-80.
24. Deutschman CS, Raj NR, McGuire EO, Kelz MB. Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med* 2013; **41**: e368-e75.
25. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *EndocrRev* 1998; **19**: 101-43.
26. Taylor T, Dluhy RG, Williams GH. beta-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J Clin Endocrinol Metab* 1983; **57**: 592-6.
27. Rittmaster RS, Cutler GB, Jr., Sobel DO, et al. Morphine inhibits the pituitary-adrenal response to ovine corticotropin-releasing hormone in normal subjects. *J Clin Endocrinol Metab* 1985; **60**: 891-5.
28. Aloisi AM, Aurilio C, Bachiocco V, et al. Endocrine consequences of opioid therapy. *Psychoneuroendocrinology* 2009; **34 Suppl 1**: S162-8.
29. Abs R, Verhelst J, Maeyaert J, et al. Endocrine consequences of long-term intrathecal administration of opioids. *J Clin Endocrinol Metab* 2000; **85**: 2215-22.
30. Taniguchi H, Sasaki T, Fujita H, et al. The effect of intraoperative use of high-dose remifentanil on postoperative insulin resistance and muscle protein catabolism: a randomized controlled study. *Int J Med Sci* 2013; **10**: 1099-107.
31. Schluger JH, Borg L, Ho A, Kreek MJ. Altered HPA axis responsivity to metyrapone testing in methadone maintained former heroin addicts with ongoing cocaine addiction. *Neuropsychopharmacology* 2001; **24**: 568-75.
32. Schlaghecke R, Kornely E, Santen RT, Ridderskamp P. The effect of long-term glucocorticoid therapy on pituitary-adrenal responses to exogenous corticotropin-releasing hormone. *N Engl J Med* 1992; **326**: 226-30.
33. Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010; **31**: 98-132.
34. Hildreth AN, Mejia VA, Maxwell RA, Smith PW, Dart BW, Barker DE. Adrenal suppression following a single dose of etomidate for rapid sequence induction: a prospective randomized study. *J Trauma* 2008; **65**: 573-9.
35. Watt I, Ledingham IM. Mortality amongst multiple trauma patients admitted to an intensive therapy unit. *Anaesthesia* 1984; **39**: 973-81.
36. Kato K, Nakagawa C, Murabayashi H, Oomori Y. Expression and distribution of GABA and GABAB-receptor in the rat adrenal gland. *J Anat* 2014; **224**: 207-15.
37. Amenta F, Collier WL, Erdo SL, Giuliani S, Maggi CA, Meli A. GABAA receptor sites modulating catecholamine secretion in the rat adrenal gland: evidence from 3H-muscimol autoradiography and in vivo functional studies. *Pharmacology* 1988; **37**: 394-402.
38. Kenyon CJ, Thomson I, Fraser R. Stimulation of aldosterone secretion by benzodiazepines in bovine adrenocortical cells. *Fundam Clin Pharmacol* 1999; **13**: 213-9.
39. al-Damluji S. Adrenergic control of the secretion of anterior pituitary hormones. *Baillieres Clin Endocrinol Metab* 1993; **7**: 355-92.
40. Ruffolo RR, Jr. The pharmacology of dobutamine. *Am J Med Sci* 1987; **294**: 244-8.
41. Seo DO, Lee S, Rivier C. Role of specific adrenergic receptors in mediating the adrenocorticotropic hormone response to increased nitric oxide levels. *J Neuroendocrinol* 2003; **15**: 530-7.

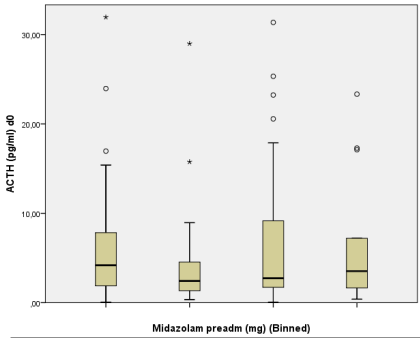
Supplementary Appendix



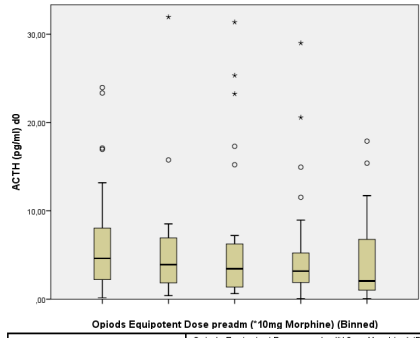
		Propofol preadm (mg) (Binned)				
		1	2	3	4	5
Propofol preadm (mg)	Minimum	,0	50,0	131,4	470,4	728,0
	Maximum	,0	110,0	455,0	722,4	4800,0
	Count	60	4	30	31	31



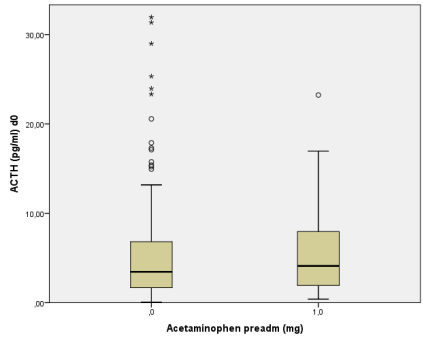
		Etomidate preadm (mg) (Binned)	
		1	2
Etomidate preadm (mg)	Minimum	,0	14,0
	Maximum	,0	65,0
	Count	94	62



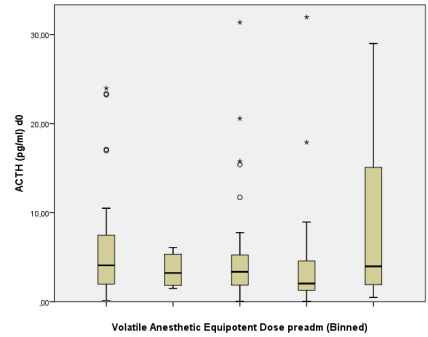
		Midazolam preadm (mg) (Binned)			
		1	2	3	4
Midazolam preadm (mg)	Minimum	,0	1,5	7,5	13,0
	Maximum	,0	7,0	12,0	80,0
	Count	68	26	36	26



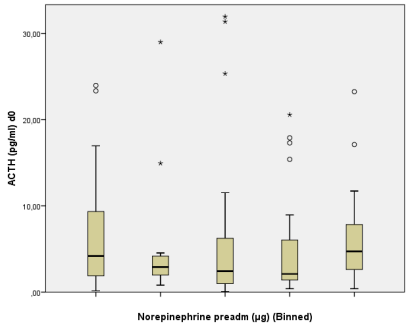
		Opioids Equipotent Dose preadm (*10mg Morphine) (Binned)				
		1	2	3	4	5
Opioids Equipotent Dose preadm (*10mg Morphine)	Minimum	,0	2,7	12,6	24,6	33,0
	Maximum	1,7	12,0	24,0	30,0	54,0
	Count	32	31	36	29	28



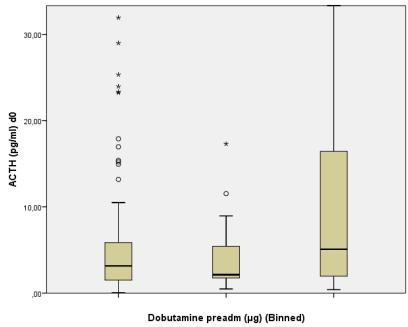
		Acetaminophen preadm (mg)	
		,0	1,0
Acetaminophen preadm (mg)	Minimum	,0	1000,0
	Maximum	,0	4000,0
	Count	139	17



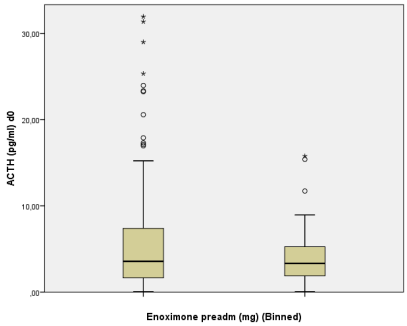
		Volatile Anesthetic Equipotent Dose preadm (Binned)				
		1	2	3	4	5
Volatile Anesthetic Equipotent Dose preadm	Minimum	,0	33,3	121,1	223,3	318,2
	Maximum	,0	113,3	217,8	316,7	720,0
	Count	54	8	32	31	31



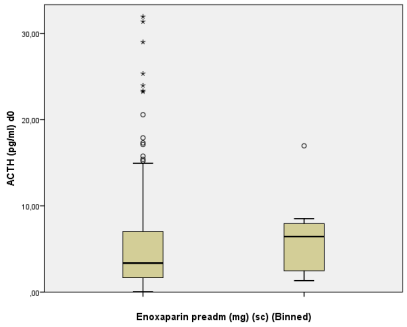
		Norepinephrine preadm (µg) (Binned)				
		1	2	3	4	5
Norepinephrine preadm (µg)	Minimum	,0	126,0	572,4	1237,5	2277,0
	Maximum	,0	561,6	1189,5	2268,0	57600,0
	Count	40	23	31	31	31



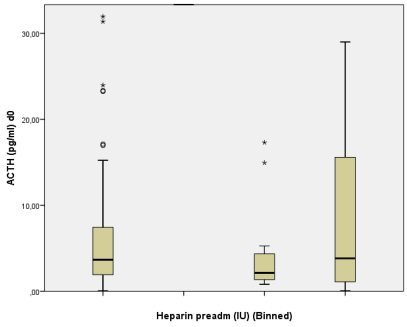
		Dobutamine preadm (µg) (Binned)		
		1	2	3
Dobutamine preadm (µg)	Minimum	,0	2500,0	21000,0
	Maximum	,0	20640,0	99600,0
	Count	106	19	31



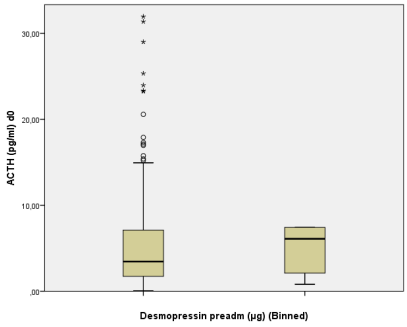
		Enoximone preadm (mg) (Binned)	
		1	2
Enoximone preadm (mg)	Minimum	,0	15,0
	Maximum	,0	55,0
	Count	138	18



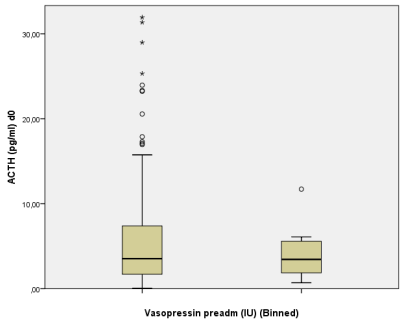
		Enoxaparin preadm (mg) (sc) (Binned)	
		1	2
Enoxaparin preadm (mg) (sc)	Minimum	,0	20,0
	Maximum	,0	100,0
	Count	148	8



		Heparin preadm (IU) (Binned)			
		1	2	3	4
Heparin preadm (IU)	Minimum	,0	2500,0	5000,0	24000,0
	Maximum	,0	2500,0	23500,0	40000,0
	Count	93	1	31	31

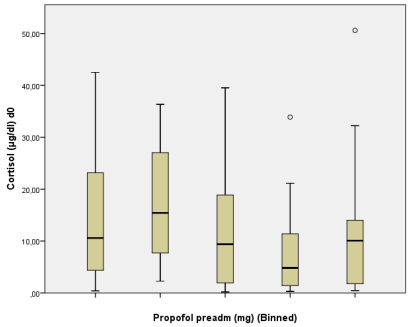


		Desmopressin preadm (µg) (Binned)	
		1	2
Desmopressin preadm (µg)	Minimum	,0	16,0
	Maximum	,0	20,0
	Count	151	5

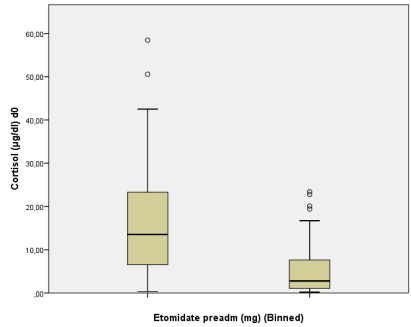


		Vasopressin preadm (IU) (Binned)	
		1	2
Vasopressin preadm (IU)	Minimum	,0	1,0
	Maximum	,0	7,0
	Count	145	11

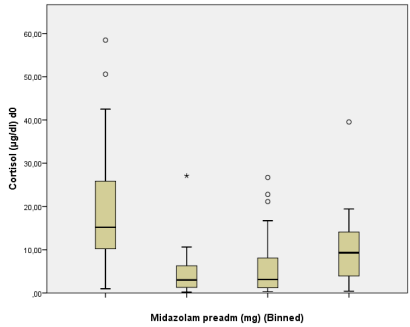
Figure S1 | Association of plasma ACTH upon admission with cumulative drug doses
The cumulative doses of each drug were automatically binned by the statistical software, to visualize the pattern of the association with the outcome of interest. For the multivariable linear regression analyses, drugs where automatically binning created 2 groups were added dichotomized to the model. Drugs displaying a J-shaped relationship were added categorized in 3 groups (norepinephrine) and the other drugs were added as continuous variables. These analyses were performed with the use of SPSS software, version 22 (IBM). Data are presented as box plots, with the central line indicating the medians, the box the interquartile ranges and the whiskers the 10th and 90th percentiles. To convert values for ACTH to SI units (pmol/l), multiply by 0.22.



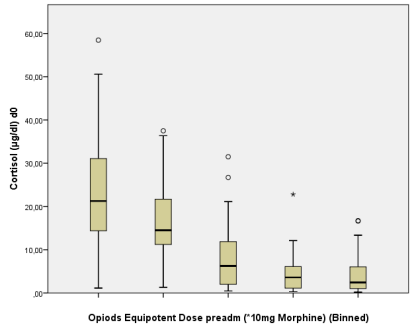
		Propofol preadm (mg) (Binned)				
		1	2	3	4	5
Propofol preadm (mg)	Minimum	,0	50,0	131,4	470,4	728,0
	Maximum	,0	110,0	455,0	722,4	4800,0
	Count	60	4	30	31	31



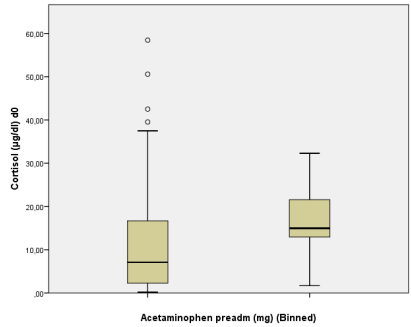
		Etomidate preadm (mg) (Binned)	
		1	2
Etomidate preadm (mg)	Minimum	,0	14,0
	Maximum	,0	65,0
	Count	94	62



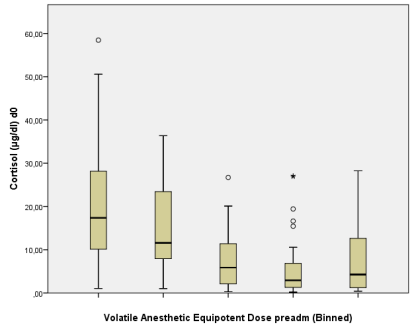
		Midazolam preadm (mg) (Binned)			
		1	2	3	4
Midazolam preadm (mg)	Minimum	,0	1,5	7,5	13,0
	Maximum	,0	7,0	12,0	80,0
	Count	68	26	36	26



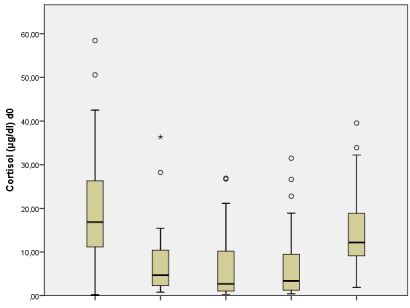
		Opiods Equipotent Dose preadm (*10mg Morphine) (Binned)				
		1	2	3	4	5
Opiods Equipotent Dose preadm (*10mg Morphine)	Minimum	,0	2,7	12,6	24,6	33,0
	Maximum	1,7	12,0	24,0	30,0	54,0
	Count	32	31	36	29	28



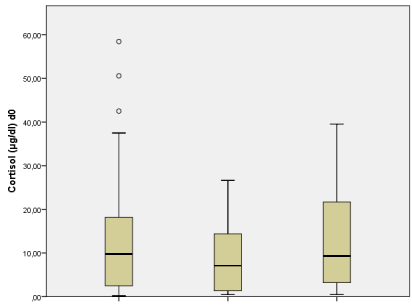
		Acetaminophen preadm (mg)	
		,0	1,0
Acetaminophen preadm (mg)	Minimum	,0	1000,0
	Maximum	,0	4000,0
	Count	139	17



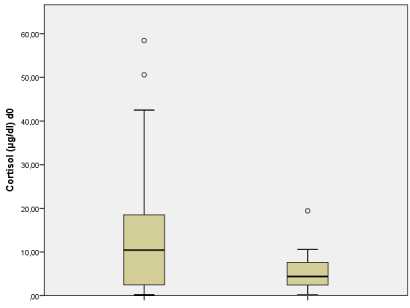
		Volatile Anesthetic Equipotent Dose preadm (Binned)				
		1	2	3	4	5
Volatile Anesthetic Equipotent Dose preadm	Minimum	,0	33,3	121,1	223,3	318,2
	Maximum	,0	113,3	217,8	316,7	720,0
	Count	54	8	32	31	31



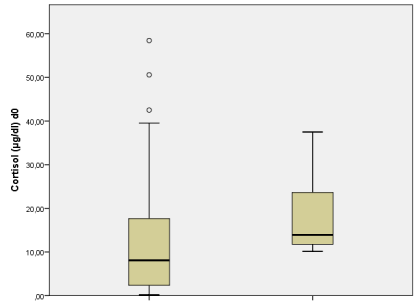
		Norepinephrine preadm (µg) (Binned)				
		1	2	3	4	5
Norepinephrine preadm (µg)	Minimum	,0	126,0	572,4	1237,5	2277,0
	Maximum	,0	561,6	1189,5	2268,0	57600,0
	Count	40	23	31	31	31



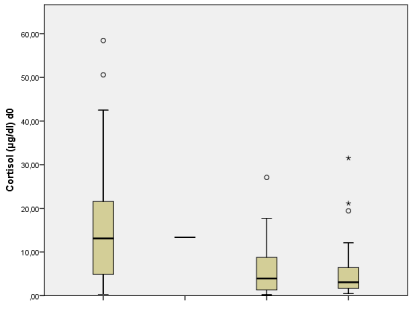
		Dobutamine preadm (µg) (Binned)		
		1	2	3
Dobutamine preadm (µg)	Minimum	,0	2500,0	21000,0
	Maximum	,0	20640,0	99600,0
	Count	106	19	31



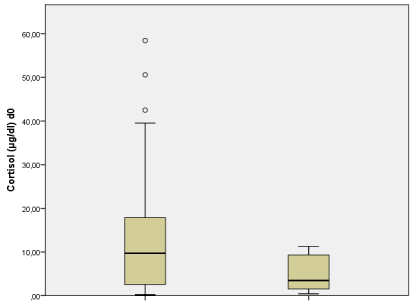
		Enoximone preadm (mg) (Binned)	
		1	2
Enoximone preadm (mg)	Minimum	,0	15,0
	Maximum	,0	55,0
	Count	138	18



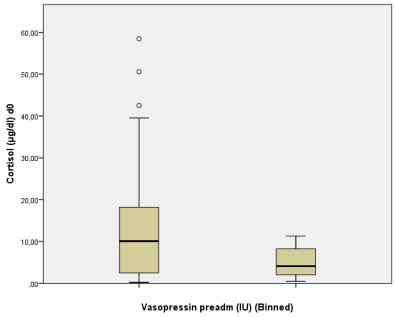
		Enoxaparin preadm (mg) (sc) (Binned)	
		1	2
Enoxaparin preadm (mg) (sc)	Minimum	,0	20,0
	Maximum	,0	100,0
	Count	148	8



		Heparin preadm (IU) (Binned)			
		1	2	3	4
Heparin preadm (IU)	Minimum	,0	2500,0	5000,0	24000,0
	Maximum	,0	2500,0	23500,0	40000,0
	Count	93	1	31	31



		Desmopressin preadm (µg) (Binned)	
		1	2
Desmopressin preadm (µg)	Minimum	,0	16,0
	Maximum	,0	20,0
	Count	151	5



		Vasopressin preadm (IU) (Binned)	
		1	2
Vasopressin preadm (IU)	Minimum	,0	1,0
	Maximum	,0	7,0
	Count	145	11

Figure S2 | Association of plasma cortisol upon admission with cumulative drug doses
The cumulative doses of each drug were automatically binned by the statistical software, to visualize the pattern of the association with the outcome of interest. For the multivariable linear regression analyses, drugs where automatically binning created 2 groups were added dichotomized to the model. Drugs displaying a J-shaped relationship were added categorized in 3 groups (midazolam, norepinephrine) and the other drugs were added as continuous variables. These analyses were performed with the use of SPSS software, version 22 (IBM). Data are presented as box plots, with the central line indicating the medians, the box the interquartile ranges and the whiskers the 10th and 90th percentiles. To convert values for cortisol to SI units (nmol/l), multiply by 27.6.

4

ADRENOCORTICAL FUNCTION DURING PROLONGED CRITICAL ILLNESS AND BEYOND

Adapted from:

- **Peeters B,** Meersseman P, Vander Perre S, Wouters P, Vanmarcke D, Debaveye Y, Billen J, Vermeersch P, Langouche L, Van den Berghe G. Adrenocortical function during prolonged critical illness and beyond: a prospective observational study. Intensive Care Med 2018; 44: 1720-1729.

Abstract

Background For patients suffering from prolonged critical illness, it is unknown whether and when the hypothalamus-pituitary-adrenal axis alterations recover, and to what extent adrenocortical function parameters relate to sepsis/septic shock, to clinical need for glucocorticoid treatment and to survival.

Methods Patients still in ICU on day 7 (N=392) and 20 matched healthy subjects were included. Morning blood and 24h-urine were collected daily and cosyntropin tests (250µg) performed weekly, repeated 1 week after ICU-discharge on the regular ward.

Results In all patients free of glucocorticoid treatment up until ICU-day 28 (N=347), plasma ACTH always remained low/normal, whereas free cortisol remained high (P≤0.002) explained by reduced binding proteins (P≤0.02) and suppressed cortisol breakdown (P≤0.001). Beyond ICU-day 28 (N=64 long-stayers), plasma (free)cortisol was no longer elevated. One week after ICU-discharge, plasma ACTH and (free)cortisol always rose to supra-normal levels (P≤0.006), most pronounced in long-stayers. Long-stayers always showed low incremental total (P≤0.001), but normal incremental free, cortisol responses to weekly cosyntropin-tests, explained by low cortisol plasma binding proteins. Sepsis/septic shock patients were not different from others, patients subsequently receiving glucocorticoids (N=45) were not different from those who did not, and non-survivors were distinguishable from survivors only by higher (free)cortisol.

Conclusions Irrespective of sepsis/septic shock, need for glucocorticoids and survival, low cortisol plasma binding proteins and suppressed cortisol breakdown determine systemic (free)cortisol availability in prolonged critical illness, the latter no longer elevated beyond ICU-day 28. The uniform rise in ACTH and cortisol to supra-normal levels 1 week after ICU-discharge indicates recovery of a central adrenocortical suppression while in ICU. Low cortisol plasma binding invalidates the cosyntropin-test.

Introduction

High plasma concentrations of total and free cortisol and low levels of cortisol binding proteins hallmark critical illness.[4] Until recently, several-fold increased ACTH-driven cortisol production was considered to be the main driver of critical illness-induced hypercortisolism. However, studies have reported low rather than high plasma ACTH throughout the first week in ICU, a cortisol production rate that was not or only slightly higher than in matched healthy subjects and an important role for suppressed cortisol breakdown in bringing about hypercortisolism in the critically ill.[4-6] Possible explanations for low plasma ACTH comprise feedback-inhibition exerted by circulating (free)cortisol that is elevated via non-ACTH dependent secretion and via suppressed breakdown,[4] or inflammation- and hypoxia-induced cellular damage to the hypothalamus and/or the pituitary gland.[7,8] Also, the integrity of the adrenal cortex could be impaired by hypoxia, inflammation, hemorrhage causing primary adrenal insufficiency or by sustained reduced ACTH signaling, evoking central hypoadrenalism.[9] It is currently unknown whether and when these alterations in ACTH and cortisol recover in critically ill patients suffering from various diseases including sepsis/septic shock.

Experts have defined "critical illness-related corticosteroid insufficiency (CIRCI)" as the condition in which patients may not, or may no longer, be able to produce the required amount of cortisol that is essential for survival.[10] Patients suffering from septic shock and long-stay ICU patients are assumed to be particularly at risk of developing CIRCI.[11-14] Experts have advised to diagnose CIRCI either by an incremental cortisol response to 250 µg of cosyntropin (Synacthen®) below 9µg/dl or by a random plasma total cortisol below 10µg/dl.[15,16] However, the concept of CIRCI as a clinical entity that should be treated remains controversial. Indeed, it is not known how many patients would fulfill these presumed criteria of CIRCI without any obvious clinical need for glucocorticoid treatment. Vice versa, it has not been investigated whether initiation of glucocorticoid treatment in the ICU is supported by abnormalities in adrenocortical function parameters.

We here investigated whether and when the central and peripheral alterations that occur within the hypothalamus-pituitary-adrenal axis during prolonged critical illness recover, and to what extent the currently used diagnostic criteria for CIRCI relate to septic shock, need for glucocorticoid treatment and survival. To this end, we documented the changes over time - from ICU-day 7 up to recovery or death - in plasma ACTH and

(free)cortisol, urinary cortisol metabolites, and in plasma total and free cortisol responses to cosyntropin, with focus on long-stay (≥4 weeks in ICU) patients in comparison with shorter-stay patients (1-2 weeks; 2-3 weeks; 3-4 weeks in ICU).

Methods

Study design, study participants, and sample size calculation

This prospective observational study was performed in 5 medical/surgical ICUs at the University Hospitals of Leuven, Belgium. Consecutive adult (age ≥18y) critically ill patients were screened for eligibility on ICU-day 6. Exclusion criteria were (details provided in the Supplementary Material) treatment with systemic glucocorticoids, etomidate, azoles or other drugs predisposing to adrenal insufficiency, no vital organ support, no arterial or central venous catheter in place, referral from another ICU, cerebral/pituitary/adrenal disorders with impact on the neuroendocrine system, enrollment in another trial, or expected death within 12h. On ICU-day 7, after written informed consent from the patient or next of kin, the study started.

The required study sample size was determined by an estimated effect size of longer duration of critical illness on adrenocortical function and plasma ACTH. To detect primary adrenal insufficiency occurring within 4 weeks in ICU (causing plasma ACTH >50% above healthy values), with 80% power and 95% certainty, 64 patients - not receiving glucocorticoid treatment up to ICU-day 28 - and 20 healthy controls matched for age, gender and BMI were needed (**Table 1**).[4] Also, within this time-window, this sample size would allow to detect an adaptive stress response, with pituitary reactivation as a result of normalized cortisol breakdown, whereby a normalization of plasma ACTH in patients who recover. Recruitment started on February 18, 2015 and continued until 64 patients fulfilled the requirements of ICU stay ≥4 weeks (ICU≥4w) without receiving glucocorticoids (July 7, 2017) (**Fig. S1**). Patients who were discharged or died before this time-point, were analyzed for comparison, divided into 3 groups based on duration of ICU-stay (between 1-2 weeks (ICU1-2w), between 2-3 weeks (ICU2-3w), between 3-4 weeks (ICU3-4w)). The day of ICU discharge was defined as the day on which patients no longer required vital organ support. Patients who received glucocorticoids after study inclusion, were compared for their last pre-treatment assessment with patients not receiving glucocorticoid treatment, selected pair-wise, matched for day of assessment and baseline risks (**Table S1**).

The study protocol was in accordance to the 1964 Declaration of Helsinki and its later amendments, was approved by the Institutional Ethical Review Board (S57249) and made available prior to study start (ISRCTN98806770).

Table 1: Participant characteristics

	Healthy Subjects (n=20)	P-value*	ICU stay 1-2w (n=164)	ICU stay 2-3w (n=75)	ICU stay 3-4w (n=44)	ICU stay ≥4w (n=64)	P-value**
Male gender - no. (%)	14 (70)	0.83	108 (66)	51 (68)	29 (66)	47 (73)	0.73
Age - yr (mean ± SEM)	64 ± 2	0.87	64 ± 1	64 ± 2	59 ± 2	65 ± 2	0.18
Malignancy - no. (%)			26 (16)	7 (9)	3 (7)	10 (16)	0.44
Diagnostic admission categories							**0.001**
Cardiac surgery - no. (%)			29 (18)	21 (28)	13 (29)	13 (20)	
Complicated other surgery - no. (%)			63 (38)	17 (23)	11 (25)	27 (42)	
Multiple trauma and burns - no. (%)			11 (7)	12 (16)	6 (14)	14 (22)	
Medical - no. (%)			61 (37)	25 (33)	14 (32)	10 (16)	
Patient characteristics at study inclusion (ICU[c] day 7)							
Infection - no. (%)			123 (75)	62 (83)	40 (91)	53 (83)	0.08
Sepsis[d] - no. (%)			103 (63)	57 (76)	38 (86)	47 (73)	**0.009**
Septic shock[d] - no. (%)			45 (27)	34 (45)	21 (47)	38 (59)	**<0.0001**
Requiring vasopressors on ICU day 7 - no. (%)			67 (41)	41 (55)	24 (55)	50 (78)	**<0.0001**
Norepinephrine infusion rate on ICU day 7 – µg/kg/min (mean ± SEM)			0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.09 ± 0.01	**<0.0001**
Treatment with inhaled glucocorticoids on ICU day 7 - no. (%)			8 (5)	1 (1)	1 (2)	1 (2)	0.38
Clinical outcomes							
Days in ICU - mean ± SEM			10 ± 0	17 ± 0	23 ± 0	49 ± 3	**<0.0001**
ICU non-survivor – no. (%)			20 (12)	8 (11)	6 (14)	13 (20)	0.34

[a]The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. [b]The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects severity of illness, with higher values indicating more severe illness, and can range from 0 to 71.[1] [c]ICU denotes intensive care unit. [d]Incidence of sepsis and septic shock was defined according to [2,3]. The * indicates the comparison between healthy subjects and all patients. The ** indicates the comparison between patient groups.

Clinical data and sample collection

Demographic, anthropometric, ICU admission characteristics, patient characteristics at study inclusion on ICU-day 7, and outcomes were documented (**Table 1** and **Table S1**). From ICU-day 7 until ICU-day 28 and/or ICU-discharge or death, morning blood and 24h-urine samples were collected daily (for details, see Supplementary Material). Thereafter, for ICU≥4w, samples were collected weekly until ICU-discharge or death, with an additional sample on the last ICU day. For all patients, a short ACTH-stimulation test [intravenous injection of 250µg cosyntropin (Synacthen®) with blood sampling before, 30 and 60 minutes after injection] was performed weekly. Seven days after ICU-discharge, an additional blood sample was taken and a cosyntropin test was performed on the ward. For the demographically matched healthy volunteers, morning blood and 24h-urine were sampled and a cosyntropin test was performed. Details on sampling procedure and sample storing are provided in the Supplementary Material.

Quantification of plasma ACTH and (free)cortisol concentrations

Plasma ACTH concentrations were measured with a double-monoclonal immunoradiometric assay (Brahms Diagnostics), cortisol (Immunotech) and cortisol-binding-globulin (CBG) (Riazen) by competitive radio-immunoassay. Plasma albumin was quantified by the bromocresol green colorimetric method (Sigma-Aldrich). Plasma free cortisol was calculated using the Coolens' formula adapted for individual albumin and CBG concentrations, previously validated as representative of measured free cortisol in the ICU-context.[17,18] Results were not available to the bedside clinicians.

Estimation of the activity of cortisol metabolizing enzymes

Liquid-chromatography-tandem-mass-spectrometry (LC-MS/MS) was used to determine absolute urinary concentrations of cortisol (F), 5α-tetrahydrocortisol (allo-THF), 5β-tetrahydrocortisol (THF), cortisone (E), 5α-tetrahydrocortisone (allo-THE), and tetrahydrocortisone (THE) after deglucuronidation.[4,19] The activity of 11β-HSD2 was estimated by the E/F ratio, activity of 5α-reductase by allo-THF/F and allo-THE/E ratios, and activity of 5β-reductase by THF/F and THE/E ratios.[4] These estimations were performed only for ICU≥4w patients weekly throughout ICU-stay. Results were not available to bedside clinicians.

Statistical analyses

Data are presented as mean±standard errors (SEM) or numbers and percentages, as appropriate. Continuous data were compared with unpaired Student's t-tests, where necessary after transformation to obtain a near-normal distribution. Proportions were compared with the use of chi-square tests. Time-series were compared with repeated-measures-ANOVA, where necessary after transformation to obtain a near-normal distribution. Statistical analyses were performed with JMP® Pro (v13.0.0, SAS Institute). Two-sided P-values ≤0.05 were considered to indicate statistical significance. No corrections for multiple comparisons were done.

Results

Patient characteristics

Three hundred and ninety-two patients were included in the study and 45 subsequently received glucocorticoids (**Fig. S1**). Of the remaining 347 included patients, 64 patients fulfilled the requirements of being critically ill for ≥4 weeks. The 283 patients who were discharged from ICU or died before day 28 were divided into 3 groups for duration of ICU-stay (1-2 weeks; 2-3 weeks; 3-4 weeks) (**Table 1**). Patients who subsequently received glucocorticoids were comparable to those who did not, with the exception of a higher admission APACHE-II score, higher proportions of patients with sepsis and septic shock, a longer ICU-stay and a higher mortality (**Table S1**).

Results for the 347 patients who did not receive treatment with glucocorticoids

Changes in plasma ACTH and cortisol concentrations over time in ICU and on the regular ward

As compared with healthy subjects, plasma ACTH concentrations were low/normal and plasma total and free cortisol concentrations were high for all patients on ICU-day 7 (**Fig.1a1-3**), with a trend for lower ACTH (P=0.09) and significantly higher free cortisol (P=0.002) for ICU≥4w patients than for shorter-stayers. There was no effect of treatment with inhaled glucocorticoids on plasma ACTH (P=0.53), on total (P=0.51) and free (P=0.77) cortisol on ICU-day 7. The suppressed ACTH gradually normalized towards ICU-discharge, whereas free cortisol progressively decreased but remained higher than normal, except for ICU≥4w patients (**Fig.1a1-3**). On ICU-day 7, plasma CBG (**Fig.1a4**) concentrations in all patients were lower than in healthy subjects. CBG gradually increased to healthy reference values towards ICU-discharge, but stayed lower than normal, in particular for ICU≥4w patients (**Fig.1a4**). Plasma albumin concentrations were always low (4.1±0.02 g/dL vs. 7.8±0.2 g/dL, P<0.0001). As compared with the last value obtained in ICU, 1 week after ICU-discharge, all surviving patients revealed a 66%-increase in plasma ACTH, a 22%-rise in plasma total cortisol, a 29%-increase in plasma free cortisol, a 4%-increase in plasma CBG, and a 9% increase in plasma albumin (P<0.0001) (**Fig.1b**). Remarkably, for ICU≥4w patients, as compared with shorter-stayers, the rise in plasma free cortisol was larger (P=0.02) and tended to be larger for total

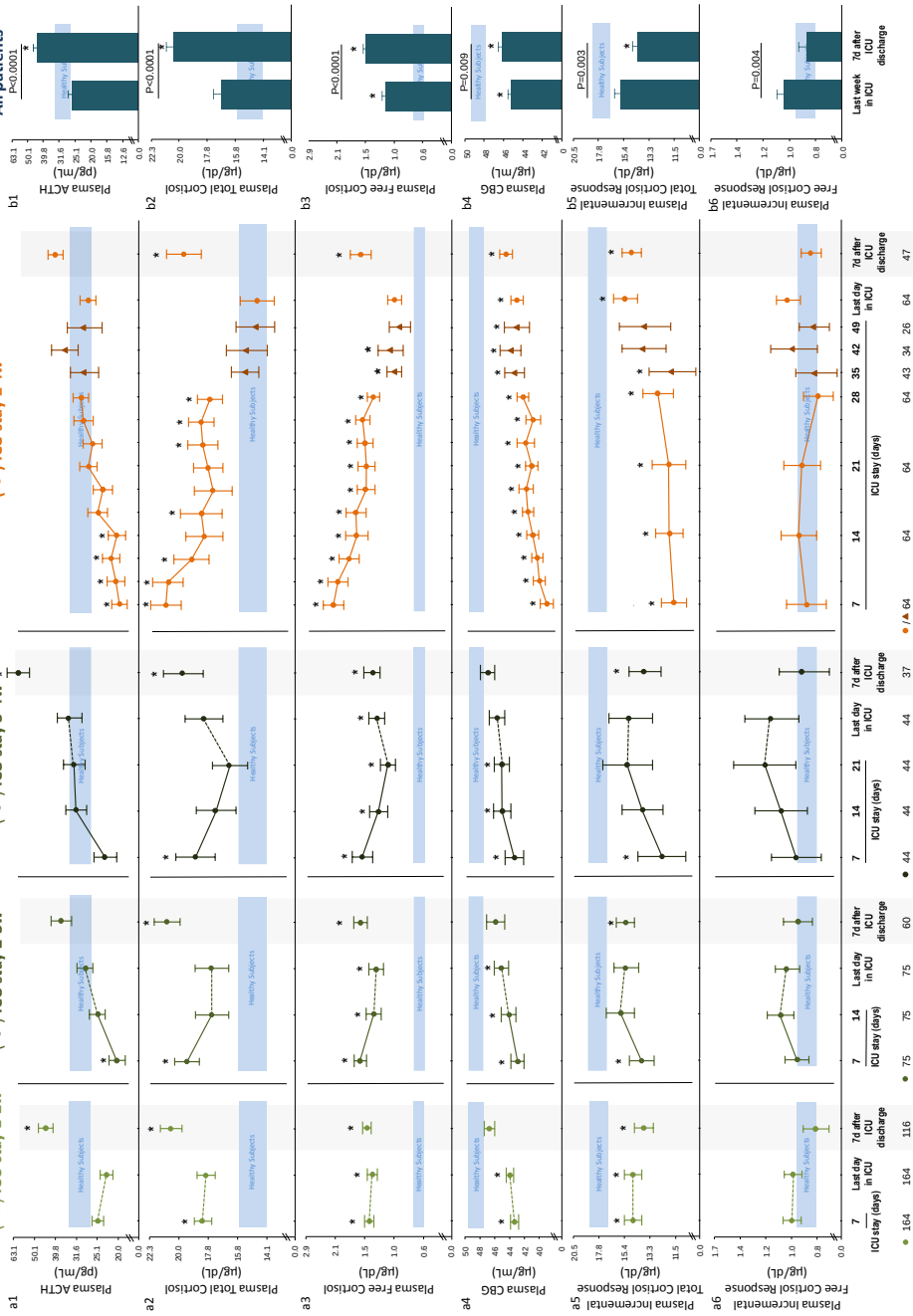

(-●-) ICU stay 1-2w
(-●-) ICU stay 2-3w
(-●-) ICU stay 3-4w
(-●-) ICU stay ≥ 4w
All patients
a1 Plasma ACTH (pg/mL)
a2 Plasma Total Cortisol (µg/dL)
a3 Plasma Free Cortisol (µg/dL)
a4 Plasma CBG (µg/mL)
a5 Plasma Incremental Total Cortisol Response (µg/dL)
a6 Plasma Incremental Free Cortisol Response (µg/dL)
b1 Plasma ACTH (pg/mL)
b2 Plasma Total Cortisol (µg/dL)
b3 Plasma Free Cortisol (µg/dL)
b4 Plasma CBG (µg/mL)
b5 Plasma Incremental Total Cortisol Response (µg/dL)
b6 Plasma Incremental Free Cortisol Response (µg/dL)
P<0.0001
P<0.0001
P<0.0001
P=0.009
P=0.003
P=0.004
Healthy Subjects
ICU stay (days)
Last day in ICU
7d after ICU discharge
Last week in ICU

Figure 1 | Adrenocortical function parameters from day 7 in ICU until ICU-discharge or death - and 7 days after ICU-discharge - for patients who did not receive glucocorticoids
Panel a. Time courses for the 347 ICU patients, divided into the 4 cohorts based on the duration of ICU stay, as compared with 20 matched healthy subjects. **Panel b.** Last assessment in ICU and assessment 7 days after ICU-discharge for all patients. Data are shown as mean±SEM on a logarithmic scale. ICU denotes intensive care unit. d denotes day. w denotes week. Circles during ICU-stay denote data points for all patients included within each time cohort, triangles denote data points of a decreasing numbers of patients. The horizontal blue-shaded area represents the mean±SEM of results from the 20 healthy subjects. The * indicates a P≤0.05 for the comparison with healthy subjects.

cortisol (P=0.06). For all patients tested 7 days post-ICU on the regular ward, plasma ACTH and plasma (free)cortisol concentrations were higher than those of healthy subjects. (**Fig.1b1-3**), whereas plasma CBG (**Fig.1b4**) and albumin (4.8±0.08 g/dL vs. 7.8±0.2 g/dL; P<0.0001) concentrations were still lower.

Changes in incremental cortisol responses to cosyntropin over time in ICU and on the regular ward

Incremental total cortisol responses to cosyntropin were either normal or low for shorter-stayers, whereas ICU≥4w patients uniformly revealed suppressed incremental total cortisol responses throughout ICU-stay (**Fig.1a5**). In contrast, incremental free cortisol responses were always normal in all patients at all times (**Fig.1a6**). Incremental total cortisol responses correlated positively with plasma CBG concentrations (P<0.0001). There was no effect of treatment with inhaled glucocorticoids on incremental total (P=0.48) and free (P=0.85) cortisol responses on ICU-day 7. As compared with the last value obtained in ICU, 1 week post-ICU, all surviving patients revealed a 9% decrease in incremental total cortisol response, and a 17% decrease in incremental free cortisol response (**Fig.1b5-6**). Incremental total cortisol responses to cosyntropin measured 1 week post-ICU, were lower than those of healthy subjects, and incremental free cortisol responses were normal (**Fig.1b5-6**).

Changes in the estimated activity of cortisol metabolizing enzymes over time during ICU-stay

For ICU≥4w patients, estimated activities of 11β-HSD2, 5α-reductase and 5β-reductase were lower than in healthy subjects at all times (**Fig.2**). From ICU-day 7 to the last ICU day, the estimated activity of 11β-HSD2 and of 5β-reductase slightly increased (P≤0.0001) but always remained lower than normal, whereas the estimated activity of 5α-reductase further decreased (P<0.0001).

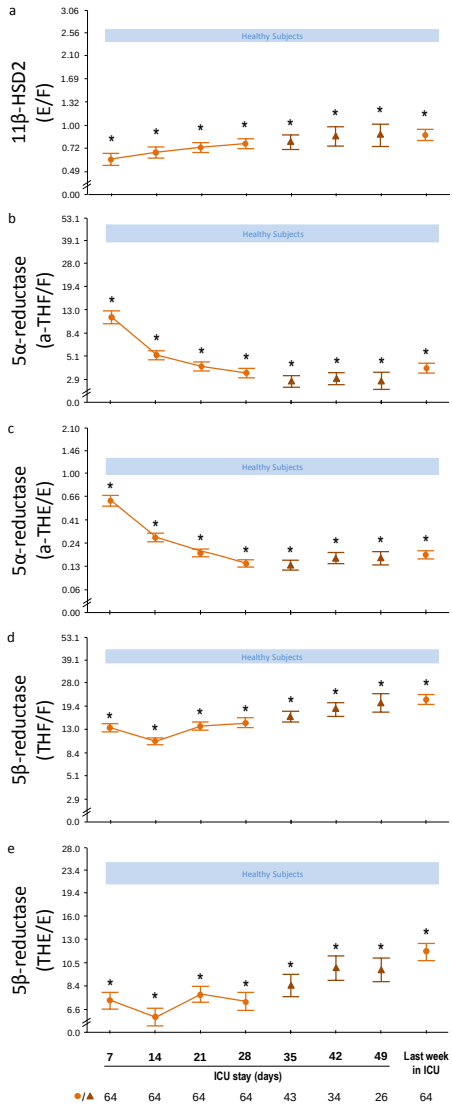


Figure 2 | Estimated activity of the cortisol metabolizing enzymes from day 7 in ICU until ICU-discharge or death among the 64 long-stay (ICU≥4w) patients who did not receive glucocorticoids

Results for the patients are shown as mean±SEM on a logarithmic scale. ICU denotes intensive care unit. Circles during ICU-stay denote data points for all patients in ICU for at least 4 weeks, triangles denote data points of a decreasing numbers of patients thereafter. The horizontal blue-shaded areas represent the mean±SEM of results from the 20 healthy subjects. The * indicates a P<0.001 for the comparisons with healthy subjects.

Comparison of ICU≥4w patients with and without sepsis/septic shock

As compared with patients not suffering from sepsis, patients with sepsis revealed similar plasma ACTH, total and free cortisol, CBG and albumin concentrations (**Fig.3a1-6**). A random plasma cortisol<10μg/dl at any time in ICU occurred in 30% of patients with sepsis and in 35% of patients without sepsis (P=0.67). An incremental total cortisol response to cosyntropin<9μg/dl at any time in ICU occurred in 59% of the patients with sepsis and in 41% of patients without sepsis (P=0.19). Similar results were obtained for the comparison of patients with and without septic shock (**Fig.3b1-6**). A random plasma cortisol<10μg/dl at any time in ICU occurred in 32% of patients with septic shock versus 31% of patients without septic shock (P=0.94). An incremental total cortisol response to cosyntropin<9μg/dl at any

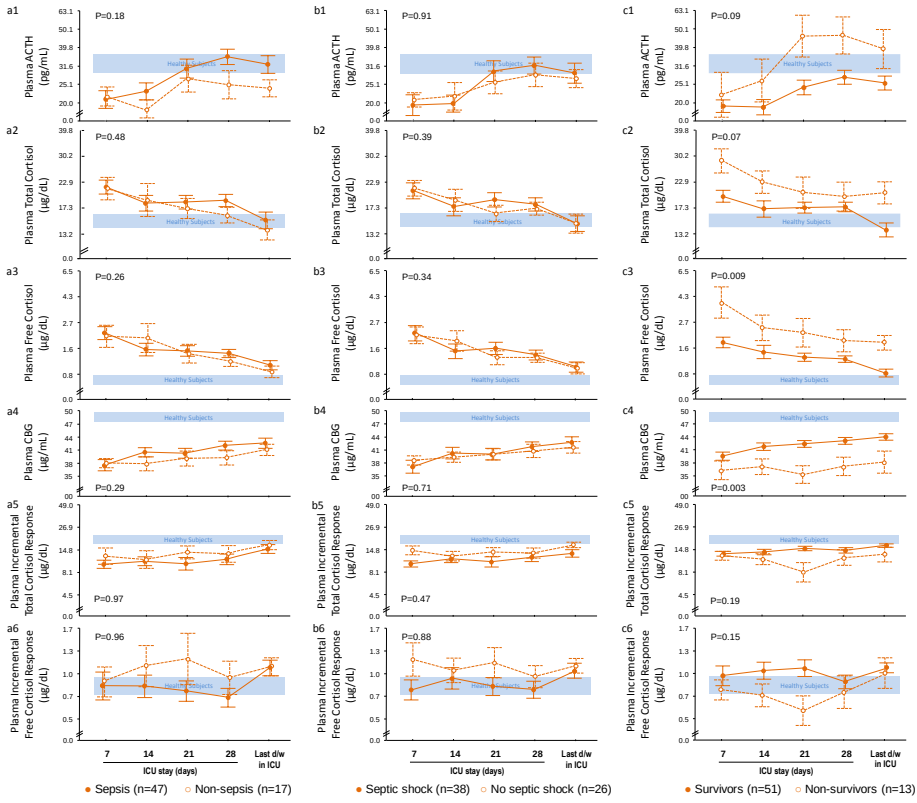


Figure 3 | Adrenocortical function parameters from day 7 in ICU until ICU-discharge or death in long-stay (ICU≥4w) patients who did not receive glucocorticoids, (a) compared for the presence or absence of sepsis, (b) compared for the presence or absence of septic shock and (c) compared for survivors and non-survivors

Data are shown as mean±SEM on a logarithmic scale. ICU denotes intensive care unit. The horizontal blue-shaded areas represent the mean±SEM of results from the 20 healthy subjects. The numerical P-values are those for the comparisons between patient groups.

time in ICU occurred in 61% of patients with septic shock versus 46% of patients without septic shock (P=0.25). Also, patients with septic shock were not different from patients with sepsis without shock (data not shown).

Comparison of ICU≥4w survivors with ICU≥4w non-survivors

Plasma free and total cortisol concentrations were always higher or tended to be higher in non-survivors than in survivors, in the face of comparable plasma ACTH (**Fig.3c1-3**). Plasma CBG concentrations were always lower in non-survivors than in survivors, whereas albumin (P=0.15) and the incremental total and free cortisol responses to cosyntropin were similar (**Fig.3c4**). A random plasma cortisol<10μg/dl at any time in ICU occurred in 35% of survivors and in 15% of non-survivors (P=0.16). An incremental total cortisol response to cosyntropin<9μg/dl at any time in ICU occurred in 53% of survivors and in 62% of non-survivors (P=0.57).

Comparison of the patients who subsequently received glucocorticoid treatment with those who did not

Of the 45 patients who were included on ICU-day 7 and who subsequently received glucocorticoids, 24 could be matched to 24 patients who were not treated withglucocorticoids, for comparison of adrenocortical function parameters on the same ICU-day (ICU-day 14±1). None of the studied adrenocortical function parameters in these patients who received glucocorticoid treatment differed from those who were not treated (Fig.4). This was also the case when only considering hydrocortisone-treated patients (data not shown). Furthermore, a random plasma cortisol<10μg/dl occurred in 13% of both glucocorticoid-treated patients on the pre-treatment day and of matched patients who did not receive glucocorticoid treatment (P>0.90). An incremental total cortisol response to cosyntropin<9μg/dl occurred in 43% of glucocorticoid-treated patients versus 40% of glucocorticoid-untreated patients (P=0.81).

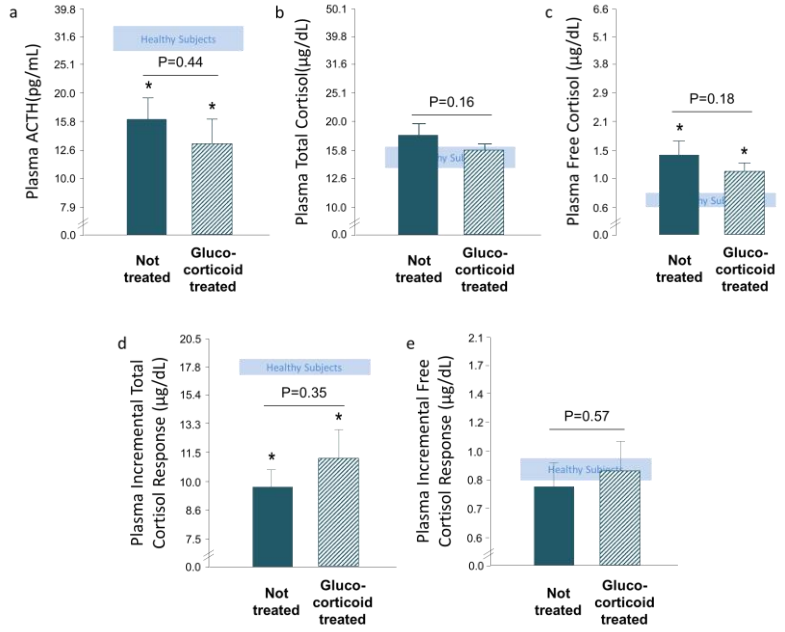


Figure 4 | Adrenocortical function parameters for patients on the last pre-glucocorticoid treatment assessment and for patients who did not receive glucocorticoids, matched for risk factors and day of assessment

Data are shown as mean±SEM on a logarithmic scale. The horizontal blue-shaded areas represent the mean±SEM of results from the 20 healthy subjects. The numerical P-values are those for the comparisons between patient groups and a * represents P≤0.01 for the comparisons with healthy subjects.

Discussion

In this prospective study of prolonged critically ill patients requiring >7 days of intensive care, plasma ACTH remained low/normal throughout ICU-stay up to day 28, whereas plasma free cortisol remained high, largely explained by low plasma binding and persistently suppressed cortisol breakdown, irrespective of the presence of sepsis/septic shock. In particular the low binding proteins among sicker patients and non-survivors determined their higher plasma free cortisol levels. Development of primary adrenal failure was not observed. However, beyond ICU-day 28, plasma free cortisol was no longer elevated and not compensated by increased ACTH, which could be due to a central (endogenous or exogenous) adrenocortical suppression. Such a central suppression was further supported by the uniform rise in plasma ACTH and (free)cortisol to supra-normal levels 1 week later on the regular ward. Low incremental total cortisol responses to cosyntropin coincided with normal free cortisol responses at all times. Hence, low responses to cosyntropin during critical illness likely reflected the increased cortisol distribution volume, which has been documented in an earlier study,[4] given low plasma binding, rather than the functional reserve of the adrenal cortex. Finally, the initiation of glucocorticoid treatment, as judged necessary by bedside clinicians, was not supported by differences in the presently measured adrenal function parameters.

A first important finding was a plasma ACTH that was never elevated and mostly low throughout ICU-stay, whereas plasma (free)cortisol remained higher than normal, and that cortisol breakdown always remained suppressed. However, with illness persisting beyond 4 weeks, plasma (free)cortisol, gradually decreased to healthy reference values, which was not accompanied by elevated plasma ACTH. This is unexpected given the high severity of illness. The observation of a clear increase in plasma ACTH, and in (free)cortisol, 7 days later on the regular ward, was striking. Indeed, at that time-point patients were recovering, which would be considered to reduce the need for high systemic cortisol availability. Instead this need appeared to be increased. Together, these findings could suggest a central suppression of ACTH-driven cortisol production in the ICU, most pronounced during the protracted phase of critical illness, which recovered after ICU-discharge. One can only speculate on the underlying mechanism of such a suppression which could be of endogenous or exogenous origin. A protracted central suppression would explain the previously observed adrenal atrophy in long-stay, but not short-stay ICU patients,

documented post-mortem.[14,20,21] The rise in systemic cortisol availability after ICU-discharge could suggest that the systemic cortisol availability during critical illness may not be sufficient for the level of stress. Although this could suggest a form of CIRCI, the diagnosis with use of advised tests is troublesome. Indeed, in this study, neither a total plasma cortisol<10µg/dl nor an incremental cortisol response to cosyntropin <9µg/dl identified patients with shock or at risk of death.

Indeed, all long-stay ICU patients alike revealed low total cortisol responses to cosyntropin throughout ICU-stay, a finding that can be explained by the very low plasma CBG concentrations that increase cortisol distribution volume. Such an explanation is supported by the much lower than normal peak total plasma cortisol concentration in response to a bolus injection of 100 mg hydrocortisone previously observed for ICU patients, with a calculated distribution volume that was ±40% higher than normal.[4] Hence, the low incremental total plasma cortisol response to cosyntropin in the critically ill could be compatible with a normal amount of cortisol released from the adrenal cortex, diluted over an increased distribution volume. This possibility is further supported by the normal to high incremental free cortisol responses. It therefore seems unlikely that an incremental total cortisol response to cosyntropin of <9µg/dl adequately points to the presence of CIRCI.

Surprisingly, those patients, for whom clinicians at the bedside decided it was appropriate to treat with glucocorticoids, could not be distinguished on the basis of the studied adrenal function parameters from patients who did not need such treatment, after careful matching for type, severity and duration of illness. Instead, this finding suggests that glucocorticoids are often given to patients with the aim to increase blood pressure or to reduce inflammation.[22-29] Whether these are justified indications remains debated.[15,16] The most recent RCTs investigating glucocorticoid treatment for septic shock showed opposite results, adding to the ongoing controversy.[28,29]

A limitation of the study is the use of single morning samples which may have precluded the detection of subtle changes within the dynamics of ACTH and cortisol secretion. However, an earlier study reported that a single morning sample correlated well with secretion as derived by deconvolution analysis of repeated sampling time series.[17] Second, we could not calculate cortisol distribution volume, and therefore relied on previous work.[4] Third, the integrity of the adrenal cortex may have been positively affected by the weekly cosyntropin injections.[30] Fourth, the number of patients requiring intensive care beyond day 28 was relatively small. Fifth, local cortisol activity is further regulated by tissue-specific alterations of glucocorticoid

signaling and we did not examine potential tissue resistance to glucocorticoids. Indeed, besides alternative splicing of the GR, also GR expression, GR affinity and GR translocation are regulated and could be tissue-specific during critical illness.[31-34] Pro-infammatory cytokines decrease the expression of the glucocorticoid receptor and increase its oxidation, which hampers both ligand and DNA binding. Vitamin C has been suggested to reverse these changes and restore glucocorticoid function, a mechanism that could explain the potential reduction in mortality of patients with severe sepsis and septic shock from glucocorticoid administration together with vitamin C.[35] The strengths of the current study are its large sample size and the longitudinal design, with adrenal function parameters documented repeatedly, up to recovery or death, for patients suffering from various diseases, not only from sepsis/septic shock. This allowed to conclude that not the presence of vasopressor-treated septic shock but rather the increased cortisol distribution volume, as previously documented in a similar patient population,[4] appeared to explain the low incremental total cortisol responses to cosyntropin, which questions the value of this test to assess the integrity of the adrenal cortex. It also reported, for the first time, on the recovery of the changes beyond ICU-discharge, which allowed to hypothesize that long-stay patients in particular may be at risk of insufficient cortisol availability. This hypothesis requires further investigation via an RCT that assesses the effect on patient-centered outcomes either of a lower dose of hydrocortisone among long-stay patients, given the persistently low cortisol breakdown, or of treatment with cosyntropin.[4,30]

In conclusion, irrespective of sepsis/septic shock, of clinical need for glucocorticoids and of survival, low cortisol plasma binding proteins and suppressed cortisol breakdown determine the systemic cortisol availability in prolonged critical illness which is no longer elevated beyond ICU-day 28. The uniform rise in ACTH and cortisol to supra-normal levels 1 week after ICU-discharge, most pronounced among very long-stayers, indicates recovery of a central adrenocortical suppression while in ICU. Low cortisol plasma binding invalidates the cosyntropin-test to investigate adrenocortical functional reserve in the ICU context.

Acknowledgements

We thank the patients and healthy volunteers for participating, the clinical staff for excellent protocol compliance and the research assistants for sample handling and data entry. This work was supported by the Research Foundation-Flanders (FWO) [Grant G091918N to GVdB, research mandate 11W9315N to BP and 1842019N to PV]; by the Methusalem Program of the Flemish Government [METH/14/06 to GVdB and LL via KU Leuven]; by a European Research Council Advanced Grant [AdvG-2017-785809 to GVdB] from European Union's Horizon 2020 research and innovation programme.

Personal contribution

Bram Peeters participated in designing the study, conducting the study, gathering and analyzing the data, and writing the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-29.
2. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644-55.
3. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; **315**: 801-10.
4. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477-88.
5. 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 Endocrinol Metab* 1995; **80**: 1238-42.
6. Peeters B, Guiza F, Boonen E, Meersseman P, Langouche L, Van den Berghe G. Drug-induced HPA axis alterations during acute critical illness: a multivariable association study. *Clin Endocrinol (Oxf)* 2017; **86**: 26-36.
7. Polito A, Sonneville R, Guidoux C, et al. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoSOne* 2011; **6**: e25905.
8. Bornstein SR. Predisposing factors for adrenal insufficiency. *N Engl J Med* 2009; **360**: 2328-39.
9. Boonen E, Bornstein SR, Van den Berghe G. New insights into the controversy of adrenal function during critical illness. *Lancet Diabetes Endocrinol* 2015; **3**: 805-15.
10. Annane D, Pastores SM, Arlt W, et al. 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). *Intensive Care Med* 2017; **43**: 1781-92.
11. Annane D, Sebille V, Troche G, Raphael JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000; **283**: 1038-45.
12. Barquist E, Kirton O. Adrenal insufficiency in the surgical intensive care unit patient. *JTrauma* 1997; **42**: 27-31.
13. Van den Berghe G. On the Neuroendocrinopathy of Critical Illness. Perspectives for Feeding and Novel Treatments. *Am J Respir Crit Care Med* 2016; **194**: 1337-48.
14. Peeters B, Langouche L, Van den Berghe G. Adrenocortical Stress Response during the Course of Critical Illness. *Compr Physiol* 2017; **8**: 283-98.
15. 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**: 1751-63.
16. 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. *Crit Care Med* 2017; **45**: 2078-88.
17. Boonen E, Meersseman P, Vervenne H, et al. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab* 2014; **306**: E883-92.
18. Vanhorebeek I, Peeters RP, Vander Perre S, et al. Cortisol response to critical illness: effect of intensive insulin therapy. *J Clin Endocrinol Metab* 2006; **91**: 3803-13.
19. Kosicka K, Siemiatkowska A, Palka D, Szpera-Gozdziewicz A, Breborowicz GH, Glowka FK. Detailed analysis of cortisol, cortisone and their tetrahydro- and allo-tetrahydrometabolites in human urine by LC-MS/MS. *J Pharm Biomed Anal* 2017; **140**: 174-81.
20. Boonen E, Langouche L, Janssens T, et al. Impact of Duration of Critical Illness on the Adrenal Glands of Human Intensive Care Patients. *J Clin Endocrinol Metab* 2014; **99**: 4214-22.
21. Deutschman CS, Raj NR, McGuire EO, Kelz MB. Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med* 2013; **41**: e368-e75.

22. Briegel J, Forst H, Haller M, et al. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. *Crit Care Med* 1999; **27**: 723-32.
23. Annane D, Sebille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002; **288**: 862-71.
24. Sprung CL, Annane D, Keh D, et al. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111-24.
25. Annane D, Bellissant E, Bollaert PE, et al. Corticosteroids in the treatment of severe sepsis and septic shock in adults: a systematic review. *JAMA* 2009; **301**: 2362-75.
26. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 1511-8.
27. Meduri GU, Bridges L, Shih MC, Marik PE, Siemieniuk RA, Kocak M. Prolonged glucocorticoid treatment is associated with improved ARDS outcomes: analysis of individual patients' data from four randomized trials and trial-level meta-analysis of the updated literature. *Intensive Care Med* 2016; **42**: 829-40.
28. Venkatesh B, Finfer S, Cohen J, et al. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. *N Engl J Med* 2018; **378**: 797-808.
29. Annane D, Renault A, Brun-Buisson C, et al. Hydrocortisone plus Fludrocortisone for Adults with Septic Shock. *N Engl J Med* 2018; **378**: 809-18.
30. Gibbison B. Diagnosing adrenal insufficiency in critical illness: Time to go back to the start. *Resuscitation* 2018; **129**: A11-A2.
31. Bergquist M, Nurkkala M, Rylander C, Kristiansson E, Hedenstierna G, Lindholm C. Expression of the glucocorticoid receptor is decreased in experimental Staphylococcus aureus sepsis. *JInfect* 2013; **67**: 574-83.
32. Guerrero J, Gatica HA, Rodriguez M, Estay R, Goecke IA. Septic serum induces glucocorticoid resistance and modifies the expression of glucocorticoid isoforms receptors: a prospective cohort study and in vitro experimental assay. *Crit Care* 2013; **17**: R107.
33. Peeters RP, Hagendorf A, Vanhorebeek I, et al. Tissue mRNA expression of the glucocorticoid receptor and its splice variants in fatal critical illness. *ClinEndocrinol(Oxf)* 2009; **71**: 145-53.
34. Siebig S, Meinel A, Rogler G, et al. Decreased cytosolic glucocorticoid receptor levels in critically ill patients. *AnaesthIntensive Care* 2010; **38**: 133-40.
35. Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. *Chest* 2017; **151**: 1229-38.

Supplementary Appendix

Study design

Exclusion criteria were administration of glucocorticoids within the last 72 hours, chronic treatment with glucocorticoids or other steroids within the last three months, use of etomidate within the last 72h, use of azoles within the last 7 days, other drugs predisposing to adrenal insufficiency (phenytoin, rifampicin, glitazones, imipramin, phenothiazine, phenobarbital), no longer requiring vital organ support, no arterial or central venous catheter in place, referral from another ICU, cerebral disease with intracranial hypertension threatening the neuroendocrine system, pituitary disorders, known adrenal disease, enrollment in another trial, or expected death within 12h.

Sample collection

In the ICU, undiluted blood samples were taken via the arterial or central venous line in place for clinical purposes. For patients investigated after ICU discharge and for healthy volunteers, blood samples were taken via venous puncture. As required for accurate quantification of plasma ACTH concentrations, blood samples were collected in pre-chilled EDTA tubes and immediately placed on ice, centrifuged at 4°C and stored at -80°C until assay. For patients who were not on renal replacement therapy and for healthy subjects, urine samples from a 24h-urine collection were stored in Vacuette® urine tubes at -80°C until assay.

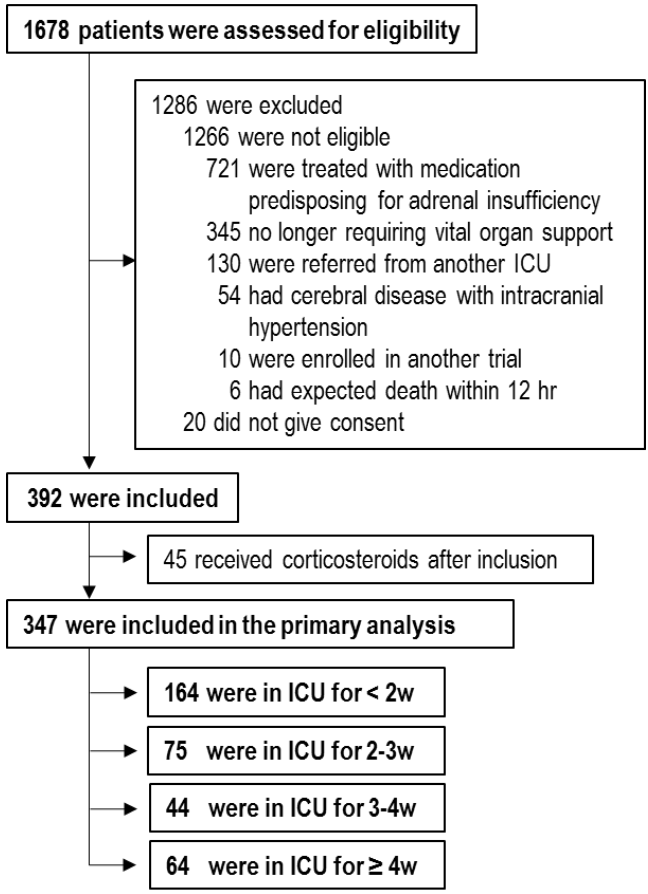


Figure S1 | Flowchart of the study participants
ICU denotes intensive care unit, hr denotes hour, w denotes weeks.

Table S1: Characteristics of corticosteroid treated and not-steroid treated patients

	Healthy Subjects	P-value*	ICU stay < 2w	ICU stay 2-3w	ICU stay 3-4w	ICU stay ≥4w	P-value**
	(n=20)		(n=164)	(n=75)	(n=44)	(n=64)	
Demography and anthropometry							
Male gender - no. (%)	14 (70)	0.83	108 (66)	51 (68)	29 (66)	47 (73)	0.73
Age - yr (mean ± SEM)	64 ± 2	0.87	64 ± 1	64 ± 2	59 ± 2	65 ± 2	0.18
BMI[a] - kg/m² (mean ± SEM)	26·4 ± 0.7	0.82	26.4 ± 0.4	26.1 ± 0.5	26.4 ± 0.8	26.9 ± 0.8	0.88
Admission characteristics							
Diabetes mellitus - no. (%)			22 (13)	20 (27)	7 (16)	11 (17)	0.17
Malignancy - no. (%)			26 (16)	7 (9)	3 (7)	10 (16)	0.44
APACHE II score[b] - (mean ± SEM)			28 ± 1	29 ± 1	31 ± 1	31 ± 1	**0.02**
Emergency admission - no. (%)			128 (78)	58 (77)	34 (77)	53 (83)	0.84
Diagnostic admission categories							**0.001**
Cardiac surgery - no. (%)			29 (18)	21 (28)	13 (29)	13 (20)	
Complicated other surgery - no. (%)			63 (38)	17 (23)	11 (25)	27 (42)	
Multiple trauma and burns - no. (%)			11 (7)	12 (16)	6 (14)	14 (22)	
Medical - no. (%)			61 (37)	25 (33)	14 (32)	10 (16)	
Patient characteristics at study inclusion (ICU[c] day 7)							
Infection - no. (%)			123 (75)	62 (83)	40 (91)	53 (83)	0.08
Sepsis - no. (%)			103 (63)	57 (76)	38 (86)	47 (73)	**0.009**
Septic shock - no. (%)			45 (27)	34 (45)	21 (47)	38 (59)	**<0.0001**
Requiring vasopressors on ICU day 7 - no. (%)			67 (41)	41 (55)	24 (55)	50 (78)	**<0.0001**
Norepinephrine infusion rate on ICU day 7 - µg/kg/min (mean ± SEM)			0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.09 ± 0.01	**<0.0001**
Clinical outcomes							
Days in ICU - (mean ± SEM)			10 ± 0	17 ± 0	23 ± 0	49 ± 3	**<0.0001**
ICU nonsurvivor - no. (%)			20 (12)	8 (11)	6 (14)	13 (20)	0.34

[a]The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. [b]The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects

severity of illness, with higher values indicating more severe illness, and can range from 0 to 71. [c]ICU denotes intensive care unit. [d]NE denotes norepinephrine. * for the comparison between all treated and untreated patients ** for the comparison between matched treated and untreated patients.

5

ACTH AND CORTISOL RESPONSES TO CRH IN ACUTE, SUBACUTE, AND PROLONGED CRITICAL ILLNESS

Adapted from:

- **Peeters B,** Meersseman P, Vander Perre S, Wouters P, Debaveye Y, Langouche L, Van den Berghe G. ACTH and cortisol responses to CRH in acute, subacute, and prolonged critical illness: a randomized, double-blind, placebo-controlled, crossover cohort study. Intensive Care Med 2018; doi: 10.1007/s00134-018-5427-y.

Abstract

Background Critically ill patients reveal high plasma free-cortisol and low plasma ACTH. The latter has been explained either by shock/inflammation-induced cell damage to hypothalamus and/or pituitary or by feedback-inhibition exerted by free-cortisol, possibly predisposing to central adrenal insufficiency. One can expect augmented/prolonged ACTH-responses to a CRH-injection with hypothalamic damage, immediately suppressed responses with pituitary damage, whereas delayed decreased responses only in prolonged critical illness with feedback-inhibition.

Methods This randomized, double-blind, placebo-controlled crossover cohort study, compared ACTH-responses to 100µg IV corticorelin (CRH) and placebo in 3 cohorts of 40 matched patients in the acute (ICU-day 3-6), subacute (ICU-day 7-16) or prolonged phase (ICU-day 17-28) of critical illness, with 20 matched healthy subjects. CRH or placebo was injected in random order on two consecutive days. Blood was sampled repeatedly over 135min and ACTH-AUC-responses to placebo were subtracted from those to CRH.

Results The order of the CRH/placebo injections did not affect the hormone-responses, hence results could be pooled. Patients in the acute phase of illness had normal mean±SEM ACTH-responses (5149±848pg/mL.min versus 4120±688pg/mL.min in healthy subjects; P=0.77), whereas those in the subacute (2333±387pg/mL.min, P=0.01) and the prolonged phases (2441±685pg/mL.min, P=0.001) were low, irrespective of the presence of sepsis/septic shock or survival.

Conclusions Suppressed ACTH-responses to CRH in the more prolonged phases, but not acute phase, of critical illness are compatible with feed-back inhibition exerted by elevated free-cortisol, rather than by cellular damage to hypothalamus and/or pituitary. Whether CRH has the potential to prevent central adrenal insufficiency in long-stay ICU-patients remains to be investigated.

Introduction

Patients suffering from critical illnesses typically reveal high plasma (free)cortisol concentrations and low-normal plasma adrenocorticotropic hormone (ACTH). Experts have interpreted the absence of elevated plasma ACTH, particularly in patients with severe infections, as caused by inflammation or hypoperfusion-induced damage to cells of the hypothalamus whereby synthesis of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) is hampered.[4-7] Shock or inflammation could also directly damage the anterior pituitary gland.[7] Also, direct inhibition at the hypothalamus and/or pituitary level by various drugs have been suggested.[5,7,8] However, an alternative explanation could be that high circulating free cortisol levels, brought about by suppressed cortisol binding proteins and by reduced cortisol breakdown,[9] exert negative feedback-inhibition at the pituitary and/or the hypothalamic level, as such lowering ACTH, CRH, and AVP-expression/secretion.[10] However, during critical illness, ACTH secretion is not completely suppressed unlike what is observed with high doses of exogenous glucocorticoids or in patients with adrenal Cushing's syndrome.[11,12] This could be explained by other concomitant central activation, such as via stress-induced AVP-increase which could potentiate CRH effects.[12-15] Also, during the first weeks of critical illness, the frequency of the ACTH and cortisol pulses was found to be normal, whereas pulse amplitudes were lower than normal.[16] However, a recent study has shown that a central suppression of ACTH is present during critical illness.[17] Suppressed ACTH sustained over an extended period of time could predispose to adrenocortical atrophy and dysfunction.[18]

Differentiation between hypothalamic lesions, damage to the pituitary corticotropes and adrenal/ectopic causes of Cushing's syndrome can be done by analyzing plasma ACTH (and cortisol) responses to an intravenous CRH bolus injection.[19] If during critical illness, the hypothalamus would be acutely damaged by shock or inflammation, and the anterior pituitary gland would be intact, one would expect augmented/prolonged ACTH-responses.[20] If the pituitary would be acutely damaged by shock or inflammation, suppressed ACTH-responses would be expected from the early phase onward.[20] Alternatively, if ACTH is suppressed by feedback-inhibition at the level of the pituitary and hypothalamus, as in patients with adrenal/ectopic Cushing's syndrome or on high doses of glucocorticoids, the ACTH-responses to a CRH injection expectedly depend on the duration of hypercortisolism,

with initially normal ACTH-responses to CRH injection followed by lowered ACTH-responses in the prolonged phase of illness.[21]

We hypothesized that sustained elevation of circulating free cortisol, brought about by suppressed cortisol binding proteins and by reduced cortisol breakdown, reduces ACTH responses to a CRH injection specifically in the prolonged phase of critical illness, irrespective of the presence of sepsis/septic shock and irrespective of survival. To test this hypothesis, we performed a randomized, double-blind, placebo-controlled crossover cohort study to compare the ACTH (and cortisol) responses to a synthetic human CRH-analogue, in the acute, subacute and prolonged phases of critical illness with those of healthy subjects, in relation to presence of sepsis/septic shock and survival.

Methods

Study participants and sample size calculation

This randomized, double-blind, placebo-controlled crossover cohort study was performed in 5 medical/surgical ICUs at the University Hospitals of Leuven, Belgium. The study aimed at comparing 3 cohorts of unique adult (age ≥18y) critically ill patients, matched for demographics, comorbidities and type/severity of critical illness upon ICU admission (**Table 1**), assessed in the acute (ICU day 3-6), subacute (ICU day 7-16) or prolonged phase (ICU day 17-28) of critical illness, with demographically matched healthy control subjects. All patients with a stabilized condition for at least 48h, and an expected stay in ICU for at least another 48h, were screened for eligibility. Exclusion criteria were (details provided in the Supplementary Material) treatment with systemic glucocorticoids, etomidate, azoles or other drugs predisposing to adrenal insufficiency, no vital organ support, no arterial or central venous catheter in place, referral from another ICU, cerebral/pituitary/adrenal disorders, enrollment in another trial, or expected death within 12h (**Fig.1a**).

The sample size of the study was determined based on an estimated effect size of a long duration of critical illness on the ACTH-responses to corticorelin, a synthetic human CRH analogue that is further referred to as CRH. Twenty unique patients per cohort would allow to detect, with an alpha error of 1% or less and a power of 80% or more, a suppression of the ACTH-response to CRH in long-stay critically ill patients of the same size (±60% decrease) as previously reported for Cushing's patients on replacement hydrocortisone treatment 7-9 days after surgical removal of the tumor, in comparison with the response of 20 healthy volunteers.[21] To further account for confounding by various illness-related aspects, the required number of patients was doubled to 40 unique patients per cohort (total of 120) (**Fig.1a**). If a patient, who had been included in a certain time cohort was still in ICU and eligible for including in a later time cohort, this patient was tested again. The results from these repeated tests within the same patient were not included in the primary analysis but were analyzed separately as a secondary, additional, longitudinal analysis of the impact of duration of illness. Screening for eligible patients started on July 1, 2016, and continued until the preset number of 40 patients in all 3 cohorts was reached (May 10, 2018), with comparable proportions of 4 diagnostic categories (**Table 1** and **Fig.1a**). The study protocol was in accordance with the 1964 Declaration of Helsinki and its later amendments, was approved by

Table 1: Participant characteristics

	Healthy subjects (n=20)	P-value*	*Acute phase of ci*[e] (n=40)	*Subacute phase of ci*[e] (n=40)	*Prolonged phase of ci*[e] (n=40)	P-value**
Demography and anthropometry						
Male gender - no. (%)	13 (65)	0.94	28 (70)	24 (60)	25 (63)	0.62
Age - yr (mean ± SEM)	63 ± 3	0.58	67 ± 2	65 ± 2	62 ± 2	0.30
BMI[a] - kg/m^2 (mean ± SEM)	26.2 ± 0.7	0.94	25.2 ± 0.8	25.6 ± 0.8	27.6 ± 1.3	0.20
Admission characteristics						
Diabetes mellitus - no. (%)			6 (15)	6 (15)	8 (20)	0.79
Malignancy - no. (%)			6 (15)	12 (30)	13 (33)	0.14
APACHE II score[b] - (mean ± SEM)			30 ± 1	31 ± 1	31 ± 1	0.78
Emergency admission - no. (%)			8 (20)	8 (20)	9 (23)	0.95
Diagnostic admission categories						1.00
Cardiac surgery - no. (%)			9 (23)	9 (23)	9 (23)	
Complicated other surgery - no. (%)			10 (25)	10 (25)	10 (25)	
Multiple trauma and burns - no. (%)			16 (40)	16 (40)	16 (40)	
Medical - no. (%)			5 (12)	5 (12)	5 (12)	
ICU[c] day on testday 1 - median(IQR)			4 (3-5)	9 (7-12)	19 (17-22)	**<0.0001**
Patient characteristics on testday 1						
Infection - no. (%)			30 (75)	32 (80)	33 (83)	0.70
Sepsis[d] - no. (%)			27 (68)	30 (75)	30 (75)	0.69
Septic shock[d] - no. (%)			21 (53)	15 (38)	19 (48)	0.38
Plasma ACTH - pg/mL (median and IQR)	21 (15-33)	0.54	14 (11-32)	19 (14-27)	27 (17-41)	**0.009**
Plasma total cortisol - µg/dL (median and IQR)	13 (11-16)	**<0.0001**	24 (16-33)	24 (20-31)	23 (17-29)	0.47
Plasma free cortisol - µg/dL (median and IQR)	5 (0.4-0.7)	**<0.0001**	2.6 (1.2-4.6)	2.1 (1.4-4.5)	2.0 (1.1-3.0)	0.26
Plasma CBG - µg/mL (mean ± SEM)	52 ± 2	**<0.0001**	37 ± 1	41 ± 1	43 ± 1	**0.002**
Plasma albumin - g/dL (mean ± SEM)	6.4 ± 0.1	**<0.0001**	3.9 ± 0.2	3.9 ± 0.1	3.8 ± 0.1	0.86
Clinical outcomes						
Days in ICU - median and IQR			11 (8-18)	18 (13-26)	30 (28-44)	**<0.0001**
ICU non-survivor - no. (%)			8 (20)	9 (23)	9 (23)	0.95

[a]The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. [b]The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects severity of illness, with higher values indicating more severe illness, and can range from 0 to 71.[1] [c]ICU denotes intensive care unit. [d]Incidence of sepsis and septic shock was defined according to.[2,3] [e]ci denotes critical illness. The * indicates the comparison between healthy subjects and all patients. The ** indicates the comparison between patient cohorts.

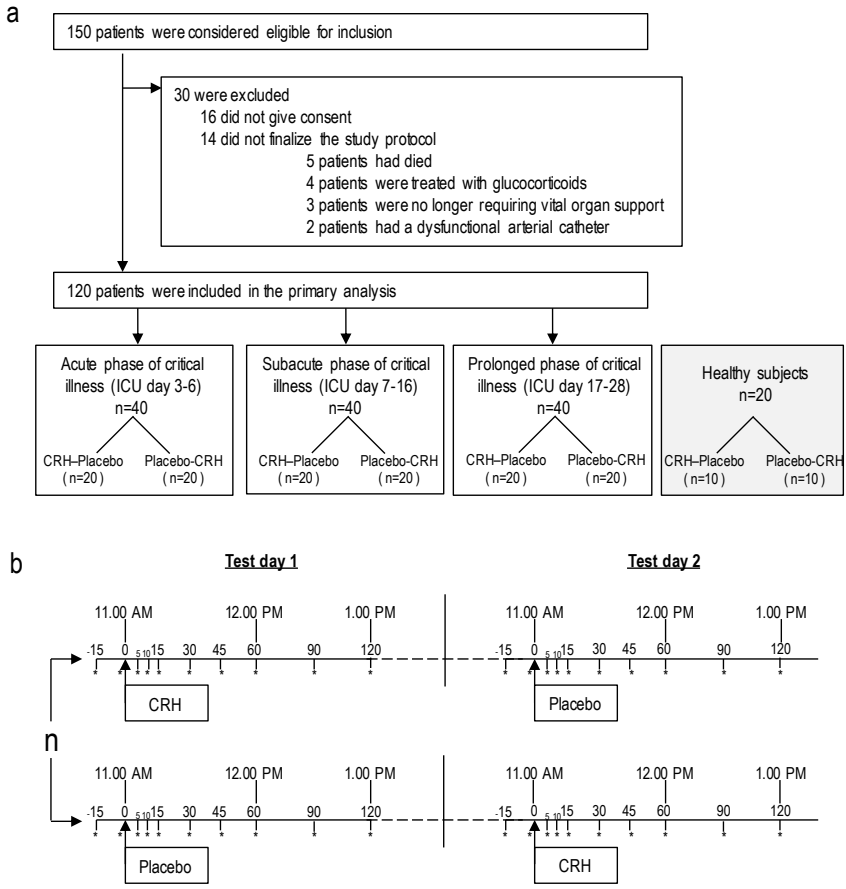


Figure 1 | Flowchart of the study participants and study design
Panel a. Flowchart of the study participants. **Panel b.** Randomization into crossover subgroups. ICU denotes intensive care unit. * blood sample.

the Institutional Ethical Review Board (S58941) and made available prior to study start (ISRCTN14587520).

Clinical data, study design, and sample collection

Demographic, ICU admission and patient characteristics at study inclusion in a time cohort, and patient outcomes were documented (**Table 1**). After obtaining written informed consent from the healthy volunteers and from the patients or the patients' next of kin, intravenous injections of either 100µg of the synthetic human CRH analogue (CRH Ferring®) in 1ml 0.9%NaCl or of placebo (1ml 0.9%NaCl) were given on two consecutive days at 11:00 AM, in a random order (**Fig.1b**). Concealment of order assignment was ensured by the use of

a central computerized randomization system. The randomization was stratified in permuted blocks of 2 according to cohort number and the 4 diagnostic admission categories. The block size was unknown to the medical and research teams. Members from the clinical staff who were not involved in the study or patient care, were responsible for preparation and blinding of study medication. Patients, healthy subjects, and the research team were blinded for CRH or placebo injection. For sample collection and quantification of plasma ACTH and (free)cortisol concentrations, see Supplementary Material.

Data and statistical analyses

Within the crossover design, each patient or healthy subject served as his/her own control. First, it was investigated whether the order of administration of placebo and CRH affected the hormonal responses and if this was not the case, the results for placebo and CRH could be pooled for further analysis. To determine the change in the area under the curve (AUC) of plasma ACTH and (free)cortisol in response to placebo or CRH, the plasma concentrations of sample 1 and 2 (before injection) were averaged and served as baseline, after which the AUC was calculated by the trapezoidal rule, on the placebo and the CRH test day. The AUC of the hormone-responses to placebo were than subtracted from the AUC of the hormone-responses to CRH, to determine the "delta AUC", which is further referred to as the "incremental hormone-response". In addition, plasma half-life of ACTH and of cortisol were estimated by dividing ln2 by the estimated elimination rate constant, calculated from the slope of the regression line of the log-transformed linear decline of the concentration over time.[22]

All data are presented as mean±standard error of the mean (SEM), median and interquartile range (IQR), or numbers and percentages. Comparisons of normally distributed data were performed with use of unpaired Student's t-tests, and Wilcoxon rank-sum test was used to compare non-normally distributed data. Proportions were compared with the use of chi-square tests. To compare time-series, repeated measures ANOVA was used, where necessary after transformation to obtain a near-normal distribution. Statistical analyses were performed with use of JMP® Pro 13.0.0 (SAS Institute, Cary, NC, USA). Two-sided P-values at or below 0.05 were considered to indicate statistical significance.

Results

Patient characteristics and baseline plasma concentrations of ACTH and (free)cortisol

One hundred and twenty critically ill patients and 20 healthy subjects were studied (**Table 1**). The 3 time cohorts (median 4 days, 9 days or 19 days in ICU) had equal proportions of patients within the 4 admission diagnostic categories and of emergency admissions and had similar admission APACHE II scores. For each time cohort, as compared with healthy subjects, patients had similar morning plasma ACTH concentrations, higher plasma (free)cortisol concentrations, lower plasma cortisol binding proteins (CBG and albumin) concentrations (**Table 1**). With increasing time in ICU, plasma ACTH and CBG concentrations increased slightly, whereas plasma (free)cortisol remained high and albumin concentrations remained low. Of the 120 patients, 87 (73%) suffered from sepsis and 55 (46%) suffered from septic shock at study inclusion, 26 (22%) patients died in the ICU, and 42 (35%) died while in hospital.

Plasma incremental ACTH-responses to CRH over time in ICU

For patients as well as healthy subjects, the order of the CRH/placebo injections did not affect the ACTH-responses (P=0.15 for the acute phase, P=0.08 for the subacute phase, P=1.00 for the prolonged phase, and P=0.16 for the healthy subjects) (**Fig.2**). Accordingly, results could be pooled for further analysis.

As compared with ACTH-responses of healthy subjects, the ACTH-responses of patients in the acute phase of critical illness were similar, whereas those in the subacute and the prolonged phases were lower (**Fig.3a**). The mean ACTH-responses to CRH decreased by 55% from the acute to the subacute phase, and remained constant from the subacute to the prolonged phase (**Fig.3a**).

Of the 120 unique patients, 30 patients were tested more than once. Of these 30 patients, 19 were tested in the acute and the subacute phase, and 14 were tested in the subacute and prolonged phase. Longitudinal analyses of these repetitive tests within patients

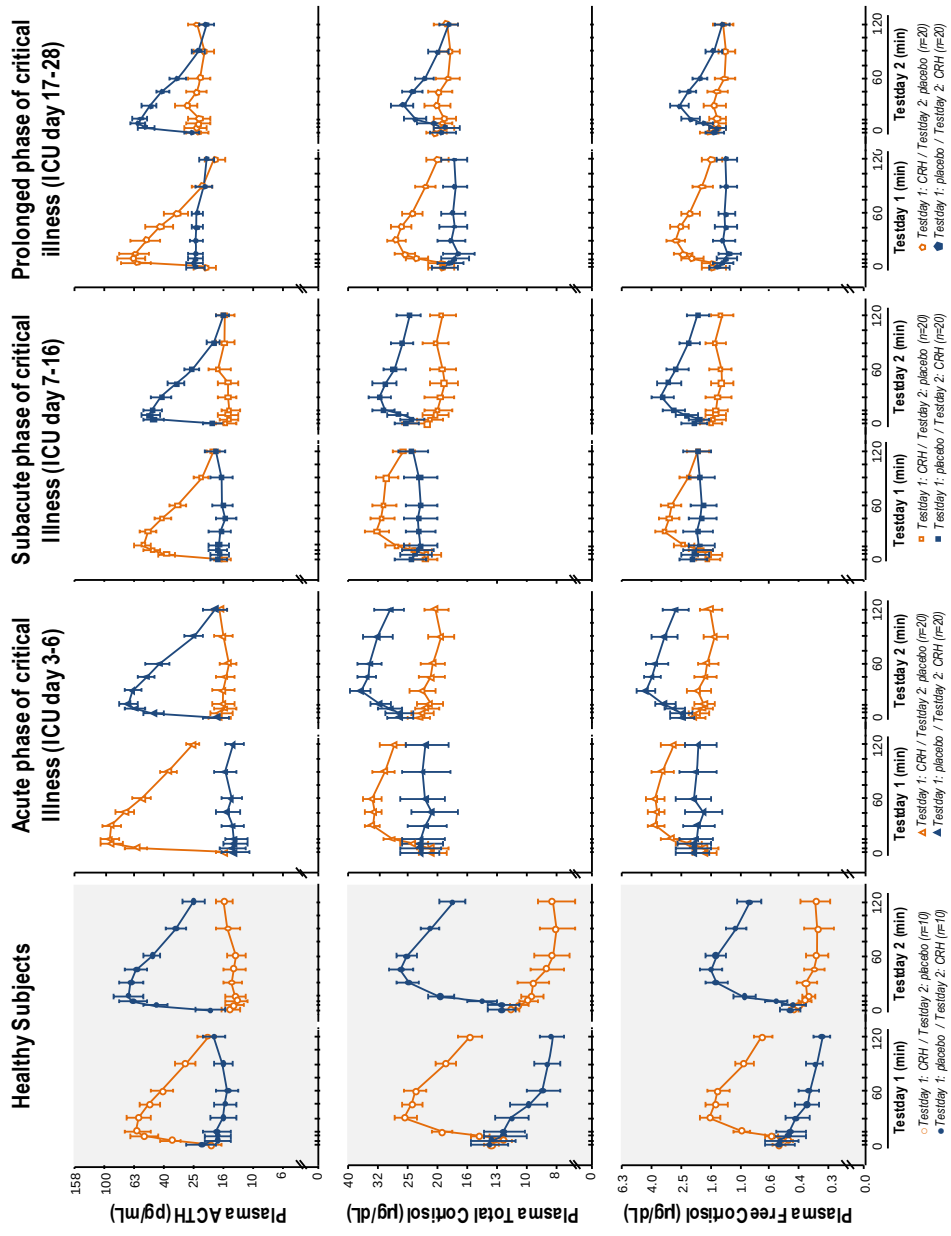


Figure 2 | Plasma ACTH, total and free cortisol concentrations after CRH or placebo injection over time in ICU

Data are shown as mean±SEM on a logarithmic scale. ICU denotes intensive care unit.

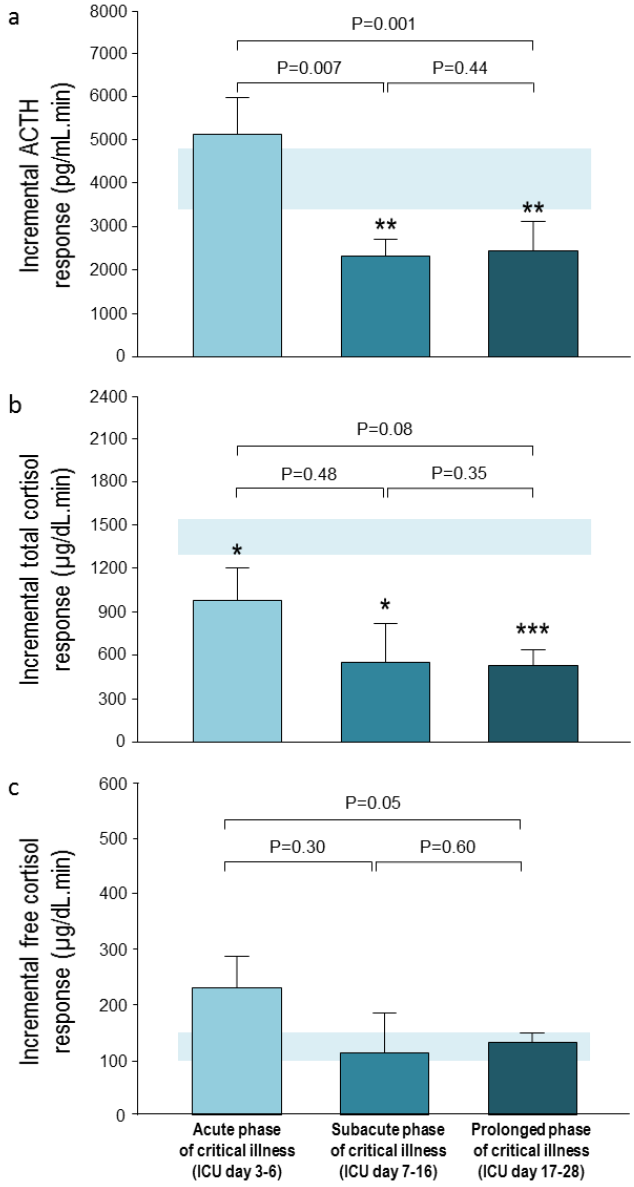


Figure 3 | Incremental (a) ACTH, (b) total cortisol and (c) free cortisol-responses to CRH and placebo in 3 patient cohorts

The AUC hormone-responses to placebo were subtracted from the AUC hormone-responses to CRH and indicate the incremental hormone-responses. Data are shown as mean±SEM on a logarithmic scale. ICU denotes intensive care unit. The horizontal blue-shaded areas represent the mean±SEM incremental hormone-responses from the 20 healthy subjects. * P≤0.05, ** P≤0.01, and *** P≤0.0001 for the comparisons with healthy subjects. The numerical P-values are those for the comparisons between patient cohorts.

confirmed the results of the unique patient cohorts, with a decrease of the mean ACTH-responses to CRH by 60% from the acute to the subacute phase (P=0.01) and no further change from the subacute to the prolonged phase (P=0.74).

Plasma incremental (free)cortisol-responses to CRH over time in ICU

As compared with total cortisol-responses to CRH of healthy subjects, total cortisol-responses of patients in the acute, subacute, and prolonged phases of critical illness were always lower (**Fig.3b**), whereas the free cortisol-responses were always normal (**Fig.3c**). As compared with the acute phase of critical illness, total cortisol-responses to CRH tended to further lower (**Fig.3b**) and free cortisol-responses further lowered (**Fig.3c**) in the prolonged phase.

In healthy subjects, the ACTH-responses to CRH correlated positively with the total cortisol-responses (P=0.001, R^2=0.43) and with free cortisol-responses (P=0.004, R^2=0.37). Patients also showed positive correlations between ACTH- and total cortisol-responses (P=0.001, R^2=0.09) and between ACTH- and free cortisol-responses (P=0.0003, R^2=0.10), but these correlations were much weaker than in healthy subjects.

Estimated half-life of plasma ACTH and (free)cortisol over time in ICU

The estimated plasma half-life of ACTH in patients was always similar to that in healthy subjects (P=0.57, **Fig. S1**). The estimated plasma half-life of total cortisol was a mean 3.25-fold longer in patients than in controls (P=0.0002), and the estimated plasma half-life of free cortisol was a mean 3.10-fold longer in patients than in controls (P=0.006).

Comparison of survivors with non-survivors, and patients with and without sepsis/septic shock

The ACTH-responses were always similar for hospital survivors and non-survivors, for patients with and without sepsis, and for patients with and without septic shock (**Fig.4**). This also applied to the (free)cortisol-responses (data not shown).

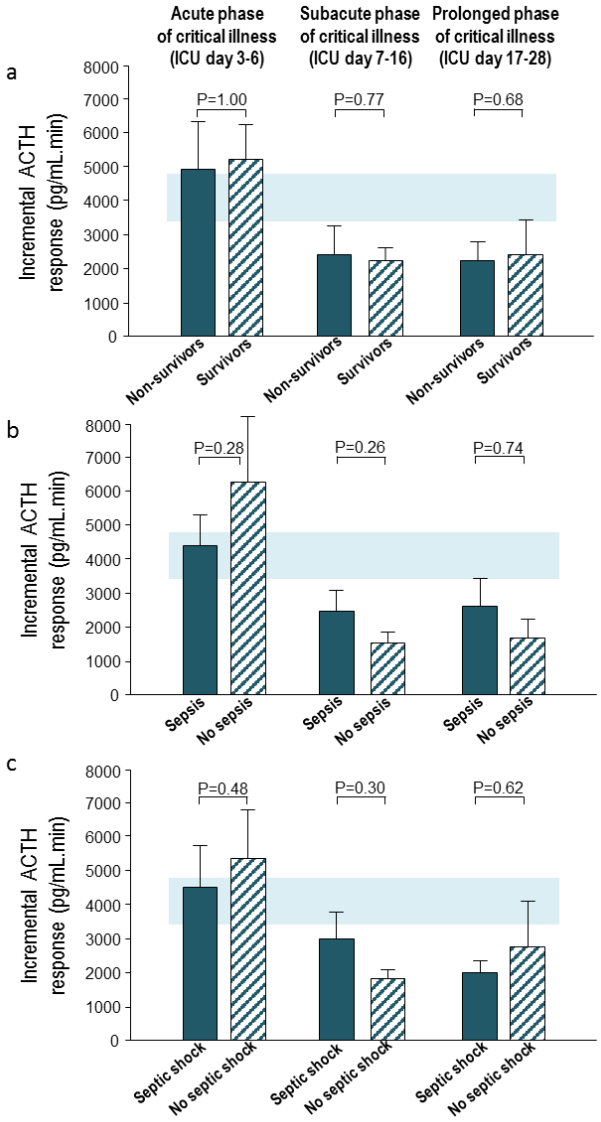


Figure 4 | Incremental ACTH-responses to CRH and placebo in 3 patient cohorts, in (a) survivors and non-survivors, (b) patients with and without sepsis, and (c) patients with and without septic shock

The AUC ACTH-responses to placebo were subtracted from the AUC ACTH-responses to CRH and indicate the incremental ACTH-responses. Data are shown as mean±SEM on a logarithmic scale. ICU denotes intensive care unit. The horizontal blue-shaded areas represent the mean±SEM incremental hormone-responses from the 20 healthy subjects. The numerical P-values are those for the comparisons between patient groups.

Side effects of CRH injection

None of the patients revealed hemodynamic instability in response to any of the test injections, whereas a sense of flushing was reported by all healthy subjects on one of the 2 study days.

Discussion

In the presence of low/normal baseline plasma ACTH and increased plasma (free)cortisol concentrations, incremental ACTH-responses to CRH in patients in the acute phase of critical illness were normal, whereas ACTH-responses became ±55% lower than normal in the later phases, irrespective of the presence of sepsis/septic shock or survival. Interestingly, the total cortisol-responses to CRH were always lower than in healthy subjects whereas the free cortisol-responses were always normal, in line with increased cortisol distribution volume during critical illness.[9,17] The time courses of the ACTH-responses to CRH were thus compatible with feed-back inhibition exerted by elevated free cortisol, rather than with hypothalamic and/or pituitary cell damage. These findings generate the hypothesis that CRH could offer potential for prevention of central hypoadrenalism in ICU patients who require intensive care for several weeks, for whom it has been shown that free cortisol levels are no longer elevated.[17] The absence of hemodynamic instability in response to the CRH injections in the patients of this study is an important safety aspect for future studies.

The observation of a normal ACTH response to CRH in the first few days of critical illness argues against a damaged hypothalamus or pituitary by hypoperfusion or inflammation.[7] The finding that presence of sepsis or septic shock did not affect ACTH-responses at any time during the course of critical illness further supports this interpretation. The 55% lowering of the ACTH-responses to CRH in the subacute and prolonged phase of critical illness corroborates sustained feedback-inhibition by elevated circulating free cortisol and is in line with the previously documented suppressed nocturnal pulsatile ACTH secretion during critical illness.[16] Indeed, a similar degree of suppression of the ACTH-response to CRH has been reported for patients after surgical treatment for Cushing's syndrome and for patients after withdrawal of ≥2 weeks of therapeutic glucocorticoid treatment.[21,23] The suppressed ACTH-responses to CRH observed in the subacute/prolonged phases of critical illness is compatible with low endogenous CRH and/or low vasopressin signaling,[24] that both can be suppressed by high circulating levels of glucocorticoids.[10] During health, hypothalamic CRH-neurons co-express CRH and AVP, which synergistically activate distinct signaling pathways within pituitary corticotropes.[25] It is well known that AVP is only a weak direct stimulator of ACTH but a much more powerful synergizer of CRH,[10] and thus AVP action may be required for a normal ACTH-response to exogenous CRH.[26] Vice versa, experiments in CRH knockout mice have shown that ACTH secretion depends on CRH.[27,28] Reactivation of

hypothalamic CRH secretion is indeed crucial for the reactivation of ACTH secretion after withdrawal of chronic glucocorticoid treatment.[28] Downregulation of CRH expression, via activating the glucocorticoid receptor, can be brought about by elevated free cortisol and/or by high circulating levels of bile acids that have previously shown to characterize subacute and prolonged critical illness.[29,30] A postmortem study of human patients who died from septic shock after an illness of approximately one week, reported reduced ACTH mRNA levels in the pituitary gland.[4] This suppressed ACTH gene expression occurred in the absence of a compensatory rise in the expression of CRH and vasopressin in the hypothalamus and without altered expression of the CRH-receptor 1 and the vasopressin-receptor (V1b), supporting our current findings.[4] The results of the current study however cannot rule out a direct pituitary defect due to effects of inflammation and/or hypoxia selectively in the more prolonged phases of illness.

Remarkably, in all patients, irrespective of the duration of illness, total cortisol-responses to CRH were lower than normal whereas free cortisol-responses were always normal. This is in line with a recent study of long-stay patients who received weekly short ACTH stimulation tests for 4 weeks in the ICU, that revealed uniformly low incremental total cortisol-responses but normal incremental free cortisol-responses, explained by low plasma binding and increased cortisol distribution volume.[17] In the current study, with increasing duration of critical illness, both total and free cortisol-responses tended to further decrease. This could be partially explained by the suppressed ACTH release in response to CRH and/or by the onset of decline of adrenocortical function. Indeed, appropriate ACTH signaling is essential to maintain integrity and function of the adrenal cortex.[31] A post-mortem study of adrenal glands harvested from patients who had been critically ill for several weeks showed loss of zonational structure, lipid droplet depletion, and suppressed ACTH-regulated gene expression.[18] Suppressed ACTH secretion could thus negatively affect adrenal function in long-stay ICU patients.[16,32] Such a negative effect of suppressed ACTH could also explain why critically ill patients beyond the fourth week in the ICU were recently shown to have circulating (free)cortisol levels that were not higher than those of healthy subjects, despite their severe illness and high risk of death.[17] One week after ICU discharge on the regular ward, survivors had higher than normal plasma ACTH and (free)cortisol levels, although they were recovering. This further suggested a central adrenocortical suppression during the ICU phase, which could predispose long-stay ICU patients to central adrenal insufficiency.

A first limitation of this study is that, for obvious reasons, no hypothalamic and pituitary tissues were available for quantification of expression of CRH, vasopressin, ACTH, and of the CRH-receptor 1 and vasopressin-receptor. This should be done in validated animal models of prolonged critical illness.[33] A second limitation is that one cannot exclude additional suppression at the hypothalamic level from analgo-sedative drugs that are used throughout ICU stay, of which opioids are the main component.[34] Indeed, intra-operative opioids and prolonged opioid use for chronic pain have shown to lower plasma ACTH concentrations.[35-39] Furthermore, in healthy subjects, morphine blunts the ACTH-response to CRH injection at a supra-pituitary level.[40] However, given the normal ACTH-responses to CRH, observed during the acute phase, when opioid doses are usually higher than in the later phases, an important role of opioids is unlikely. Also other sedative drugs can have an additional suppressive effects, such as the benzodiazepine alprazolam, which may inhibit the central HPA axis in patients with panic disorder, in which a central overdrive may play a role, and thereby exert its anti-panic effect.[41] Dexmedetomidine is a new and highly selective α2-adrenergic receptor agonist, with sedative, analgesic, and anxiolytic functions, which in theory can inhibit the HPA axis.[42] It is widely used in various surgical anesthesias and during ICU stay, but does not appear to influence HPA axis activity.[43,44] The strengths of the study were the randomized, double-blind, placebo-controlled crossover design, which allowed to compare matched patients in different phases of critical illness while minimizing confounders.

Our findings open perspectives for novel strategies to protect long-stay ICU patients against the risk of developing adrenal insufficiency. If the lack of priming of the corticotropes by CRH would be responsible for reduced ACTH expression and secretion, providing CRH could potentially allow (re)activation of ACTH synthesis and release in response to any fall in cortisol and could hereby prevent adrenal atrophy in the prolonged phase of illness.[45] It has been shown that continuous infusion of CRH can reactivate ACTH secretion with preservation of circadian rhythmicity and pulsatility.[46] Studies of CRH infusion in the critically ill should probably initiate this intervention rather early, when the corticotropes are still fully responsive to CRH. If corticotropes remain sensitive to feedback-inhibition, CRH infusion may not result in too high plasma cortisol and would respect any eventual tissue-specific regulation of cortisol action, which are important safety aspects. In the current study, no side effects of a CRH bolus were noted. However, caution is warranted given that CRH has also been involved in anxiety disorders, depression, memory and

learning,[47,48] and is able to increase catecholamines and heart rate.[49] If a direct pituitary defect would be present in the prolonged phases of illness, which we could not exclude, CRH will not be able to prevent this.

In conclusion, the results of the CRH tests did not support the presence of shock/inflammation-induced hypothalamic and/or pituitary damage in critically ill patients. Instead, the consequences of prolonged feedback-inhibition exerted by elevated (free)cortisol are compatible with suppressed ACTH-responses to CRH in the prolonged phases of critical illness. These findings raise the hypothesis that CRH infusion could prevent the development of a central adrenal insufficiency in long-stay ICU patients, which should be further investigated.

Acknowledgements

We thank the patients and healthy volunteers for participating, the clinical staff for excellent protocol compliance and the research assistants for sample handling and data entry. We thank Ferring International Center SA, Switzerland, for kindly providing the study drug (CRH Ferring), to support scientific research in septic shock, and we thank dr. Johan Masure (Ferring Pharmaceuticals) for facilitating this support. This work was supported by the Research Foundation-Flanders (FWO) [Grant G091918N to GVdB, research mandate 11W9315N to BP]; by the Methusalem Program of the Flemish Government [METH/14/06 to GVdB and LL via KU Leuven]; by a European Research Council Advanced Grant [AdvG-2017-785809 to GVdB] from European Union's Horizon 2020 research and innovation programme.

Personal contribution

Bram Peeters participated in designing the study, conducting the study, gathering and analyzing the data, and writing the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-29.
2. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644-55.
3. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016; **315**: 801-10.
4. Polito A, Sonneville R, Guidoux C, et al. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoSOne* 2011; **6**: e25905.
5. Bornstein SR. Predisposing factors for adrenal insufficiency. *N Engl J Med* 2009; **360**: 2328-39.
6. Deutschman CS, Raj NR, McGuire EO, Kelz MB. Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med* 2013; **41**: e368-e75.
7. Annane D. The Role of ACTH and Corticosteroids for Sepsis and Septic Shock: An Update. *Front Endocrinol (Lausanne)* 2016; **7**: 70.
8. Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010; **31**: 98-132.
9. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477-88.
10. Jameson J, De Groot L. Endocrinology: Adult and Pediatric, 7th Edition. Philadelphia: Elsevier; 2016: 142-3.
11. Wagner-Bartak NA, Baiomy A, Habra MA, et al. Cushing Syndrome: Diagnostic Workup and Imaging Features, With Clinical and Pathologic Correlation. *AJR Am J Roentgenol* 2017; **209**: 19-32.
12. Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocrine reviews* 1984; **5**: 1-24.
13. Raff H, Sharma ST, Nieman LK. Physiological basis for the etiology, diagnosis, and treatment of adrenal disorders: Cushing's syndrome, adrenal insufficiency, and congenital adrenal hyperplasia. *Compr Physiol* 2014; **4**: 739-69.
14. Hauger RL, Aguilera G. Regulation of pituitary corticotropin releasing hormone (CRH) receptors by CRH: interaction with vasopressin. *Endocrinology* 1993; **133**: 1708-14.
15. Watanabe T, Oki Y, Orth DN. Kinetic actions and interactions of arginine vasopressin, angiotensin-II, and oxytocin on adrenocorticotropin secretion by rat anterior pituitary cells in the microperifusion system. *Endocrinology* 1989; **125**: 1921-31.
16. Boonen E, Meersseman P, Vervenne H, et al. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab* 2014; **306**: E883-92.
17. Peeters B, Meersseman P, Vander Perre S, et al. Adrenocortical function during prolonged critical illness and beyond: a prospective observational study. *Intensive Care Med* 2018.
18. Boonen E, Langouche L, Janssens T, et al. Impact of Duration of Critical Illness on the Adrenal Glands of Human Intensive Care Patients. *J Clin Endocrinol Metab* 2014; **99**: 4214-22.
19. Schulte HM, Chrousos GP, Avgerinos P, et al. The corticotropin-releasing hormone stimulation test: a possible aid in the evaluation of patients with adrenal insufficiency. *J Clin Endocrinol Metab* 1984; **58**: 1064-7.
20. Jameson J, De Groot L. Endocrinology: Adult and Pediatric, 7th Edition. Philadelphia: Elsevier; 2016: 2655-88.
21. Gomez MT, Magiakou MA, Mastorakos G, Chrousos GP. The pituitary corticotroph is not the rate limiting step in the postoperative recovery of the hypothalamic-pituitary-adrenal axis in patients with Cushing syndrome. *J Clin Endocrinol Metab* 1993; **77**: 173-7.
22. Charmandari E, Hindmarsh PC, Johnston A, Brook CG. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: alterations in cortisol pharmacokinetics at puberty. *J Clin Endocrinol Metab* 2001; **86**: 2701-8.
23. Graber AL, Ney RL, Nicholson WE, Island DP, Liddle GW. Natural History of Pituitary-Adrenal Recovery Following Long-Term Suppression with Corticosteroids. *J Clin Endocrinol Metab* 1965; **25**: 11-6.
24. Beurel E, Nemeroff CB. Interaction of stress, corticotropin-releasing factor, arginine vasopressin and behaviour. *Curr Top Behav Neurosci* 2014; **18**: 67-80.

25. Herman JP, McKlveen JM, Ghosal S, et al. Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Compr Physiol* 2016; **6**: 603-21.
26. Levin N, Shinsako J, Dallman MF. Corticosterone acts on the brain to inhibit adrenalectomy-induced adrenocorticotropin secretion. *Endocrinology* 1988; **122**: 694-701.
27. Muglia LJ, Jacobson L, Luedke C, et al. Corticotropin-releasing hormone links pituitary adrenocorticotropin gene expression and release during adrenal insufficiency. *J Clin Invest* 2000; **105**: 1269-77.
28. Paragliola RM, Papi G, Pontecorvi A, Corsello SM. Treatment with Synthetic Glucocorticoids and the Hypothalamus-Pituitary-Adrenal Axis. *Int J Mol Sci* 2017; **18**.
29. Vanwijngaerden YM, Wauters J, Langouche L, et al. Critical illness evokes elevated circulating bile acids related to altered hepatic transporter and nuclear receptor expression. *Hepatology* 2011; **54**: 1741-52.
30. McMillin M, Frampton G, Quinn M, et al. Suppression of the HPA Axis During Cholestasis Can Be Attributed to Hypothalamic Bile Acid Signaling. *Mol Endocrinol* 2015; **29**: 1720-30.
31. Ferreira JG, Cruz CD, Neves D, Pignatelli D. Increased extracellular signal regulated kinases phosphorylation in the adrenal gland in response to chronic ACTH treatment. *J Endocrinol* 2007; **192**: 647-58.
32. Stavreva DA, Wiench M, John S, et al. Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. *Nat Cell Biol* 2009; **11**: 1093-102.
33. Jenniskens M, Weckx R, Dufour T, et al. The Hepatic Glucocorticoid Receptor Is Crucial for Cortisol Homeostasis and Sepsis Survival in Humans and Male Mice. *Endocrinology* 2018; **159**: 2790-802.
34. Pascoe JE, Williams KL, Mukhopadhyay P, Rice KC, Woods JH, Ko MC. Effects of mu, kappa, and delta opioid receptor agonists on the function of hypothalamic-pituitary-adrenal axis in monkeys. *Psychoneuroendocrinology* 2008; **33**: 478-86.
35. Taylor T, Dluhy RG, Williams GH. beta-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J Clin Endocrinol Metab* 1983; **57**: 592-6.
36. Aloisi AM, Aurilio C, Bachiocco V, et al. Endocrine consequences of opioid therapy. *Psychoneuroendocrinology* 2009; **34 Suppl 1**: S162-8.
37. Abs R, Verhelst J, Maeyaert J, et al. Endocrine consequences of long-term intrathecal administration of opioids. *J Clin Endocrinol Metab* 2000; **85**: 2215-22.
38. Shinoda T, Murakami W, Takamichi Y, Iizuka H, Tanaka M, Kuwasako Y. Effect of remifentanil infusion rate on stress response in orthopedic surgery using a tourniquet application. *BMC Anesthesiol* 2013; **13**: 14.
39. Watanabe K, Kashiwagi K, Kamiyama T, et al. High-dose remifentanil suppresses stress response associated with pneumoperitoneum during laparoscopic colectomy. *J Anesth* 2014; **28**: 334-40.
40. Rittmaster RS, Cutler GB, Jr., Sobel DO, et al. Morphine inhibits the pituitary-adrenal response to ovine corticotropin-releasing hormone in normal subjects. *J Clin Endocrinol Metab* 1985; **60**: 891-5.
41. Abelson JL, Curtis GC, Cameron OG. Hypothalamic-pituitary-adrenal axis activity in panic disorder: effects of alprazolam on 24 h secretion of adrenocorticotropin and cortisol. *J Psychiatr Res* 1996; **30**: 79-93.
42. al-Damluji S. Adrenergic control of the secretion of anterior pituitary hormones. *Baillieres Clin Endocrinol Metab* 1993; **7**: 355-92.
43. Gu H, Zhang M, Cai M, Liu J. Comparison of Adrenal Suppression between Etomidate and Dexmedetomidine in Children with Congenital Heart Disease. *Med Sci Monit* 2015; **21**: 1569-76.
44. Kim MH, Lee KY, Bae SJ, Jo M, Cho JS. Intraoperative dexmedetomidine attenuates stress responses in patients undergoing major spine surgery. *Minerva Anestesiol* 2018.
45. Ur E, Capstick C, McLoughlin L, Checkley S, Besser GM, Grossman A. Continuous administration of human corticotropin-releasing hormone in the absence of glucocorticoid feedback in man. *Neuroendocrinology* 1995; **61**: 191-7.
46. Schulte HM, Chrousos GP, Gold PW, et al. Continuous administration of synthetic ovine corticotropin-releasing factor in man. Physiological and pathophysiological implications. *J Clin Invest* 1985; **75**: 1781-5.
47. Behan DP, Grigoriadis DE, Lovenberg T, et al. Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders. *Mol Psychiatry* 1996; **1**: 265-77.

48. Smith SR, de Jonge L, Pellymounter M, et al. Peripheral administration of human corticotropin-releasing hormone: a novel method to increase energy expenditure and fat oxidation in man. *J Clin Endocrinol Metab* 2001; **86**: 1991-8.
49. Nijsen MJ, Croiset G, Stam R, et al. The role of the CRH type 1 receptor in autonomic responses to corticotropin- releasing hormone in the rat. *Neuropsychopharmacology* 2000; **22**: 388-99.
50. Vanhorebeek I, Peeters RP, Vander Perre S, et al. Cortisol response to critical illness: effect of intensive insulin therapy. *J Clin Endocrinol Metab* 2006; **91**: 3803-13.

Supplementary Appendix

Study participants

Exclusion criteria were administration of glucocorticoids within the last 72 hours, chronic treatment with glucocorticoids or other steroids within the last three months, use of etomidate within the last 72h, use of azoles within the last 7 days, other drugs predisposing to adrenal insufficiency (phenytoin, rifampicin, glitazones, imipramin, phenothiazine, phenobarbital), no longer requiring vital organ support, no arterial or central venous catheter in place, referral from another ICU, cerebral disease with intracranial hypertension threatening the neuroendocrine system, pituitary disorders, known adrenal disease, enrollment in another trial, or expected death within 12h.

Sample collection and quantification of plasma ACTH and (free) cortisol concentrations

Undiluted blood was sampled 15 minutes before injection of placebo or CRH, immediately prior to injection, as well as 5, 10, 15, 30, 45, 60, 90, and 120 minutes after injection. Samples were taken via an arterial catheter in place for clinical purposes for ICU patients and via a venous puncture for the healthy subjects. As required for accurate quantification of plasma ACTH concentrations, blood samples were collected in pre-chilled EDTA tubes and immediately placed on ice, centrifuged at 4°C and stored at -80°C until assay.

Plasma ACTH concentrations were measured with a double-monoclonal immunoradiometric assay (Brahms Diagnostics, Berlin, Germany). Total plasma cortisol concentrations (Immunotech, Prague, Czech Republic) and plasma cortisol-binding-globulin (CBG) concentrations (Riazen, Louvain-La-Neuve, Belgium) were quantified by competitive radio-immunoassay. Plasma albumin was quantified by the bromocresol green colorimetric method (Sigma-Aldrich, St. Louis, Missouri, USA). Plasma free cortisol was calculated using the Coolens' formula adapted for albumin and CBG concentrations, which has been previously validated as representative of measured free cortisol concentrations in the ICU context.[16,50]

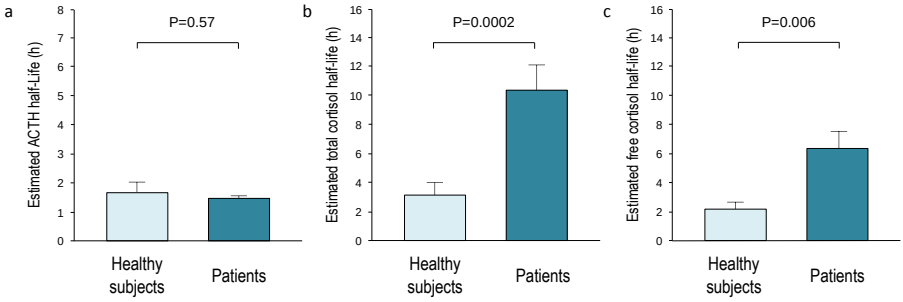


Figure S1 | Estimated half-life of plasma (a) ACTH, (b) total cortisol and (c) free cortisol in patients compared to healthy subjects

The plasma half-lives were estimated by dividing ln2 by the estimated elimination rate constant, calculated from the slope of the regression line of the log-transformed linear decline of the concentration over time. h denotes hour. The numerical P-values are those for the comparisons between patients and healthy subjects.

6

GENERAL DISCUSSION AND PERSPECTIVES

Patients suffering from critical illness requiring treatment in an intensive care unit (ICU) typically reveal elevated plasma cortisol concentrations, in proportion to illness severity. This was traditionally attributed exclusively to a central activation of the hypothalamus-pituitary-adrenal (HPA) axis. However, low rather than high plasma ACTH concentrations have been reported in critically ill patients during the first 7 days in ICU, with loss of diurnal ACTH and cortisol rhythm. Low ACTH together with high cortisol is referred to as 'ACTH-cortisol dissociation'. Although cortisol production is somewhat increased with inflammation, a reduced cortisol breakdown explains to a larger extent the hypercortisolism during critical illness. Inflammation-driven decrease in cortisol binding proteins further increase the active free cortisol fraction. However, sustained low circulating ACTH might contribute to adrenal atrophy and dysfunction in the prolonged phase of critical illness. These findings have revived the ongoing debate about which level of cortisol availability is sufficient in the struggle for survival of the critically ill, about the concept of "critical illness related adrenal insufficiency", and about how to correctly interpret diagnostic laboratory tests. The ongoing controversy clearly indicates the need for further research on this important clinical problem.

The **general aim** of this doctoral thesis was thus to gain more insight into the regulation of the HPA axis response during the course of critical illness, from admission to recovery or death, in order to understand the so-called "ACTH-cortisol dissociation" and the pathophysiology of "critical illness related adrenal insufficiency". We hypothesized that during critical illness the pharmacological effects of frequently used drugs may partly explain the acute "ACTH-cortisol dissociation", we hypothesized that prolonged deprivation of trophic ACTH-effects on the adrenal cortex may contribute to adrenal insufficiency whereas reactivation of the pituitary with increased plasma ACTH occurs in those patients who recover, and we hypothesized that sustained elevated free cortisol levels, via feedback inhibition, may cause a central suppression of the HPA axis, specifically in the prolonged phase.

In **chapter 3** of this doctoral thesis, we investigated the pharmacological effects of frequently used drugs on the acute "ACTH-cortisol dissociation" in ICU patients in an **observational association study**. We used a multivariable linear regression analysis to investigate any independent association between these drugs and the concentrations of ACTH and cortisol in plasma collected upon ICU admission from a mixed set of surgical and

medical ICU patients.[1] We documented low plasma ACTH concentrations upon admission to the ICU in the face of normal total and elevated free cortisol plasma concentrations. A further lowering of plasma ACTH and a steep rise in plasma total/free cortisol were shown from the morning after admission to the ICU onwards. We showed that none of the drugs administered 24h prior to ICU admission independently affected plasma ACTH on ICU admission, adjusting for other known determinants of the stress response. However, we demonstrated a suppressive effect for opioids, etomidate, and propofol on admission plasma cortisol, and a stimulatory effect for dobutamine on admission plasma cortisol. These associations were independent of the medical or surgical diagnostic category, severity of illness, sepsis, or other patient characteristics. The presence of a subsequent rebound effect on plasma total and free cortisol concentrations was investigated for each suppressive drug, and we were able to demonstrate a steep rise in plasma total and free cortisol on the morning following ICU admission, when the transient drug effect of these suppressive drugs waned off.

In **chapter 4**, we investigated whether and when the central and peripheral alterations that occur within the HPA axis during prolonged critical illness recover, and to what extent the currently used diagnostic criteria for CIRCI relate to presence of sepsis/septic shock, need for glucocorticoid treatment or survival, in a **prospective observational study**. To this end, we documented the changes over time - from ICU-day 7 up to recovery or death - in plasma ACTH and total and free cortisol, urinary cortisol metabolites, and in plasma total and free cortisol responses to cosyntropin (a synthetic ACTH-analogue), with focus on long-stay (≥4 weeks in ICU) patients in comparison with shorter-stay patients (1-2 weeks; 2-3 weeks; 3-4 weeks in ICU). We showed that plasma ACTH remained low/normal throughout ICU-stay up to day 28, whereas plasma free cortisol remained high, largely explained by low plasma binding and persistently suppressed cortisol breakdown, irrespective of the presence of sepsis/septic shock. In particular the low binding proteins among sicker patients and non-survivors determined their higher plasma free cortisol levels. Development of primary adrenal failure was not observed. However, beyond ICU-day 28, plasma free cortisol was no longer elevated and not compensated by increased ACTH, which could be due to a central (endogenous or exogenous) adrenocortical suppression. Such a central suppression was further supported by the uniform rise in plasma ACTH and (free)cortisol to supra-normal levels 1 week later on the regular ward. Low incremental total cortisol responses to cosyntropin coincided with normal free cortisol responses at all times. Hence, low responses

to cosyntropin during critical illness likely reflected the increased cortisol distribution volume, which has been documented in an earlier study,[2] given low plasma binding, rather than the functional reserve of the adrenal cortex. Finally, the initiation of glucocorticoid treatment, as judged necessary by bedside clinicians, was not supported by differences in the presently measured adrenal function parameters.

In **chapter 5** of this research project, we performed a **randomized, double-blind, placebo-controlled crossover cohort study** to compare the ACTH (and cortisol) responses to a synthetic human CRH-analogue (corticorelin), in the acute, subacute and prolonged phases of critical illness with those of healthy subjects, in relation to presence of sepsis/septic shock and survival. We demonstrated that in the presence of low/normal baseline plasma ACTH and increased plasma total and free cortisol concentrations, incremental ACTH-responses to CRH in patients in the acute phase of critical illness were normal, whereas ACTH-responses became ±55% lower than normal in the later phases, irrespective of the presence of sepsis/septic shock or survival. Interestingly, the total cortisol-responses to CRH were always lower than in healthy subjects whereas the free cortisol-responses were always normal, in line with increased cortisol distribution volume during critical illness.[2] The time courses of the ACTH-responses to CRH were thus compatible with feed-back inhibition exerted by elevated free cortisol.

In summary, the findings of these 3 clinical studies add important information to correctly understand the pathophysiology of the HPA axis response during critical illness from admission to recovery and death. These dynamic changes in adrenocortical parameters during the course of critical illness, each time in comparison with those in matched healthy control subjects, are summarized in **Fig. 1**.

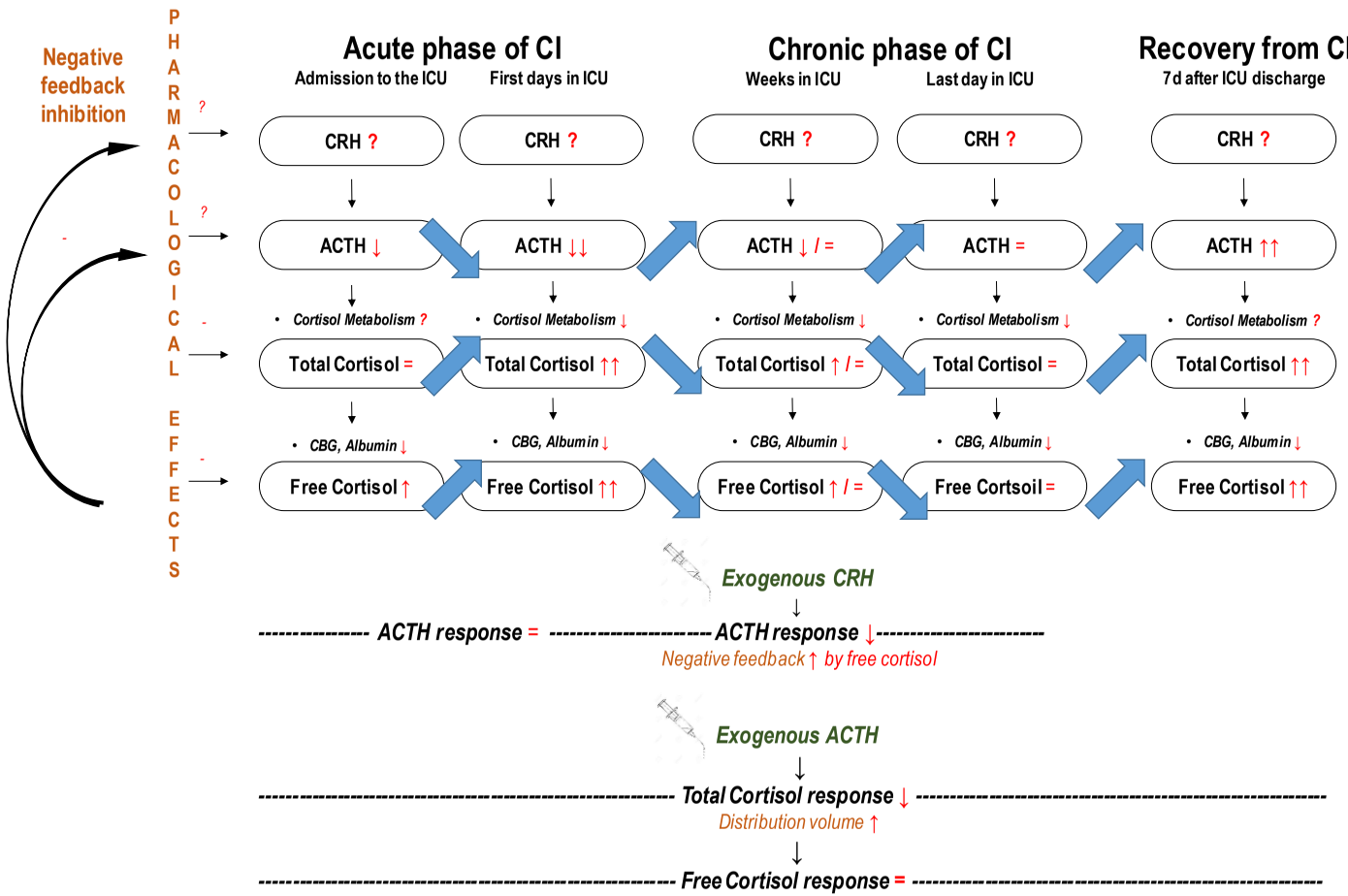

Negative feedback inhibition
PHARMACOLOGICAL EFFECTS
Acute phase of CI
Admission to the ICU
First days in ICU
Chronic phase of CI
Weeks in ICU
Last day in ICU
Recovery from CI
7d after ICU discharge
CRH ?
ACTH ↓
ACTH ↓↓
ACTH ↓ / =
ACTH =
ACTH ↑↑
• Cortisol Metabolism ?
• Cortisol Metabolism ↓
Total Cortisol =
Total Cortisol ↑↑
Total Cortisol ↑ / =
Total Cortisol =
Total Cortisol ↑↑
• CBG, Albumin ↓
Free Cortisol ↑
Free Cortisol ↑↑
Free Cortisol ↑ / =
Free Cortsoil =
Free Cortisol ↑↑
Exogenous CRH
ACTH response =
ACTH response ↓
Negative feedback ↑ by free cortisol
Exogenous ACTH
Total Cortisol response ↓
Distribution volume ↑
Free Cortisol response =

Figure 1 | Changes in adrenocortical function parameters during the course of critical illness
All changes in plasma hormone concentrations are in comparison with healthy matched control subjects. ↑, elevated plasma concentrations; ↓, decreased plasma concentrations; =, similar plasma concentrations; ?, unknown; -, inhibits; CI, critical illness; ICU intensive care unit; d, days; CRH, corticotropin releasing hormone; ACTH, adrenocorticotropic hormone; CBG, corticosteroid-binding globulin.

In the 3 studies, we observed a suppression, endogenous or exogenous, at some level of the HPA axis, during the course of critical illness. In **chapter 3**, a clear **iatrogenic suppressive effect from opioids, etomidate, and propofol on plasma total and free cortisol** in the acute phase of critical illness (admission to the ICU) was demonstrated, but not on plasma ACTH. This was surprising, as we had hypothesized that the pharmacological effects of drugs used during surgery or the acute phase of critical illness would (at least partly) explain the 'ACTH-cortisol dissociation'. More specifically, these effects would explain the low plasma ACTH concentrations as observed from day 4 in patients suffering from severe trauma and sepsis,[3] in patients during septic shock,[4] and from the first day in ICU in a mixed population of medical and surgical critically ill patients[2]. These suppressive effects on plasma ACTH would be in line with animal experiments,[5-7] and with small interventional studies in surgical[8-10] and ICU patients[11]. Notwithstanding the fact that we indeed documented low plasma ACTH, even from admission to the ICU onwards with even a further lowering during the following days in ICU, none of the administered drugs was identified as affecting plasma ACTH in the multivariable linear regression model. At first sight, these findings seem to contradict previous studies. However, this does not completely rule out the possibility of an effect at the hypothalamic and/or pituitary level from the analgo-sedative drugs, which are generally thought to mainly act centrally.[7] There are 2 reasons to mention. First, plasma ACTH was only measured at one single time point and higher plasma ACTH values may have occurred prior to the ICU admission, as documented in other studies shortly after surgery.[12,13] A potential initial suppression of plasma ACTH by analgo-sedative drugs before ICU admission, when plasma free cortisol was not yet elevated, can therefore not be ruled out. Second, during admission to the ICU, plasma total cortisol was in fact normal, but plasma free cortisol was already elevated, due to the instant fall in plasma CBG and albumin concentrations, and thus not by an activated HPA axis (as plasma total cortisol was not elevated). An increase in plasma free cortisol, the unbound cortisol fraction that exerts its biological effects, exerts immediate negative feedback inhibition at

the hypothalamic/pituitary level within minutes, and reduces CRH and ACTH release.[14] This inhibition of ACTH release through negative feedback inhibition by elevated free cortisol may have dominated during this phase of critical illness, and may have hidden any additional central pharmacological suppression on ACTH release. The steep rise in plasma total and free cortisol from the morning after admission to the ICU onwards together with the further lowering of plasma ACTH, seems to support the important role of negative feedback inhibition in the acute phase.

In contrast with ACTH, a clear iatrogenic suppressive effect of etomidate on plasma cortisol was demonstrated. Indeed, etomidate is a well-known strong suppressor of adrenocortical cortisol production by inhibiting the enzyme 11-beta-hydroxylase.[15] Besides etomidate, also propofol and more strongly opioids were associated with a large and dose-dependent lowering of plasma cortisol, probably by binding to the GABA-A-receptor and to specific opioid binding sites in the adrenal gland respectively.[16-19] The possibility that this suppression of plasma cortisol by these drugs would be exerted by a hidden additional central pharmacological suppression on ACTH release is unlikely, given the observation that the following morning plasma ACTH concentrations further lowered, while plasma cortisol concentrations rose to supranormal levels. Before admission to the ICU, the doses of opioids and propofol that were administered in the emergency department and the operating room were much higher than those which were administered during the following days in ICU, and the suppressing effects were shown to be dose-dependent. This suggests that the drug effects on the adrenal cortex especially played a role during the onset or the very acute phase of critical illness, when administered drug doses were high, with a steep rise in plasma cortisol when the transient drug effect wore off. The driving force of the steep increase in plasma cortisol between admission to the ICU and the following morning remains, however, unclear. A possible explanation is the increase in circulating inflammatory mediators, such as TNF, IL-1, and IL-6, that can activate cortisol secretion by potentiating the effects of ACTH on the adrenal gland and by a direct effect on steroidogenesis,[13,20-23] but also inhibitory effects have been described.[24,25] Alternatively, an acute reduced rate of conversion of plasma cortisol to cortisone via 11β-HSD2 in kidney and an acute reduced breakdown of cortisol via A-ring reductases in liver, as has been shown in patients beyond the onset of critical illness, could also apply during the first hours of critical illness.[2]

In conclusion of the first part, during the onset of critical illness, plasma ACTH seems suppressed mainly due to negative feedback inhibition by elevated free cortisol, plasma total cortisol seems suppressed due to the pharmacological effects of analog-sedative drugs, and plasma free cortisol seems elevated due to the instant lowering of cortisol binding proteins. Observational studies in which plasma ACTH and cortisol and cortisol metabolites are measured repeatedly, even earlier than admission to the ICU, from admission to the emergency department or the operating room onwards, in relation with the pharmacological effects of the administered drugs would help to better understand the dynamics of the acute HPA axis response during critical illness.

In **chapter 4**, an **adrenocortical suppression** was suggested only in **patients necessitating intensive care for more than 28 days**, in which plasma total and free cortisol were no longer elevated and not compensated by an increase in ACTH, despite their severe illness and high risk of death. On the contrary, throughout ICU-stay up to day 28, plasma total and free cortisol remained high, largely explained by low plasma binding and persistently suppressed cortisol breakdown, and development towards primary adrenal insufficiency was not observed. Furthermore, the evolution in plasma ACTH and plasma total and free cortisol were similar in patients with and without sepsis or septic shock. This observation was striking, as until now it is generally assumed that in particular patients suffering from sepsis and septic shock were at risk of a failing HPA axis response with insufficiently elevated plasma cortisol.[26-28] Our findings, however, strongly argue against the role of an inadequate HPA axis response in the pathophysiology and outcome of sepsis, as suggested by experts in the field.[29] Moreover, this longitudinal set-up of the study not only in patients with sepsis, but in a mixed population of medical, surgical, trauma, and burn patients, with and without sepsis, allowed to conclude that plasma ACTH remained low/normal throughout ICU-stay, whereas plasma free cortisol remained high, both in patients with and without sepsis/septic shock. Also, our findings contradict studies in which lower plasma cortisol levels were shown in non-survivors in comparison with survivors.[30-32] Indeed, in our study it was shown that the sicker patients were, the lower plasma cortisol binding protein concentrations and the higher plasma free cortisol concentrations rose. Non-survivors also showed lower plasma binding proteins and thus higher plasma free cortisol concentrations than survivors.

Strikingly, the longitudinal set-up of the study identified that, if critical illness sustained beyond 28 days, a lack of elevated total and free cortisol was not compensated by increased ACTH, which could be due to a central (endogenous or exogenous) HPA axis suppression, possibly concomitantly with the onset of decline of adrenocortical function. It has been shown that appropriate ACTH signaling is essential to maintain integrity and function of the adrenal cortex.[33] A post-mortem study of adrenal glands harvested from patients who had been critically ill for several weeks showed loss of zonational structure, lipid droplet depletion, and suppressed ACTH-regulated gene expression.[34] Suppressed ACTH secretion could thus negatively affect adrenocortical function in long-stay ICU patients.[35,36] However, given that in all patients, plasma ACTH and cortisol one week after ICU discharge increased to values higher than those of healthy subjects, this further supports the presence of a central HPA axis suppression during ICU stay, which could be mal-adaptive in prolonged (>4weeks) critically ill patients. Indeed, if one would expect a return to normal after an "adaptive" stress response, the values documented one week after ICU discharge would expectedly be "normal". Furthermore, the rise in cortisol seemed to be the largest in the long-stay patients (>4weeks) in comparison with shorter stayers, suggesting that the central HPA axis suppression during ICU stay is the largest in long-stay patients.

These findings are in line with the results from **chapter 5**, in which a **suppression of the ACTH-responses to CRH only in the more prolonged phases of critical illness** was observed. Also in this study, the presence of sepsis or septic shock did not affect the ACTH-responses during the course of critical illness. This again argues against the role of an inadequate HPA axis response in the pathophysiology of sepsis and septic shock.[29] Furthermore, augmented/prolonged ACTH-responses are expected when the hypothalamus would be acutely damaged by shock or inflammation, and immediately suppressed ACTH-responses are expected if the pituitary would be acutely damaged by shock or inflammation, which both were not observed.[37] The time courses of the ACTH-responses to CRH were thus compatible with feed-back inhibition exerted by continuously elevated free cortisol. Also, with increasing duration of critical illness, cortisol-responses tended to further decrease, partially explained by the suppressed ACTH release, but potentially also by the onset of a decline of the adrenocortical function, as a loss of ACTH signaling can negatively influence the integrity and function of the adrenal cortex, as previously mentioned.[35,36]

Low plasma ACTH already from admission to the ICU and throughout the ICU stay was confirmed in our 3 studies. Combining the findings of our 3 studies, ACTH appears mainly suppressed by free cortisol-induced feedback-inhibition. Indeed, in an earlier study, critically ill patients show suppressed pulsatile ACTH secretion, but with a normal ACTH-cortisol dose response, and thus normal feedback-inhibition.[36] Most likely, this suppression during the course of critical illness is thus due to negative feedback inhibition by elevated free cortisol. However, in **chapter 4**, we observed an increase in plasma ACTH, together with a rise in (free)cortisol, 7 days after ICU discharge, when the need for high systemic cortisol availability would thought to be reduced. This suggests presence of a central HPA axis suppression during critical illness, which is lifted during recovery. Other endogenous or exogenous suppressors can play a role. Although in **chapter 3** the inhibition of ACTH through negative feedback inhibition may have hidden any central pharmacological suppression by analgo-sedative drugs, one cannot exclude additional central suppression from these drugs that are used throughout ICU stay. Indeed, intra-operative opioids and prolonged opioid use for chronic pain have shown to lower plasma ACTH concentrations.[9,10,38-40] In experimental models of sepsis, it was shown that pituitary ACTH expression levels were suppressed in the more chronic phase of critical illness, which was attributed to nitric oxide and/or by suppressed orexin.[41,42] The findings in **chapter 5** ruled out a direct pituitary defect in the acute phase. Although our results cannot rule out a pituitary defect due to effects of inflammation and/or hypoxia selectively in the more prolonged phases of illness, this is not supported by the observed supranormal ACTH response during recovery. Another hypothesis that needs to be investigated is a potential inhibitory effect of bile acids on the HPA axis, similarly as has been observed during cholestasis.[43] Elevated circulating levels of bile acids indeed hallmark subacute and prolonged critical illness.[44] Recently it has been shown *in vitro* and *in vivo* that plasma bile acids can gain entry into the brain via transporters and a leaky blood brain barrier.[43] Bile acids can bind and activate the glucocorticoid receptor, which in turn can suppress the expression of CRH and ACTH.[45]

Our findings have important **diagnostic implications**. Considering the suggested primary adrenocortical suppression together with a central HPA axis suppression in these studies, this could suggest a form of CIRCI. In particular, long-stay patients may be at risk of insufficient cortisol availability, and would potentially benefit from glucocorticoid treatment. Based on **chapter 4**, the generally proposed diagnosis with use of an incremental cortisol response to 250 µg of cosyntropin below 9 µg/dl or by a random plasma total cortisol

below 10µg/dl were invalidated.[46,47] These findings can explain the wide variety in prevalence of CIRCI, which is reported in studies to be up to 77%, depending on the definition and criteria used.[48] Nonetheless, the currently proposed diagnostic tests do not seem to adequately point to the presence of CIRCI.

Our new insights may also have important implications for **possible treatment strategies**. In **chapter 3**, during the acute phase of critical illness, the steep rise in plasma cortisol observed on the morning following ICU admission, when the transient drug effect waned off, could be an indication that patients need higher cortisol availability during critical illness. However, the study was not powered to demonstrate an adverse outcome. However, the main clinical interest of the study was to inform physicians on the potential iatrogenic suppressive effects of commonly used drugs. Specifically, the data suggest that prior to considering treatment with hydrocortisone based on a low plasma cortisol, avoidable iatrogenic suppressive drugs should be discontinued and the effects on plasma cortisol documented. In **chapter 4**, we found that the adrenocortical function parameters did not differ between patients for whom clinicians at the bedside decided it was appropriate to treat with glucocorticoids and patients who did not need such treatment. Also presence of sepsis or septic shock did not alter adrenocortical function parameters. This finding was striking, and suggests that during sepsis and septic shock physicians do not use glucocorticoid treatment for a failing HPA axis, but rather for increasing blood pressure or reducing inflammation.[49-56] Indeed, an increase in blood pressure might be related to a pharmacological effect of these doses of hydrocortisone on the vasculature rather than indicating a successful treatment of CIRCI.[51,57] Whether these are justified indications remains debated.[46,47] Subsequent RCTs during the last 4 decades, reporting 28-day mortality of prolonged course of the so called low-dose glucocorticoid treatment (usually 200-300mg hydrocortisone during 5-7 days) in patients with sepsis and septic shock, showed opposite results.[49-56] Of note, doses of hydrocortisone of 200 to 300 mg per day are considered high in endocrine literature.[58] Recently, 2 large RCTs that have also investigated hydrocortisone treatment for septic shock showed opposite results, with the largest multicenter trial showing no benefit.[55,56] Patients enrolled in the smaller RCT had more severe septic shock than those enrolled in the larger trial. This observation made some experts conclude that glucocorticoid therapy during septic shock might reduce mortality only in the sickest subgroup of patients with septic shock.[59] To identify these patients, we maybe need precision medicine, also referred to as individualized or personalized medicine, which has

recently gained traction, and is frequently mentioned as the next model of healthcare delivery. Its goal is to integrate unique information obtained from a given patient to customize the care provided to achieve the best possible outcome. For example, the endotoxin activity assay, a rapid diagnostic tool for endotoxin activity in human whole blood, might be useful for recognizing patients who have an increased risk of mortality due to severe sepsis.[60] Furthermore, local cortisol activity is also further regulated by tissue-specific alterations of glucocorticoid signaling.[61,62] Evidence from animal and human studies indicate that, besides alternative splicing of the GR, also GR expression, GR affinity and GR translocation are regulated and could be tissue-specific during critical illness.[63-66] A tissue-specific regulation of glucocorticoid signaling may limit undue cortisol exposure in vulnerable vital organs that would suffer from an excess of cortisol and increase it in cells that might require more cortisol action. Furthermore, among patients with a variety of inflammatory disorders, there are functional polymorphisms of the GR gene, which are associated with either increased or decreased sensitivity to glucocorticoids, and have been associated with clinical outcomes and response to exogenous glucocorticoids.[67] In the future, based on these GR polymorphisms, it might therefore be possible to identify the right patient for the right treatment. However, further research regarding tissue-specific changes and GR polymorphisms is needed.

Recently, in a systematic review of 22 RCTs, assessing the effect of low dose glucocorticoids on outcomes in patients with septic shock, short- and longer-term mortality were unaffected, and duration of shock, mechanical ventilation and ICU stay were reduced.[68] These improvements in secondary outcomes can be considered beneficial to patients and the health-care system.[59] However, caution is warranted with hydrocortisone therapy, as it can induce side effects such as myopathy or muscle wasting, whereby extending the intensive care dependency.[69,70] Other symptoms that result from an exogenous excess in glucocorticoid activity are depression, mood changes, psychological, cognitive and behavioral disturbances, impaired wound healing, and glucose intolerance and insulin insensitivity.[71] The immunosuppressive properties of glucocorticoid treatment have been extensively described, but also enhancing of inflammation and immunity have been described.[71,72] High doses of glucocorticoids can inhibit the activation of inflammatory genes in macrophages, activated by endotoxins, whereas low doses enhance inflammatory gene expression.[73] Furthermore, glucocorticoids were shown to impair innate antimicrobial autophagy, a process by which damaged cellular components are degraded.[74] This can be a

relevant effect during critical illness, given its importance for innate immunity and for quality control in cells with a long half-life, such as myofibers.[75,76] In the systematic review of 22 RCTs, assessing the effect of low dose glucocorticoids on outcomes in patients with septic shock, glucocorticoid treatment was not associated with secondary infections, delirium or encephalopathy, but was associated with increased reporting of hypernatraemia and hyperglycemia.[68] However, health-related quality of life has not been reported by any trial, and more information on the long-term effects of glucocorticoids use during critical illness are needed. Recently, in a study assessing the long-term impact of withholding supplemental parenteral nutrition for one week in the pediatric ICU on physical and neurocognitive development, the use of glucocorticoids was independently associated with poorer long-term outcomes.[77]

Another potential strategy to protect long-stay ICU patients against the risk of developing adrenal insufficiency, is therapy with CRH infusion. Indeed, administering CRH may (re)activate ACTH synthesis in the more prolonged phase of critical illness and could hereby prevent adrenal atrophy.[78] An potential advantage of CRH treatment for adrenal failure is the preservation of the circadian rhythmicity and pulsatility of ACTH and cortisol, with a conservation of the negative feedback-inhibition loop.[79] This in turn can potentially prevent too high plasma cortisol levels, which are responsible for its side-effects. However, also elevated CRH levels have been associated with the pathophysiology of anxiety disorders, depression, memory and learning,[80,81] and is able to increase catecholamines and heart rate.[82] This hypothesis requires further investigation via an RCT that assesses the effect on patient-centered outcomes of treatment with CRH among long-stay ICU patients.

References

1. Meersseman P, Boonen E, Peeters B, et al. Effect of Early Parenteral Nutrition on the Hpa Axis and on Treatment with Corticosteroids in Icu Patients. *The Journal of clinical endocrinology and metabolism* 2015: jc20151846.
2. Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477-88.
3. 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 Endocrinol Metab* 1995; **80**: 1238-42.
4. Annane D, Maxime V, Ibrahim F, Alvarez JC, Abe E, Boudou P. Diagnosis of adrenal insufficiency in severe sepsis and septic shock. *AmJRespirCrit Care Med* 2006; **174**: 1319-26.
5. Dispersyn G, Sage D, Challet E, Pain L, Touitou Y. Plasma corticosterone in rats is specifically increased at recovery from propofol anesthesia without concomitant rise of plasma ACTH. *Chronobiol Int* 2009; **26**: 697-708.
6. Kostopanagiotou G, Kalimeris K, Christodoulaki K, et al. The differential impact of volatile and intravenous anaesthetics on stress response in the swine. *Hormones (Athens)* 2010; **9**: 67-75.
7. Pascoe JE, Williams KL, Mukhopadhyay P, Rice KC, Woods JH, Ko MC. Effects of mu, kappa, and delta opioid receptor agonists on the function of hypothalamic-pituitary-adrenal axis in monkeys. *Psychoneuroendocrinology* 2008; **33**: 478-86.
8. Raff H, Flemma RJ, Findling JW. Fast cortisol-induced inhibition of the adrenocorticotropin response to surgery in humans. *J Clin Endocrinol Metab* 1988; **67**: 1146-8.
9. Shinoda T, Murakami W, Takamichi Y, Iizuka H, Tanaka M, Kuwasako Y. Effect of remifentanil infusion rate on stress response in orthopedic surgery using a tourniquet application. *BMC Anesthesiol* 2013; **13**: 14.
10. Watanabe K, Kashiwagi K, Kamiyama T, et al. High-dose remifentanil suppresses stress response associated with pneumoperitoneum during laparoscopic colectomy. *J Anesth* 2014; **28**: 334-40.
11. Aitkenhead AR, Pepperman ML, Willatts SM, et al. Comparison of propofol and midazolam for sedation in critically ill patients. *Lancet* 1989; **2**: 704-9.
12. Roth-Isigkeit AK, Schmucker P. Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids* 1997; **62**: 695-9.
13. Gibbison B, Angelini GD, Lightman SL. Dynamic output and control of the hypothalamic-pituitary-adrenal axis in critical illness and major surgery. *BrJAnaesth* 2013; **111**: 347-60.
14. Russell GM, Henley DE, Leendertz J, et al. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci* 2010; **30**: 6106-15.
15. Hildreth AN, Mejia VA, Maxwell RA, Smith PW, Dart BW, Barker DE. Adrenal suppression following a single dose of etomidate for rapid sequence induction: a prospective randomized study. *J Trauma* 2008; **65**: 573-9.
16. Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010; **31**: 98-132.
17. Kato K, Nakagawa C, Murabayashi H, Oomori Y. Expression and distribution of GABA and GABAB-receptor in the rat adrenal gland. *J Anat* 2014; **224**: 207-15.
18. Amenta F, Collier WL, Erdo SL, Giuliani S, Maggi CA, Meli A. GABAA receptor sites modulating catecholamine secretion in the rat adrenal gland: evidence from 3H-muscimol autoradiography and in vivo functional studies. *Pharmacology* 1988; **37**: 394-402.
19. Kenyon CJ, Thomson I, Fraser R. Stimulation of aldosterone secretion by benzodiazepines in bovine adrenocortical cells. *Fundam Clin Pharmacol* 1999; **13**: 213-9.
20. Gibbison B, Spiga F, Walker JJ, et al. Dynamic pituitary-adrenal interactions in response to cardiac surgery. *Crit Care Med* 2015; **43**: 791-800.
21. Spiga F, Lightman SL. Dynamics of adrenal glucocorticoid steroidogenesis in health and disease. *Mol Cell Endocrinol* 2015; **408**: 227-34.
22. Engstrom L, Rosen K, Angel A, et al. Systemic immune challenge activates an intrinsically regulated local inflammatory circuit in the adrenal gland. *Endocrinology* 2008; **149**: 1436-50.
23. Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends EndocrinolMetab* 2008; **19**: 175-80.

24. Jaattela M, Ilvesmaki V, Voutilainen R, Stenman UH, Saksela E. Tumor necrosis factor as a potent inhibitor of adrenocorticotropin-induced cortisol production and steroidogenic P450 enzyme gene expression in cultured human fetal adrenal cells. *Endocrinology* 1991; **128**: 623-9.
25. Jaattela M, Carpen O, Stenman UH, Saksela E. Regulation of ACTH-induced steroidogenesis in human fetal adrenals by rTNF-alpha. *Mol Cell Endocrinol* 1990; **68**: R31-6.
26. Annane D, Pastores SM, Arlt W, et al. 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). *Intensive Care Med* 2017; **43**: 1781-92.
27. Annane D, Sebille V, Troche G, Raphael JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000; **283**: 1038-45.
28. Barquist E, Kirton O. Adrenal insufficiency in the surgical intensive care unit patient. *JTrauma* 1997; **42**: 27-31.
29. Annane D. The Role of ACTH and Corticosteroids for Sepsis and Septic Shock: An Update. *Front Endocrinol (Lausanne)* 2016; **7**: 70.
30. Sibbald WJ, Short A, Cohen MP, Wilson RF. Variations in adrenocortical responsiveness during severe bacterial infections. Unrecognized adrenocortical insufficiency in severe bacterial infections. *Ann Surg* 1977; **186**: 29-33.
31. Finlay WE, McKee JI. Serum cortisol levels in severely stressed patients. *Lancet* 1982; **1**: 1414-5.
32. McKee JI, Finlay WE. Cortisol replacement in severely stressed patients. *Lancet* 1983; **1**: 484.
33. Ferreira JG, Cruz CD, Neves D, Pignatelli D. Increased extracellular signal regulated kinases phosphorylation in the adrenal gland in response to chronic ACTH treatment. *J Endocrinol* 2007; **192**: 647-58.
34. Boonen E, Langouche L, Janssens T, et al. Impact of Duration of Critical Illness on the Adrenal Glands of Human Intensive Care Patients. *J Clin Endocrinol Metab* 2014; **99**: 4214-22.
35. Stavreva DA, Wiench M, John S, et al. Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. *Nat Cell Biol* 2009; **11**: 1093-102.
36. Boonen E, Meersseman P, Vervenne H, et al. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab* 2014; **306**: E883-92.
37. Jameson J, De Groot L. Endocrinology: Adult and Pediatric, 7th Edition. Philadelphia: Elsevier; 2016: 2655-88.
38. Taylor T, Dluhy RG, Williams GH. beta-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J Clin Endocrinol Metab* 1983; **57**: 592-6.
39. Aloisi AM, Aurilio C, Bachiocco V, et al. Endocrine consequences of opioid therapy. *Psychoneuroendocrinology* 2009; **34 Suppl 1**: S162-8.
40. Abs R, Verhelst J, Maeyaert J, et al. Endocrine consequences of long-term intrathecal administration of opioids. *J Clin Endocrinol Metab* 2000; **85**: 2215-22.
41. Deutschman CS, Raj NR, McGuire EO, Kelz MB. Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med* 2013; **41**: e368-e75.
42. Polito A, Sonneville R, Guidoux C, et al. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoSOne* 2011; **6**: e25905.
43. McMillin M, Frampton G, Quinn M, et al. Suppression of the HPA Axis During Cholestasis Can Be Attributed to Hypothalamic Bile Acid Signaling. *Mol Endocrinol* 2015; **29**: 1720-30.
44. Vanwijngaerden YM, Wauters J, Langouche L, et al. Critical illness evokes elevated circulating bile acids related to altered hepatic transporter and nuclear receptor expression. *Hepatology* 2011; **54**: 1741-52.
45. Miura T, Ouchida R, Yoshikawa N, et al. Functional modulation of the glucocorticoid receptor and suppression of NF-kappaB-dependent transcription by ursodeoxycholic acid. *J Biol Chem* 2001; **276**: 47371-8.
46. 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**: 1751-63.
47. 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. *Crit Care Med* 2017; **45**: 2078-88.
48. 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**: 1937-49.
49. Briegel J, Forst H, Haller M, et al. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. *Crit Care Med* 1999; **27**: 723-32.
50. Annane D, Sebille V, Charpentier C, et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002; **288**: 862-71.
51. Sprung CL, Annane D, Keh D, et al. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111-24.
52. Annane D, Bellissant E, Bollaert PE, et al. Corticosteroids in the treatment of severe sepsis and septic shock in adults: a systematic review. *JAMA* 2009; **301**: 2362-75.
53. Blum CA, Nigro N, Briel M, et al. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 1511-8.
54. Meduri GU, Bridges L, Shih MC, Marik PE, Siemieniuk RA, Kocak M. Prolonged glucocorticoid treatment is associated with improved ARDS outcomes: analysis of individual patients' data from four randomized trials and trial-level meta-analysis of the updated literature. *Intensive Care Med* 2016; **42**: 829-40.
55. Venkatesh B, Finfer S, Cohen J, et al. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. *N Engl J Med* 2018; **378**: 797-808.
56. Annane D, Renault A, Brun-Buisson C, et al. Hydrocortisone plus Fludrocortisone for Adults with Septic Shock. *N Engl J Med* 2018; **378**: 809-18.
57. Loriaux DL, Fleseriu M. Relative adrenal insufficiency. *Curr Opin Endocrinol Diabetes Obes* 2009; **16**: 392-400.
58. Buttgereit F, da Silva JA, Boers M, et al. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: current questions and tentative answers in rheumatology. *Ann Rheum Dis* 2002; **61**: 718-22.
59. Marik PE. The role of glucocorticoids as adjunctive treatment for sepsis in the modern era. *Lancet Respir Med* 2018.
60. Yaroustovsky M, Plyushch M, Popov D, et al. Prognostic value of endotoxin activity assay in patients with severe sepsis after cardiac surgery. *J Inflamm (Lond)* 2013; **10**: 8.
61. Chapman K, Holmes M, Seckl J. 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev* 2013; **93**: 1139-206.
62. Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. *Mol Cell Endocrinol* 2009; **300**: 7-16.
63. Bergquist M, Nurkkala M, Rylander C, Kristiansson E, Hedenstierna G, Lindholm C. Expression of the glucocorticoid receptor is decreased in experimental Staphylococcus aureus sepsis. *JInfect* 2013; **67**: 574-83.
64. Guerrero J, Gatica HA, Rodriguez M, Estay R, Goecke IA. Septic serum induces glucocorticoid resistance and modifies the expression of glucocorticoid isoforms receptors: a prospective cohort study and in vitro experimental assay. *Crit Care* 2013; **17**: R107.
65. Peeters RP, Hagendorf A, Vanhorebeek I, et al. Tissue mRNA expression of the glucocorticoid receptor and its splice variants in fatal critical illness. *ClinEndocrinol(Oxf)* 2009; **71**: 145-53.
66. Siebig S, Meinel A, Rogler G, et al. Decreased cytosolic glucocorticoid receptor levels in critically ill patients. *AnaesthIntensive Care* 2010; **38**: 133-40.
67. Cvijanovich NZ, Anas N, Allen GL, et al. Glucocorticoid Receptor Polymorphisms and Outcomes in Pediatric Septic Shock. *Pediatr Crit Care Med* 2017; **18**: 299-303.
68. Rygard SL, Butler E, Granholm A, et al. Low-dose corticosteroids for adult patients with septic shock: a systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med* 2018; **44**: 1003-16.

69. Hermans G, Wilmer A, Meersseman W, et al. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. *Am J Respir Crit Care Med* 2007; **175**: 480-9.
70. Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G. Clinical review: Critical illness polyneuropathy and myopathy. *Crit Care* 2008; **12**: 238.
71. Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. *Nat Rev Immunol* 2017; **17**: 233-47.
72. Cain DW, Cidlowski JA. Specificity and sensitivity of glucocorticoid signaling in health and disease. *Best Pract Res Clin Endocrinol Metab* 2015; **29**: 545-56.
73. Lim HY, Muller N, Herold MJ, van den Brandt J, Reichardt HM. Glucocorticoids exert opposing effects on macrophage function dependent on their concentration. *Immunology* 2007; **122**: 47-53.
74. Wang J, Wang R, Wang H, et al. Glucocorticoids Suppress Antimicrobial Autophagy and Nitric Oxide Production and Facilitate Mycobacterial Survival in Macrophages. *Sci Rep* 2017; **7**: 982.
75. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* 2011; **469**: 323-35.
76. Masiero E, Agatea L, Mammucari C, et al. Autophagy is required to maintain muscle mass. *Cell Metab* 2009; **10**: 507-15.
77. Verstraete S, Verbruggen SC, Hordijk JA, et al. Long-term developmental effects of withholding parenteral nutrition for 1 week in the paediatric intensive care unit: a 2-year follow-up of the PEPaNIC international, randomised, controlled trial. *Lancet Respir Med* 2018.
78. Ur E, Capstick C, McLoughlin L, Checkley S, Besser GM, Grossman A. Continuous administration of human corticotropin-releasing hormone in the absence of glucocorticoid feedback in man. *Neuroendocrinology* 1995; **61**: 191-7.
79. Schulte HM, Chrousos GP, Gold PW, et al. Continuous administration of synthetic ovine corticotropin-releasing factor in man. Physiological and pathophysiological implications. *J Clin Invest* 1985; **75**: 1781-5.
80. Behan DP, Grigoriadis DE, Lovenberg T, et al. Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders. *Mol Psychiatry* 1996; **1**: 265-77.
81. Smith SR, de Jonge L, Pellymounter M, et al. Peripheral administration of human corticotropin-releasing hormone: a novel method to increase energy expenditure and fat oxidation in man. *J Clin Endocrinol Metab* 2001; **86**: 1991-8.
82. Nijsen MJ, Croiset G, Stam R, et al. The role of the CRH type 1 receptor in autonomic responses to corticotropin- releasing hormone in the rat. *Neuropsychopharmacology* 2000; **22**: 388-99.

SUMMARY

Critical illness represents any condition, evoked by major surgery, severe medical illnesses, or multiple trauma, that requires pharmacological and/or mechanical support of vital organ functions without which death would ensue. As such, it represents an extreme example of physical stress. The traditional concept of the stress response comprises hypothalamic release of corticotropin-releasing hormone (CRH) that activates pituitary adrenocorticotropic hormone (ACTH), which drives cortisol synthesis and secretion. Cortisol controls the activation status of the hypothalamic-pituitary-adrenal (HPA) axis via negative feedback inhibition at the level of the hypothalamus and the pituitary. Interestingly, low rather than high plasma ACTH concentrations have been reported in critically ill patients during the first 7 days in ICU, and a reduced cortisol breakdown largely explains the high plasma cortisol concentrations during critical illness. Inflammation-driven decrease in cortisol binding proteins further increases the active free cortisol fraction. Low ACTH together with high cortisol is referred to as 'ACTH-cortisol dissociation'. Which level of cortisol availability is sufficient in the struggle for survival of the critically ill and interpreting diagnostic laboratory tests remain unclear.

The general aim of this doctoral thesis was to gain more insight into the regulation of the HPA axis response during the course of critical illness, from admission to recovery or death, in order to understand the so-called "ACTH-cortisol dissociation" and the pathophysiology of "critical illness related adrenal insufficiency". We hypothesized that during critical illness the pharmacological effects of frequently used drugs may partly explain the acute "ACTH-cortisol dissociation", we hypothesized that prolonged deprivation of trophic ACTH-effects on the adrenal cortex may contribute to adrenal insufficiency whereas reactivation of the pituitary with increased plasma ACTH occurs in those patients who recover, and we hypothesized that sustained elevated free cortisol levels, via feedback inhibition, may cause a central suppression of the HPA axis, specifically in the prolonged phase.

In a first part, we investigated the pharmacological effects of frequently used drugs on the acute "ACTH-cortisol dissociation" in surgical and medical ICU patients. We documented low plasma ACTH concentrations upon admission to the ICU in the face of normal total and elevated free cortisol plasma concentrations. A further lowering of plasma ACTH and a steep rise in plasma total/free cortisol were shown from the morning after admission to the ICU onwards. We showed that none of the drugs administered 24h prior to ICU admission independently affected plasma ACTH on ICU admission. However, we demonstrated a suppressive effect for opioids, etomidate, and propofol on admission plasma cortisol, and a stimulatory effect for dobutamine on admission plasma cortisol. These associations were independent of the medical or surgical diagnostic category, severity of illness, sepsis, and all other patient characteristics. The presence of a subsequent rebound effect on plasma total and free cortisol concentrations was investigated for each suppressive drug, and we were able to demonstrate a steep rise in plasma total and free cortisol on the morning following ICU admission, when the transient drug effect of these suppressive drugs waned off.

In a second part, we investigated whether and when the alterations that occur within the HPA axis during critical illness recover, and to what extent the currently used diagnostic criteria for CIRCI relate to presence of sepsis/septic shock, need for glucocorticoid treatment or survival. We documented the changes over time - from ICU-day 7 up to recovery or death - in plasma ACTH and total and free cortisol, urinary cortisol metabolites, and in plasma total and free cortisol responses to cosyntropin (a synthetic ACTH-analogue), with focus on long-stay (≥4 weeks in ICU) patients in comparison with shorter-stay patients (1-2 weeks; 2-3 weeks; 3-4 weeks in ICU). We showed that plasma ACTH remained low/normal throughout ICU-stay up to day 28, whereas plasma free cortisol remained high, largely explained by low plasma binding and persistently suppressed cortisol breakdown, irrespective of the presence of sepsis/septic shock. The low binding proteins among sicker patients and non-survivors determined their higher plasma free cortisol levels. Development of primary adrenal failure was not observed. However, beyond ICU-day 28, plasma free cortisol was no longer elevated and not compensated by increased ACTH, which could be due to a central (endogenous or exogenous) adrenocortical suppression. Such a central suppression was further supported by the uniform rise in plasma ACTH and (free)cortisol to supra-normal levels 1 week later on the regular ward. Low incremental total cortisol responses to cosyntropin coincided with normal free cortisol responses at all times. Hence,

low responses to cosyntropin during critical illness likely reflected the increased cortisol distribution volume, which has been documented in an earlier study, given low plasma binding, rather than the functional reserve of the adrenal cortex. Finally, the initiation of glucocorticoid treatment, as judged necessary by bedside clinicians, was not supported by differences in the presently measured adrenal function parameters.

In a last part, we compared the ACTH (and cortisol) responses to a synthetic human CRH-analogue (corticorelin), in the acute, subacute and prolonged phases of critical illness with those of healthy subjects, in relation to presence of sepsis/septic shock and survival. We demonstrated that incremental ACTH-responses to CRH in patients in the acute phase of critical illness were normal, whereas ACTH-responses became ±55% lower than normal in the later phases, irrespective of the presence of sepsis/septic shock or survival. Interestingly, the total cortisol-responses to CRH were always lower than in healthy subjects whereas the free cortisol-responses were always normal, in line with increased cortisol distribution volume during critical illness. The time courses of the ACTH-responses to CRH were compatible with feed-back inhibition exerted by elevated free cortisol.

In summary, the findings of these 3 clinical studies add important information to correctly understand the pathophysiology of the HPA axis response during critical illness from admission to recovery and death. These novel insights help to better understand the "ACTH-cortisol dissociation" and the pathophysiology of "critical illness related adrenal insufficiency", and advocate change in clinical practice.

SAMENVATTING

Kritieke ziekte is een toestand die veroorzaakt wordt door majeure chirurgie, een ernstige medische ziekte, of een polytrauma, en die farmacologische en/of mechanische ondersteuning vergt, zonder dewelke een onmiddellijk overlijden zou volgen. Als zodanig is het een extreem voorbeeld van fysieke stress. Het traditioneel concept van de stressrespons omvat een hypothalame vrijzetting van corticotropine-releasing hormoon (CRH) dat het hypofysaire adrenocorticotrope hormoon (ACTH) vrijzet, dewelke op zijn beurt cortisolsynthese en -secretie in de bijnier aandrijft. Cortisol controleert de activiteit van de hypothalame-hypofysaire-bijnieras(HPA-)as d.m.v. negatieve feedbackinhibitie t.h.v. de hypothalamus en de hypofyse. In kritiek zieke patiënten werden echter gedaalde (en geen gestegen!) ACTH-concentraties in het bloed (plasma) vastgesteld gedurende de eerste 7 dagen op intensieve zorgen. Een gedaalde afbraak samen met een nauwelijks gestegen productie van cortisol verklaarde daarentegen de gestegen cortisolconcentraties. Een door inflammatie gedreven daling in de bindingseiwitten van cortisol in het bloed vergrootte dan weer de actieve vrije cortisolfractie. Het fenomeen van lage plasma ACTH-concentraties samen met hoge plasma cortisolconcentraties wordt de "ACTH-cortisol-dissociatie" genoemd. Hoeveel cortisol er moet beschikbaar zijn in de strijd van de kritiek zieke patiënt om te overleven en hoe traditionele testen voor de bijnierfunctie moeten geïnterpreteerd worden in de context van kritieke ziekte blijven onduidelijk.

De algemene doelstelling van dit doctoraal onderzoek was het verwerven van meer inzicht in het antwoord van de HPA-as tijdens kritieke ziekte, en dit vanaf opname op intensieve zorgen tot herstel of eventueel overlijden, om zo de "ACTH-cortisol-dissociatie" en de pathofysiologie van bijnierfalen tijdens kritieke ziekte te begrijpen. De eerste hypothese was dat de farmacologische effecten van veel gebruikte medicijnen tijdens de ontwikkeling van kritieke ziekte deels de "ACTH-cortisol-dissociatie" kunnen verklaren. In een tweede hypothese stelden we dat een langdurig tekort aan trofisch ACTH-effect op de bijniercortex bijdraagt aan bijnierfalen en dat er reactivatie is van de hypofyse met gestegen

ACTH-concentraties in patiënten die herstellen. Ten slotte stelden we in een derde hypothese dat continu gestegen vrij cortisolconcentraties, via negatieve feedbackinhibitie, een centrale onderdrukking van de HPA-as veroorzaken in de verlengde fase van kritieke ziekte.

In een eerste deel onderzochten we de farmacologische effecten van veel gebruikte medicijnen op de "ACTH-cortisol-dissociatie" in chirurgische en medische patiënten op intensieve zorgen. Tijdens de opname op intensieve zorgen, documenteerden we lage ACTH-concentraties, normale totale cortisolconcentraties, en gestegen vrije cortisolconcentraties in het plasma. Vanaf de ochtend na de opname toonden we een verdere daling in ACTH aan en een sterke stijging in totaal en vrij cortisol. We stelden vast dat geen enkel medicijn dat gedurende een periode van 24u voor opname werd toegediend een effect had op plasma ACTH. Daarentegen konden we een onderdrukkend effect van opiaten, etomidaat, en propofol, en een stimulerend effect van dobutamine op de cortisolconcentraties aantonen. Deze associaties waren onafhankelijk van de opnamediagnose, de ernst van ziekte, de aanwezigheid van sepsis, en van alle andere patiëntkarakteristieken. De aanwezigheid van een rebound effect op de cortisolconcentraties gedurende de volgende dagen werd onderzocht voor elk voornoemd medicijn. We konden een sterke stijging waarnemen in totaal en vrij cortisol op de ochtend na opame, wanneer de transiënte effecten van de onderdrukkende medicijnen waren uitgedoofd.

In een tweede deel onderzochten we of, en zo ja wanneer, de veranderingen in de HPA-as tijdens kritieke ziekte zich herstellen, en in welke mate de gebruikte diagnostische criteria voor bijnierfalen tijdens kritieke ziekte in verband staan met de aanwezigheid van sepsis en septische shock, de nood aan behandeling met glucocorticoïden, en de overlevingskansen. We documenteerden de veranderingen in de tijd – van dag 7 op intensieve zorgen tot herstel of overlijden - in plasma ACTH, totaal, en vrij cortisol, de cortisolmetabolieten in de urine, en de totale en vrije cortisolantwoorden op de injectie van cosyntropine, een synthetische ACTH-analoog. We focusten ons op de "langliggers" (≥4 weken op intensieve zorgen) en vergeleken deze met "kortliggers" (1-2 weken; 2-3 weken; 3-4 weken op intensieve zorgen). We toonden aan dat plasma ACTH laag/normaal bleef tot dag 28 op intensieve zorgen, daar waar vrij plasma cortisol hoog bleef, wat grotendeels verklaard werd door een lage concentratie aan bindingseiwitten en door een continu

onderdrukte afbraak van cortisol. Dit was onafhankelijk van de aanwezigheid van sepsis of septische shock. Een verdere daling in de concentratie van bindingseiwitten verklaarde de verdere stijging van vrij plasma cortisol in de meest zieke patiënten en in de patiënten die zouden overlijden. We konden geen ontstaan van primair bijnierfalen vastellen in onze patiënten. Na dag 28 op intensieve zorgen was vrij plasma cortisol echter niet meer gestegen, en dit werd niet gecompenseerd door een stijging in plasma ACTH. Dit kon wellicht verklaard worden door een centrale ondrukking, van endogene of exogene origine. Deze stelling van een centrale onderdrukking tijdens kritieke ziekte werd verder ondersteund door een uniforme stijging in plasma ACTH, totaal, en vrij cortisol naar supranormale waarden 1 week na ontslag van intensieve zorgen op de gewone patiëntenafdeling. De antwoorden van totaal cortisol op een injectie van cosyntropine waren verlaagd in alle patiënten op alle testmomenten, maar de vrij-cortisol-antwoorden waren telkens normaal. De lage totaal-cortisol-antwoorden weerspiegelden hiermee het gestegen cortisoldistributievolume (door de daling in concentratie van bindingseiwitten), wat in een eerdere studie werd gedocumenteerd, en dus niet de bijnierfunctie. Tenslotte toonden we aan dat het starten van een therapie met glucocorticoïden door de behandelende arts niet ondersteund werd door verschillen in bijnierfunctieparameters.

In een laatste deel vergeleken we de ACTH- (en totaal en vrij cortisol) antwoorden op een injectie met een synthetische humane CRH-analoog (corticoreline) in patiënten in de acute, subacute, en chronische fase van kritieke ziekte en in gezonde personen. We onderzochten of er een relatie was met de aanwezigheid van sepsis of septische shock en met de overlevingskansen. We toonden aan dat de incrementele ACTH-antwoorden op CRH in patiënten in de acute fase van kritieke ziekte normaal waren, en dat ze ±55% kleiner werden in de latere fasen. Deze antwoorden waren onafhankelijk van de aanwezigheid van sepsis, septische shock, of de overlevingskansen. Het tijdsverloop van de ACTH-antwoorden was compatibel met een centrale onderdrukking van de HPA-as in de verlengde fase van kritieke ziekte, veroorzaakt door negatieve feedbackinhibitie door een continu gestegen vrij-cortisolconcentratie. De totaal-cortisol-antwoorden op CRH waren altijd verlaagd en de vrij-cortisol-antwoorden op CRH waren altijd normaal, wat een gestegen cortisoldistributievolume tijdens kritieke ziekte weerspiegelde.

Samengevat, de bevindingen van deze 3 klinische studies voegen belangrijke informatie toe aan de kennis van de pathofysiologie van de HPA-as tijdens kritieke ziekte.

Deze nieuwe inzichten helpen om de "ACTH-cortisol-dissociatie" en de pathofysiologie van bijnierfalen tijdens kritieke ziekte beter te begrijpen, en pleiten voor verandering in de klinische praktijk.

DANKWOORD

Appreciatie is een mooie zaak. Het *maakt dat datgene wat excellent is in de andere, ook bij u gaat horen*. Deze woorden van Voltaire zijn passend, wanneer ik de laatste hand aan dit manuscript leg. De appreciatie is dit dankwoord, maar reikt veel verder. De andere is elke persoon die zijn of haar excellente kennis en kunde heeft aangeboden tijdens mijn doctoraat, en dat zijn er velen.

Ik dank prof. Luc Sels, rector van de KU Leuven, en prof. Rik Torfs, zijn voorganger, om dit doctoraat binnen de muren van deze universiteit te mogen verrichten. Ik dank het Fonds Wetenschappelijk Onderzoek - Vlaanderen (FWO) voor het toekennen van het aspirantenmandaat.

I thank the members of my jury, prof. Mirjam Christ-Crain, prof. Manu Malbrain, prof. Brigitte Decallonne, and prof. Steffen Rex, for the critical appraisal of this manuscript, prof. Kathleen Freson for chairing the examining committee, and prof. Joost Schymkowitz for chairing the public defence.

Mijn grootste dank gaat uit naar mijn promotor, prof. Greet Van den Berghe, en co-promotor, prof. Lies Langouche. Jullie boden mij de mogelijkheid om dit wetenschappelijk project te starten, de klinische studies te leiden, en tot een goed einde te brengen. Jullie waren mijn 2 mentoren die me onder jullie vleugels hebben genomen en me alles hebben geleerd. Jullie deuren stonden altijd letterlijk en figuurlijk open. Geen vraag was te triviaal om te stellen, geen probleem te banaal om voor te leggen. Na dagen staren op nieuw verworven data, konden jullie ogenschijnlijk moeiteloos, in enkele seconden, en met een aanstekelijk enthousiasme een gepaste analyse en interpretatie voorstellen en, en passant, werd er al een nieuwe onderzoeksvraag gesuggereerd. Terwijl ik nog aan het proberen was de data normaal te verdelen. Niet alleen was dit zeer motiverend, het deed je vooral beseffen hoeveel geluk een eenvoudige jongen uit Zichem had om getuige te mogen zijn van het ontstaan van

nieuwe kennis en deel te mogen uitmaken van dit hoogwetenschappelijke team. Jullie tandem van een clinicus en een basiswetenschapper is werkelijk een vruchtbare en synergetische combinatie om grensverleggend biomedisch onderzoek te verrichten.

De klinische studies kwamen tot stand door een uitstekende samenwerking tussen de klinische dienst en het labo. Met het klinisch research team hebben we gedurende 4 jaren bergen werk verzet waar we zeer fier op mogen zijn. Vanaf het eerste jaar van het doctoraat tot de laatste maanden hebben we elke dag patiënten geïncludeerd, getest, stalen afgenomen, verwerkt, en gegevens ingevoerd. Een bijzondere en onmetelijke dank hiervoor is op zijn plaats voor Heidi, Jan, Liese, Pieter, Sandra, Sylvia, Tim, en voor Anneleen en Helga. De artsen, verpleegkundigen, kinesisten, logistiek medewerkers, secretaresses, en IT'ers van de dienst Intensieve Geneeskunde en de dienst Medisch Intensieve Geneeskunde wil ik danken voor hun hulp en hun bereidheid om hun reeds overladen klinisch werk te vergroten en te laten verstoren voor de wetenschap. Dit is een extra inspanning die niet vanzelfsprekend is, maar waar de dienst trots op mag zijn en die van een AZ een UZ maakt.

In het labo van intensieve geneeskunde ben ik veel dank verschuldigd aan Sarah Vander Perre. Met een niet-aflatende inzet, een grote werklust en met grote precisie slaagde je er in om duizenden stalen te analyseren. Je stond steeds paraat voor kleine en grote problemen, en je was altijd een luisterend oor voor wanneer het moeilijker ging. Daarnaast wil ik alle collega's op het labo danken voor de aangename samenwerking, de collegiale sfeer, de wetenschappelijke discussies, en het vermogen om mij 4 jaar in jullie midden te verdragen: An, Chloë, Fabian, Giorgia, Ilse, Ineke, Ines, Inge, Lies P, Lisa, Marc, Marine, Nathalie, Ruben, Sam, Sarah D, Sören, Steven, Thomas D, Thomas J, en Wouter. Jullie hebben me geleerd om lankmoedig te zijn wanneer het nodig is, en ik wens het iedereen toe.

Daarnaast had ik het voorrecht om samen te werken met de LC-MS-experten in ons huis, en kon ik samen met Ivo Jans, dr. Jaak Billen, ir. Nele Peersman, en prof. Pieter Vermeersch de cortisolmetabolieten bepalen. De studies zouden ook niet mogelijk zijn geweest zonder het baanbrekende werk van mijn voorganger, dr. Eva Boonen, en zonder de hulp van dr. Philippe Meersseman, prof. Yves Debaveye, en Dimitri Vanmarcke.

Ik dank ook Ferring Pharmaceuticals om het onderzoek naar septische shock te ondersteunen en de studiemedicatie te voorzien. Een speciale dank hierbij gaat naar dr. Johan Masure om dit te faciliteren.

Naast het wetenschappelijk werk had ik ook de eer om mijn collega's te mogen vertegenwoordigen in de Academische Raad en het Groepsbestuur Biomedische Wetenschappen van de KU Leuven. Dit liet me toe om contacten te leggen buiten de Faculteit Geneeskunde. Ik dank de medebestuurders, mijn collega-vertegenwoordigers Elke Debroye, Isolde Buysse, Jens Hermans, Wannes Vandenbussche, en de collega's van de Vereniging van het Academisch Personeel KU Leuven (VAPL) voor de zeer verrijkende debatten en de soms hoogoplopende discussies, die steeds een grote loyauteit en passie voor onze Alma Mater kenmerkten.

Ik dank de dienst Anesthesie, en in het bijzonder prof. Arne Neyrinck, prof. Steffen Rex, prof. Marc Van de Velde, en wijlen em. prof. Eugene Vandermeersch, om mij als student-onderzoeker en stagiair de eerste contacten met klinsch onderzoek te bieden en om mij de kans te geven mijn klinische opleiding te onderbreken voor het schrijven van een doctoraat.

Ik wil graag mijn familie en vrienden bedanken, en in de eerste plaats mijn ouders en wijlen mijn grootouders. Door de combinatie van jullie genen, mijn opvoeding, jullie ondersteuning, en de kansen die ik telkens heb gekregen, was ik in de mogelijkheid om dit werk af te leveren.

Klinisch onderzoek lukt niet zonder patiënten en gezonde vrijwilligers. Ik ben een grote dank verschuldigd aan alle gezonde vrijwilligers die telkens weer opdraafden voor het ondergaan van mijn wetenschappelijke testen. Ten slotte gaat mijn groot respect uit naar de kritiek zieke patiënten en hun families, die ik telkens in de meest kritieke fase van hun leven kwam storen. Meer dan 500 keer heb ik met de lijdende patiënt of zijn/haar familie gesproken, en wat me bijblijft is de grote bereidheid om deel te nemen aan wetenschappelijk onderzoek. Het was een altruïstische bijdrage die ze leverden voor de medemens, voor onze welvaart en welzijn in de kennismaatschappij van morgen. Ik draag dan ook deze thesis op aan hen.

Bram
December 2018

CURRICULUM VITAE

PERSONALIA

Address	Adolphe Mathieustraat 1 – bus A1, 1050 Elsene, Belgium
Telephone	0032 476 70 69 15
E-mail	bram.peeters@kuleuven.be
	bram.peeters@uzleuven.be
Date of birth	June 30th 1988
Place of birth	Asse, Belgium
Nationality	Belgian

EDUCATION

2006 – 2009	**Bachelor of Medicine -** *Magna cum laude* *KU Leuven, Leuven, Belgium*
2009 – 2013	**Master of Medicine -** *Magna cum laude* *KU Leuven , Leuven, Belgium*
2013 – 2014	**Master of Health Care Management and Policy -** *Magna cum laude* *Master thesis: Use of Global Trigger Tool to detect adverse events.* *Promotor: prof. dr. Kris Vanhaecht* *Co-promoter: prof. dr. Marc Van de Velde, prof. dr. Johan Van Eldere* *KU Leuven, Leuven, Belgium*
2013 – present	**Master of Specialist Medicine - Medical Residency** *Department of Anesthesiology, UZ Leuven, Leuven, Belgium* *Department of Intensive Care Medicine, UZ Leuven, Leuven, Belgium* *Department of Anesthesiology, Europe Hospitals, Brussels, Belgium*
2014 – 2018	**Doctoral Training in Mechanisms of Human Disease** **FWO Aspirant Fellowship** *PhD thesis: HPA axis alterations in critically ill patients.* *Promotor: prof. dr. Greet Van den Berghe* *Co-promotor: prof. dr. Lies Langouche* *Laboratory of Intensive Care Medicine, KU Leuven, Leuven, Belgium*

RESEARCH EXPERIENCE

2008 – 2013 **Student-Researcher**
Department of Neurosciences - Prof. dr. Philip Joris
Department of Cardiovascular Sciences - Prof. dr. Eugene Vandermeersch
Paper: Study of unexplained neurologic deficit following neuraxial anesthesia or analgesia strategies.
Department of Clinical and Experimental Medicine - Prof. dr. Arne Neyrinck
Paper: Should lungs be cooled again, after normothermic ex vivo lung perfusion?

2014 – 2018 **PhD fellowship - Aspirant FWO**
Laboratory of Intensive Care Medicine, KU Leuven, Leuven, Belgium

2014 **Laboratory Animal Science Course**

2016 **Good Clinical Practice Training Course for Experienced Researchers**

MANAGEMENT EXPERIENCE

2010 – 2011 **Member of the Board of Biomedical Sciences, KU Leuven**
Member of the Academic Council, KU Leuven
Student Representative, Medica, LOKO

2013 – 2018 **Member of the Medical Council, UZ Leuven**
Medical Resident Representative

2014 – present **Chairman of the Society of Medical Residents Leuven**
LVGA (Leuvense Vereniging van Geneesheer-Assistenten) – www.lvga.be

2014 – present **Member of the National Society of Medical Residents**
NVASO (Nationale Vereniging van ASO's) – www.vaso-amsf.be

2016 – 2018 **Member of the Board of Biomedical Sciences, KU Leuven**
Member of the Academic Council, KU Leuven
Member of the Advisory Commission Honorary Doctorates, KU Leuven
Member of the Research Policy Council, KU Leuven
Member of the Doctoral School Committee, KU Leuven
(Post)Doctoral Fellow Representative

2018 **Participant McKinsey & Company 2018 Insight Program**
United Kingdom, on behalf of the Brussels office

HONORS AND AWARDS

2006 **2nd prize Flemish Mathematical Olympiad**
Vlaamse Wiskunde Olympiade, Brussels, Belgium

2016 **1st prize Scientific Paper Presentation Award**
Belgian Society of Intensive Care Medicine, Brussels, Belgium

2018 **Winner Presidential Poster Competition**
Endocrine Society's 100th Annual Meeting, Chicago, USA

LANGUAGES / SOFTWARE SKILLS

Languages	Dutch: native
	French: fluent
	English: fluent
Software	Microsoft Office Word, Excel, PowerPoint
	Matlab
	JMP (Statistical Software)

HOBBIES

1996 – present	**Piano, clarinet**
	Hagelandse Academie voor Muziek en Woord, Diest
2002 – 2007	**Scouting Member, Leader**
	Scouting Sint-Jan, Diest
2005 – present	**Glider Pilot**
	Royal Belgian Air Cadets; Diest Aero Club
2016	**Finisher New York City Marathon, as a participant of Run for Life**
	Fundraising initiative for KWF Kankerbestrijding

PUBLICATIONS

1. A. Téblick, **B. Peeters**, L. Langouche, G. Van den Berghe. Adrenal function and dysfunction in critically ill patients. *(Submitted)*
2. **B. Peeters**, P. Meersseman, S. Vander Perre, P. Wouters, Y. Debaveye, L. Langouche, G. Van den Berghe. ACTH and cortisol responses to CRH in acute, subacute, and prolonged critical illness: a randomized, double-blind, placebo-controlled, crossover cohort study. Intensive Care Med 2018; doi: 10.1007/s00134-018-5427-y.
3. **B. Peeters**, P. Meersseman, S. Vander Perre, P. Wouters, D. Vanmarcke, Y. Debaveye, J. Billen, P. Vermeersch, L. Langouche, G. Van den Berghe. Adrenocortical function during prolonged critical illness and beyond: a prospective observational study. Intensive Care Med 2018; 44:1720-29.
4. **B. Peeters**, L. Langouche, G. Van den Berghe. Adrenocortical Stress Response during the Course of Critical Illness. Compr Physiol 2017; 8:283-298.
5. **B. Peeters**, F. Guiza, E. Boonen, P. Meersseman, L. Langouche, G. Van den Berghe. Drug-induced HPA axis alterations during acute critical illness: a multivariable association study. Clinical Endocrinology 2017; 86:26–36.
6. P. Meersseman, E. Boonen, **B. Peeters**, S. Vander Perre, PJ. Wouters, L. Langouche, G. Van den Berghe. Effect of Early Parenteral Nutrition on the HPA Axis and on Treatment With Corticosteroids in Intensive Care Patients. J Clin Endocrinol Metab 2015; 100:2613-20.

7. **B. Peeters**, E. Boonen, L. Langouche, G. Van den Berghe. The HPA axis response to critical illness: New study results with diagnostic and therapeutic implications. Mol Cell Endocrinol 2015; 408:235-40.
8. Stanzi, A. Neyrinck, H. Cauwenberghs, J. Nijs, **B. Peeters**, M. Brusseleers, V. Leung, A. Colle, L. Costardi, J. Somers, J. Van Puyvelde, E. Verbeken, L. Santambrogio, D. Van Raemdonck. Should lungs be cooled again, after normothermic ex vivo lung perfusion (evlp)? Transplant International 2013; 26:116-116.
9. **B. Peeters**, T. Phlips, H. Vanden Bosch. The role of radiofrequency catheter ablation in patients with Wolff-Parkinson-White syndrome: analysis based on two case reports. Tijdschr voor Geneeskunde 2013; 22:1093-1102.

ORAL AND POSTER PRESENTATIONS

- **Endocrine Society's Annual Meeting**. April, 2016, **Boston, USA**: Drug-induced HPA axis alterations during acute critical illness: a multivariable association study. (Poster)
- **European Anaesthesiology Congress (Euranaesthesia)**. May, 2016, **London, UK**: Drug-induced HPA axis alterations during acute critical illness: a multivariable association study. (Poster)
- **Belgian Society of Intensive Care medicine (SIZ) 36th Annual Meeting**. June, 2016, **Brussels, Belgium**: Drug-induced HPA axis alterations during acute critical illness. (Oral)
- **Endocrine Society's Annual Meeting**. March, 2018, **Chicago, USA**: The HPA-axis responses to prolonged critical illness: when do these normalize again, if at all? (Poster)