



Endocrine and metabolic considerations in critically ill patients 3

New insights into the controversy of adrenal function during critical illness

Eva Boonen, Stefan R Bornstein, Greet Van den Berghe*

Critical illness represents a life-threatening disorder necessitating recruitment of defence mechanisms for survival. Herein, the hypothalamic–pituitary–adrenal axis is essential. However, the relevance of a relative insufficiency of the hypothalamic–pituitary–adrenal axis in critical illness, which is diagnosed by a suppressed cortisol response to exogenous adrenocorticotrophic hormone (ACTH) irrespective of the plasma cortisol concentration, is controversial. Findings from several studies have provided insights that clarify at least part of this controversy. Rather than an activated hypothalamic–pituitary–adrenal axis, ACTH-independent regulators have been reported to contribute to increased cortisol availability during critical illness. One of these regulators is reduced cortisol breakdown, mediated by suppressed expression and activity of cortisol metabolising enzymes in the liver and kidneys. This downstream mechanism increases concentrations of plasma cortisol, but the ensuing feedback-inhibited ACTH release, when sustained for more than 1 week, has been shown to negatively affect adrenocortical integrity and function. Reduced adrenocortical ACTH signalling could explain reduced cortisol responses to exogenous ACTH. Whether such reduced cortisol responses in the presence of raised plasma (free) cortisol identifies adrenal failure needing treatment is unlikely. Additionally, reduced cortisol breakdown affects the optimum dose of hydrocortisone treatment during critical illness. Identification of patients with an insufficient hypothalamic–pituitary–adrenal axis response and the optimum treatment for this disorder clearly need more well designed preclinical and clinical studies.

Introduction

The human organism is constantly exposed to variable levels of stress exerted by external and internal stimuli.¹ Critical illness is defined as any life-threatening disorder that needs support of vital organ function, without which death would be imminent. Thus, this disorder represents physical stress of such a severity and magnitude that it poses a major challenge to the human body. Mechanisms to cope with such severe stress are mediated by complex endocrine responses. Herein, the hypothalamic–pituitary–adrenal axis (HPA) plays a key part because the increased exposure to cortisol is essential to acutely provide energy, retain fluid, increase cardiac output and blood pressure, and induce an appropriate immune response while protecting against excessive inflammation.^{2,3} Failing of this stress response can have rapid and lethal results, which, for example, is the case for patients with pre-existing adrenal failure who develop an Addisonian crisis (acute deficiency of the glucocorticoid cortisol) when undergoing surgery without adequate coverage with hydrocortisone. Absolute and relative adrenal failure also occur during critical illness and are most often reported in patients who have sepsis.⁴ However, the relevance of such a relative adrenal insufficiency during critical illness, which is defined as a suppressed cortisol response to an injection of adrenocorticotrophic hormone (ACTH) irrespective of the baseline concentration of plasma cortisol,⁴ is controversial.

Several studies have provided new insights that clarify part of this controversy, and will be discussed in this

Series paper. Results of these studies suggest that the HPA axis stress response to critical illness might differ in several aspects from the response to less severe stressors, which could have major consequences for the function of the adrenal glands. In view of previous knowledge, we review these novel insights. Additionally, we propose new research questions to redirect future research and to investigate novel treatments to improve outcomes in patients with this life-threatening disorder.

HPA axis activation to acute and chronic stress: the traditional concept

The adrenal gland, a key organ to cope with stress, unites steroid-producing adrenocortical cells and catecholamine-producing chromaffin cells. Stress-induced activation of the HPA-axis starts with the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which, via the hypophyseal portal system, reaches the anterior pituitary corticotrophs to induce ACTH secretion (panel 1). ACTH is the main regulator of adrenal glucocorticoid production, stimulating steroidogenesis by binding to the melanocortin 2 receptor (MC2R; figure 1). Responses to several stressors in the different components of the HPA axis have been studied in the context of major surgery, acute and chronic infections, autoimmune diseases, the metabolic syndrome, and affective and mood disorders.^{11–13} Some studies^{14,15} had previously measured the concentrations of plasma ACTH during and shortly after surgery and noted that these were transiently raised and returned to normal on

Lancet Diabetes Endocrinol 2015; 3: 805–15

Published Online
June 11, 2015
[http://dx.doi.org/10.1016/S2213-8587\(15\)00224-7](http://dx.doi.org/10.1016/S2213-8587(15)00224-7)

This is the third in a Series of four papers about endocrine and metabolic considerations in critically ill patients

Clinical Division and Laboratory of Intensive Care Medicine, Department of Cellular and Molecular Medicine, KU Leuven University, Leuven, Belgium (E Boonen MD, Prof G Van den Berghe MD); Department of Medicine III, Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Dresden, Germany (Prof S R Bornstein MD); and Diabetes and Nutritional Sciences, Rayne Institute, Denmark Hill Campus, King's College London, London, UK (Prof S R Bornstein)

Correspondence to: Prof Greet Van den Berghe, Clinical Division and Laboratory of Intensive Care Medicine, KU Leuven, B-3000 Leuven, Belgium greet.vandenbergh@med.kuleuven.be

Panel 1: Physiological information about cortisol production and metabolism during health

The occurrence of stress activates the hypothalamus to release corticotropin-releasing hormone (CRH). CRH reaches the anterior pituitary corticotrophs via the hypophyseal portal system to induce secretion of adrenocorticotrophic hormone (ACTH). Consequently, the release of ACTH causes cortisol production in the adrenal gland. ACTH is the main regulator of adrenal glucocorticoid production and release; it stimulates steroidogenesis by binding to its receptor, the melanocortin 2 receptor present in the cell membrane of the adrenocortical cells, which, when activated, stimulates adenylate cyclase. ACTH upregulates the expression of its own receptor, mediates the release of cholesterol from lipid droplets while increasing the expression of genes encoding the proteins for cholesterol uptake (such as the LDL receptor and scavenger-receptor class B member 1), and for cholesterol synthesis (via 3-hydroxy-3-methylglutaryl-coenzyme A reductase). ACTH also increases expression of genes encoding key steroidogenic enzymes, such as steroidogenic acute regulatory protein and cytochrome P450 cholesterol side-chain cleavage enzyme. Besides this feed-forward activation of cortisol secretion, feed-back inhibition of CRH and ACTH by cortisol regulates its (ie, cortisol) own release. This negative feedback inhibition occurs at both the pituitary and hypothalamic level and involves fast and delayed forms of inhibition circuits. In healthy resting conditions, ACTH and cortisol are released in a tightly coupled pulsatile manner after a characteristic circadian rhythm. The normal patterns in the early morning and afternoon show some interindividual variation, which is further modified by sleep, any shift in light-dark responses, feeding, and by physical and mental stress or illnesses.

In the circulation, cortisol is predominantly transported when bound to cortisol-binding globulin. Only free cortisol can enter a cell. In specific cells, cortisol can be inactivated to cortisone via 11 β -hydroxysteroid dehydrogenase (11 β -HSD)-2, which can be activated back to cortisol via 11 β -HSD1. Cortisol and cortisone are metabolised via 5 α -reductases and 5 β -reductases mostly in the liver.

Only cortisol can bind to the glucocorticoid receptor or the mineralocorticoid receptor, located in the cytosol of many cells. After binding to the receptor, this complex exerts its function by binding in the nucleus to DNA, or by exerting non-genomic effects. The molecular basis of cell-specific glucocorticoid responsiveness is not fully understood, but comprises of a differential expression of the receptor isoforms, coreceptor proteins functioning as coactivators, and corepressors of transcription.

the first day after surgery. Raised concentration of circulating ACTH is thus the typical clinically measurable response to such stressors, which within the adrenal cortex, dose-dependently activates the MC2R-mediated post-receptor effects (figure 1). The adrenal gland is characterised by a remarkable capacity to adapt to acute or chronic stressors. Beside an immediate effect on glucocorticoid production, ACTH also increases the longer-term steroidogenic capacity of adrenal cells by upregulating proteins important for steroidogenesis. Furthermore, in-vitro and in-vivo animal studies noted structural changes in the adrenal gland, such as hypervascularisation of the already highly vascularised adrenal glands and cellular adrenocortical hypertrophy or hyperplasia.¹⁶ These changes might, in part, explain the increased vascular susceptibility of the adrenal tissue during extreme conditions of acute stress, such as with meningococcal sepsis, which can evoke adrenal haemorrhage and failure.¹⁷ Additionally, results from studies of corticotropin-releasing hormone (CRH)

injection in rats, showed activation of the HPA axis evokes important ultrastructural changes in adrenocortical cells, including an increased number of mitochondria, expansion of the smooth endoplasmic reticulum and filopodia, and decreased liposomes that store cholesterol—the substrate for glucocorticoid biosynthesis.¹⁸ Furthermore, the adrenal gland has the highest anti-oxidative capacity of all tissues in the human body, which seems necessary to cope with the increased production of radical oxygen species due to steroidogenesis, as illustrated by the fact that mutations in antioxidant defence genes lead to glucocorticoid deficiency in patients.¹⁹

In conditions of chronic stress, a continued stimulation of the adrenal gland and thereby adrenal hypertrophy can be regarded to be an adaptive response essential for the continued provision of cortisol in proportion with the sustained higher requirements of glucocorticoid effects. Inferentially, hyperplasia and nodular transformation of adrenal cortical tissue could result from such chronic hyperstimulation of the adrenal cortex. In this context, it is of interest that patients who have metabolic syndrome—particularly the form with inflammatory vascular complications—and also patients who have depression, have been shown to have hyperplastic adrenal glands.^{20–22} Patients with metabolic syndrome also present with an increased incidence of adrenal nodules or incidentally discovered adrenal masses.²² In turn, such adrenal hyperplasia or adenomas with manifest or subclinical production of excess steroids, especially cortisol and aldosterone, can contribute to the symptoms and complications of the metabolic syndrome, diabetes, obesity, and depression.²² By analogy, continuously raised plasma ACTH and cortisol concentrations could inferentially result in adrenal hypertrophy and hyperplasia in other conditions of sustained and severe stress, such as extended critical illness.

HPA axis response to critical illness: activated or not?

As critical illness is an extreme example of sustained and severe physical stress, the raised plasma concentrations of cortisol would be expected to also be accompanied by high concentrations of plasma ACTH that mediate increased production of adrenocortical cortisol. However, only a few studies have reported the plasma concentrations of ACTH during critical illness. Most studies reported only ACTH measured at one timepoint, which holds restricted information in view of the pulsatile secretory pattern and the circadian rhythm of this hormone.^{4,23–25} Vermes and colleagues²⁶ reported daily plasma ACTH and cortisol concentrations measured during the first week of critical illness in patients with trauma or sepsis. Acutely raised plasma ACTH and cortisol concentrations were noted followed by a steep fall in plasma ACTH after 3 days of critical illness, whereas concentrations of plasma cortisol remained

high.²⁶ In the past 2 years in a more heterogeneous patient population who were critically ill, plasma concentrations of ACTH were uniformly much lower than normal values from the first day onward of the intensive care unit (ICU) admission, and remained below the lower limit of normality throughout the first week of critical illness.^{27,28} This so-called ACTH-cortisol dissociation of critical illness is not expected in the context of an activated HPA axis in response to such a high and sustained level of physical stress. What drives the low ACTH concentrations in the face of high plasma cortisol is unclear. Interestingly, a small study by Polito and colleagues²⁹ reported reduced ACTH mRNA concentrations in nine human pituitary glands collected postmortem from patients who died after septic shock by comparison with patients who died suddenly from other diseases, in the absence of a compensatory rise in the expression of CRH or vasopressin in the hypothalamus. Experimental models of sepsis have shown that expression of pituitary ACTH was suppressed in the more chronic phase of critical illness,³⁰ which could be evoked by nitric oxide or by suppressed orexin (also known as hypocretin).^{29,30} However, if such sepsis-induced suppression of pituitary ACTH expression was a primary manifestation of organ damage due to shock, this would inferentially cause abnormally low concentrations of plasma cortisol, which is not usually noted in patients. Another possible explanation could be an increased adrenocortical sensitivity to ACTH.³¹ However, the secretory response of the adrenocortical cells to any given endogenous concentration of plasma ACTH is normal during critical illness,³² and in response to exogenous ACTH, the cortisol response is often low, as was shown by the ACTH stimulation test. Furthermore, a recent study³³ of human adrenal glands showed that ACTH signalling was unaltered during the first week of critical illness, but was severely suppressed in the prolonged phase. The high plasma cortisol concentrations, the concomitantly low concentrations of plasma ACTH, and subsequently low ACTH-regulated gene expression in the adrenal cortex during critical illness could be caused by a negative feedback mechanism whereby inhibition is exerted by raised plasma cortisol and in turn evoked by alternative, pathways independent of ACTH.

Alternative activators of the adrenal cortex during critical illness

The dissociation between plasma ACTH and cortisol concentrations in the body suggest that other ACTH-independent activators of adrenal cortisol production, comprising the sympathetic-adrenergic system, the immune system, and adipokines, might play a part.^{32,34,35}

The adrenal gland provides a complex micro-environment of close cellular interactions between the two endocrine stress systems: the sympathetic-adrenomedullary system and the adrenal cortex.¹⁶ Furthermore,

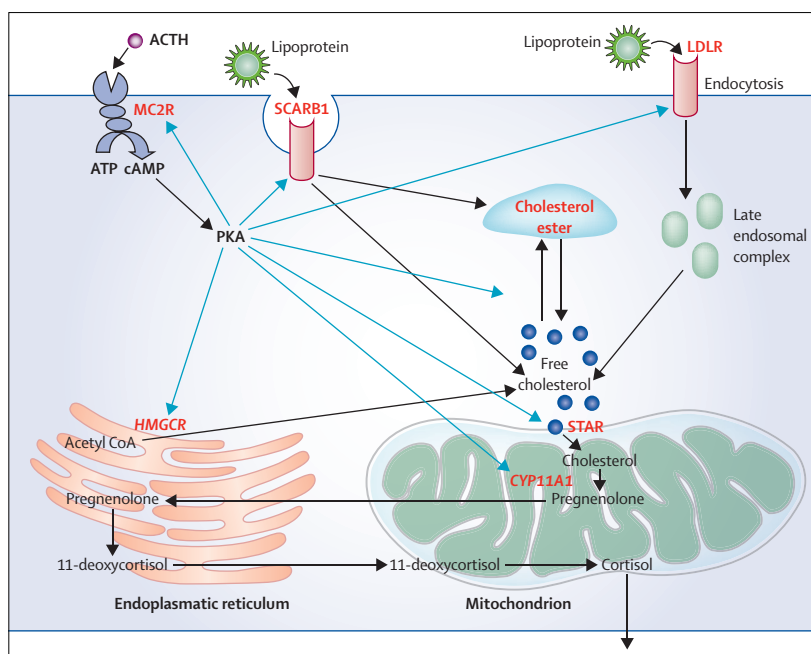


Figure 1: Overview of the effect of ACTH on the adrenal gland

ACTH binds to its receptor MC2R, present in the cell membrane of the adrenocortical cells, which stimulates adenylate cyclase, and, thereby, initiates steroidogenesis. ACTH upregulates the expression of its own receptor, and mediates the release of cholesterol from lipid droplets while increasing the expression of genes encoding the proteins for cholesterol uptake (such as LDLR and SCARB1) and cholesterol synthesis (via HMGCR). ACTH increases the expression of genes encoding key steroidogenic enzymes, such as STAR and CYP11A1.⁵⁻¹⁹ Blue lines represent ACTH effects. ACTH=adrenocorticotropic hormone. LDLR=LDL-receptor. MC2R=melanocortin 2 receptor. SCARB1=scavenger-receptor class B member 1. cAMP=cyclic AMP. PKA=protein kinase A. Acetyl CoA=acetyl coenzyme A. HMGCR=3-hydroxy-3-methylglutaryl-coenzyme-A reductase. STAR=steroidogenic acute regulatory protein. CYP11A1=cytochrome P450 cholesterol side-chain cleavage enzyme.

the splanchnic nervous system can directly activate the neuro-adrenocortical axis. Additionally, a close interaction exists between adrenocortical cells and resident macrophages, and blood immune cells and the vasculature (figure 2). CRH can activate the sympathetic-adrenomedullary system, accounting for its ability to prevent adrenocortical atrophy in animals with hypophysectomy.³⁶ Chromaffin cells might play a key part as evidenced by results from coculture systems which showed that the addition of chromaffin cells to adrenocortical cells increased the release of cortisol by up to ten times.^{37,38} Vice versa, intra-adrenal glucocorticoids induce the expression of catecholaminergic enzymes, particularly phenylethanolamine N-methyltransferase, and stimulate catecholamine release from chromaffin cells.¹⁶ Exogenous glucocorticoids can induce adrenal atrophy through negative feedback inhibition of ACTH, leading to low concentrations of intra-adrenal cortisol which, in turn, might lead to a decrease in adrenal catecholamine release. This inhibitory feedback pathway is further supported by patients treated with glucocorticoids and with Addison's disease or congenital adrenal hyperplasia, who will have low circulating adrenaline.^{39,40} Adrenomedullary dysfunction in these patients correlated with cardiovascular instability and

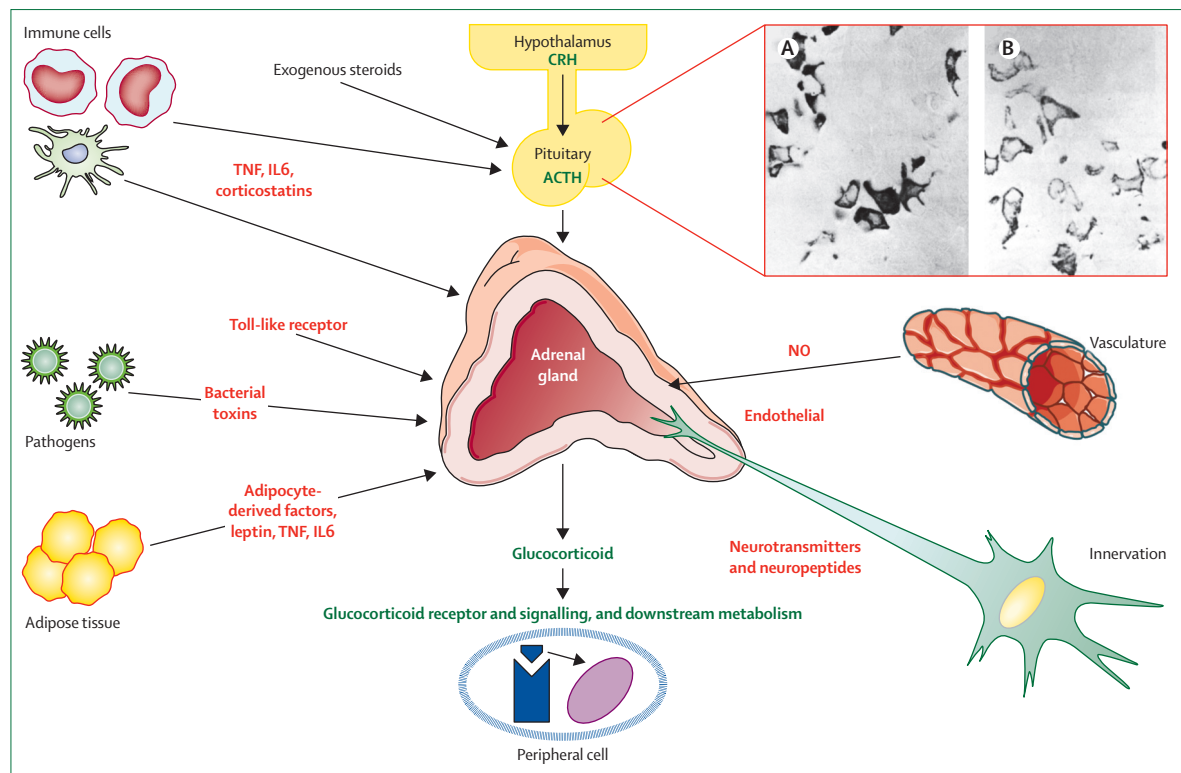


Figure 2: Current concepts of alternative activators of adrenal stress response during critical illness

Anti-inflammatory and pro-inflammatory cytokines derived from immune cells differentially regulate adrenal cortisol secretion during critical illness, especially during critical illness induced by sepsis. Bacterial and viral toxins mediated by toll-like receptors modify pituitary-adrenal hormone synthesis, glucocorticoid tissue sensitivity, and activation of peripheral cortisol metabolism indirectly via the immune system and directly through action on the cells of the adrenal cortex. Adipokines, such as leptin, IL6, and TNF, released from subcutaneous, visceral, or peri-adrenal and intra-adrenal adipocytes, directly modify adrenal steroidogenesis. Similarly, the hypothalamic-pituitary-adrenal axis function is affected by blood flow, endothelial-derived factors, neurotransmitters, and neuropeptides. Original rat corticotrophic pituitary cells stained with ACTH before (A) and after (B) stimulation with CRH. Thick arrows represent standard hypothalamic-pituitary-adrenal axis regulation. Thin arrows represent the non-ACTH regulators. Reproduced from Bornstein,³³ by permission of *New England Journal of Medicine*. CRH=corticotropin-releasing hormone. ACTH=adrenocorticotropic hormone. TNF=tumour necrosis factor. IL6=interleukin 6. NO=nitric oxide.

hypoglycaemia.^{39,40} This close functional interdependence of the two endocrine systems in the adrenal gland is further corroborated by the in-vivo phenotype of knockout animals with specific defects in either system and by patients with defects in either the function of the adrenal cortex or the medulla, such as Addison's disease or congenital adrenal hyperplasia.^{40,41} Results from patients with psychiatric illnesses, such as depression, who have high cortisol concentrations have shown that classic feed-forward overdrive and impaired feedback theories of hypercortisolaemia might not apply, and that the hypercortisolaemia of depression could result from alternative mechanisms consisting of the irregular basal hypersecretion of cortisol, which is possibly driven by splanchnic sympathetic activation.^{42,43}

In animal models of inflammatory bowel disease and viral infection, immune mediators, such as interleukin 6, and other ACTH-independent immune-adrenal pathways account for a chronic hyperstimulation of the adrenal cortex in the absence of an increased pituitary secretion of ACTH.⁴⁴ Hence, many studies in both animals and people have reported a so-called dissociation between ACTH and

cortisol release both in settings of physiological stress responses and pathophysiology. Examples of such settings include fetal stress, early postnatal life stress, ageing, inflammation and infection, psychiatric disorders, Alzheimer's disease, chronic pulmonary disease, bone fractures, alcohol dependence, and metabolic diseases.³⁵ More recently, studies have also shown that cytokines and immune mediators released from macrophages, monocytes, or other immune cells can not only directly stimulate or inhibit cortisol release from human adrenocortical cells, but also that a direct interaction exists between viral or bacterial pathogens and the adrenocortical cells.⁴⁵ Adrenocortical cells express toll-like receptors that can directly respond to the presence of Gram-negative or Gram-positive bacterial pathogens. However, findings of a detailed analysis of the mechanisms of hypothalamic-pituitary and immune-dependent adrenal regulation during systemic inflammation in genetically modified mice models suggested that the primary activation of the HPA axis in such conditions seemed to occur via immune cells.⁴⁶ Toll-like receptor signalling in immune cells, but not in adrenocortical cells, has been shown to mediate

lipopolysaccharide-induced adrenal inflammation and HPA axis stimulation.⁴⁷

Finally, adipokines released from adipose tissue, and neuropeptides and immune mediators secreted from endothelial cells, including the production of local morphogens (eg, *sonic hedgehog* and *WNT*), have been implicated in an ACTH-independent activation of adrenal cortisol regulation.^{47–49} Irrespective of the type of driver of adrenal function during life-threatening critical illnesses in patients, cortisol production is inferred to be substantially increased.

Cortisol production and metabolism during critical illness

Although the rate of cortisol production is generally accepted to increase to generate and maintain hypercortisolaemia during critical illness, the rate was only quantified in patients recently. Boonen and colleagues²⁷ documented that the morning rate of cortisol production, quantified by the stable isotope infusion technique, was only moderately increased (less than double) in patients who were critically ill with systemic inflammation response syndrome (SIRS) and unchanged in patients who were critically ill without SIRS by comparison with the rates of cortisol production in healthy matched control participants (figure 3); these results were noted even when all critically ill patients had increased concentrations of plasma total and free cortisol. This finding was quite unexpected. The stable isotope technique also allowed the quantification of cortisol plasma clearance, which was suppressed to less than half the usual rate in all critically ill patients, irrespective of inflammation status. Three subsequent studies further explored this finding.²⁷ Cortisol half-life and plasma clearance during critical illness was quantified after the administration of a 100 mg bolus of hydrocortisone. Similar results were obtained; plasma cortisol clearance was to 40% of that in participants in the matched control group, and cortisol half-life was a median of five times longer in patients who were critically ill. This reduction in cortisol breakdown was attributed to the reduced expression and activity of cortisol-metabolising enzymes, predominantly the 5 α -reductases and 5 β -reductases in the liver and 11 β -hydroxysteroid dehydrogenase 2 (11 β -HSD2) in the kidneys—as suggested by urinary steroid ratios, tracer kinetics, and assessment of human liver biopsy samples. Importantly, in the study by Boonen and colleagues,²⁷ reduced cortisol clearance was noted in all tested critically ill patients irrespective of type and severity of illness, duration of stay in the ICU, and prognosis. This finding suggests that a pronounced suppression of cortisol breakdown might be a key mechanism, together with the ongoing normal or slightly increased cortisol production, which contributes to increased plasma cortisol in conditions of sustained severe stress in critical illness. A reduced rate of cortisol breakdown has been

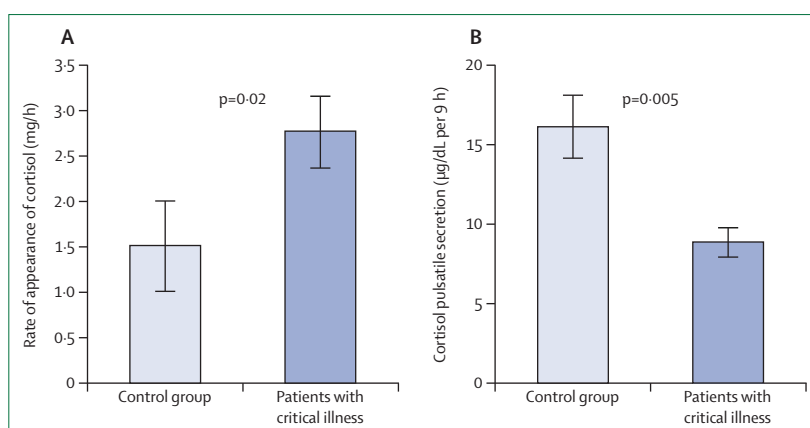


Figure 3: Cortisol production in patients who are critically ill

(A) Morning cortisol production calculated via a continuous infusion of deuterated cortisol isotope tracer between 1000 h to 1300 h in 11 patients who were critically ill and nine healthy control participants; production is higher in critically ill patients compared with control participants.²⁷ (B) Nocturnal pulsatile cortisol production calculated from time concentration profile series, sampled every 10 min from 2100 h to 0600 h in 40 patients who were critically ill and eight healthy control participants, by use of deconvolution analysis; production is lower in critically ill patients compared with control participants.³² Taken together, these data suggest that overall the 24 h cortisol production rate in patients who are critically ill is not different from that in healthy matched control participants. Bar charts show mean and standard errors.

described in patients with anorexia nervosa, post-traumatic stress disorder, and depression,^{50–52} suggesting that it could be a fundamental part of the body's general response to stress.

Knowledge that cortisol half-life and plasma clearance is uniformly reduced during critical illness allowed the further study of ACTH and cortisol secretion rates and the interaction between ACTH concentrations and cortisol secretion. A time series of plasma concentrations measured every 10 min for 9 h during the night in patients who were critically ill and in healthy control participants was constructed.³² Such a time series for plasma concentrations can be transformed into hormonal secretion profiles with the use of deconvolution analysis, which takes into account the elimination half-life of the hormone and allows the quantification of pulsatile and non-pulsatile (basal) secretion of the hormone.⁵³ Of note, two studies^{15,54} had previously investigated repeated blood samples of ACTH and cortisol to assess pulsatile secretion during surgery and critical illness, but these did not apply a deconvolution analysis or account for the fact that half-life of cortisol in critical illness is much longer than normal. Findings from the time series study³² showed that both nocturnal (2100 h to 0600 h) ACTH and cortisol pulsatile and total secretion rates were reduced in patients who were critically ill, which was attributed to reductions of the hormonal pulse masses, whereas the number of pulses were unchanged. Dose-responses between a specific ACTH concentration and cortisol secretory response were preserved, suggesting that the term ACTH-cortisol dissociation might not be entirely correct. Indeed, the cortisol secretion was still associated with the amount of circulating ACTH, but both were suppressed in patients who were critically ill in the presence of high concentrations

of total and free plasma cortisol. Hence, after the very acute phase of critical illness, high nocturnal concentrations of plasma cortisol seemed to be predominantly maintained by reduced cortisol breakdown, a conclusion that was supported by increased entropy values for ACTH and cortisol time series, suggesting increased irregularity.³² Together, the results from the nocturnal deconvolved ACTH and cortisol secretion study and from the stable isotope study,^{27,32} which reported that daytime cortisol secretion is ACTH-independent and not even double that of healthy participants, show that overall 24 h rates of cortisol production during critical illness might not be, or at best only moderately, higher than production during health (figure 3).

Effect of cortisol at the tissue level during critical illness

Increased concentrations of plasma cortisol during critical illness do not necessarily result in increased activation of the cortisol receptor at the level of the many tissues expressing these receptors. Indeed, 90% of the total cortisol concentration in circulation is bound to corticosteroid-binding globulin (CBG). Therefore, changes in the binding of cortisol to CBG could affect availability of free cortisol, the form responsible for the biological and clinical effect of the endogenous hormone. In previously published work,⁵⁵ CBG concentrations have been shown to be significantly decreased in patients in the early stage of septic shock and multiple trauma. This decrease results in much higher concentrations of free circulating cortisol than of total circulating cortisol, suggesting that CBG plays an important part in the regulation of cortisol availability to target tissues during the severe stress of critical illness.⁵⁵ Furthermore, CBG in plasma is present in two forms: intact CBG and CBG cleaved by neutrophil elastase, which has a lower affinity for cortisol. Cleavage by neutrophil elastase increases the concentration of free cortisol at the site of neutrophil action, which might target an increased cortisol bioavailability to sites of inflammation during critical illness.^{56,57} Furthermore, in the case of a high fever of 42°C, free cortisol concentration in the blood increases by three times.⁵⁸

Free cortisol has been proposed to be a better parameter to assess hypercortisolaemia in critical illness than are total cortisol concentrations, particularly in patients with systemic infection, since it provides a better correlation with disease severity.⁵⁹ Salivary cortisol concentrations have been proposed as a possible surrogate of the circulating concentrations of free cortisol in patients with septic shock.⁶⁰ However, difficulties to adequately sample enough saliva without contamination via the local conversion of cortisol to cortisone through expression of 11 β -HSD2 in the salivary gland, restrict the use of this technique.

The real meaning of any form of measurable cortisol in the circulation during critical illness should be

assessed in view of regulated glucocorticoid receptor (GR) expression and signalling. Evidence from both animal and human experimental studies suggests that alternative splicing of the GR mRNA, GR expression, GR affinity, and GR translocation are regulated and could be tissue specific during critical illness.⁶¹⁻⁶⁸ Children with critical illness due to sepsis or traumatic brain injury have lower total and cytoplasmatic GR concentrations in white blood cells than do healthy controls.⁶³ Other suggested mechanisms of corticosteroid resistance during critical illness include an increased expression of GR β , the dominant negative isoform of the receptor,⁶³ and downregulation of GR α (mediated by microRNA124).⁶⁴ Furthermore, reduced translocation to the nucleus or the presence of less functional polymorphisms might have a role in corticosteroid resistance. However, the expression of GR in different tissues warrants additional research in patients who are critically ill and the clinical relevance of these cellular changes is yet to be further elucidated.

Inadequate adrenocortical response to critical illness

Absolute adrenal failure

During critical illness, so-called absolute adrenal failure can be present for two reasons: known primary or secondary adrenocortical insufficiency (eg, due to autoimmune Addison's disease, pituitary tumours, or trauma); and critical illness-associated acquired loss of adrenal function. For patients with either of these disorders, appropriate and immediate diagnosis and treatment is essential to prevent life-threatening shock.

The acquired loss of adrenal function during critical illness could have several causes. It might be due to haemorrhage in the adrenal gland, adrenocortical ischaemia or apoptosis, or the effect of drugs that interfere with and impair cortisol production. Alternatively, the ACTH suppression noted after the very acute phase of critical illness seems to have important negative consequences on the integrity and function of the adrenal glands of patients who are critically ill, predominantly in the extended phase of illness where the effects of ACTH in the adrenal cortex were reduced.³³ Furthermore, increasing evidence emphasises the importance of ACTH secretion pulsatility to maintain normal cellular function in both the adrenal gland and the target tissues of cortisol, by preventing desensitisation of transcriptional responses. Both pulsatile ACTH secretion and pulsatile cortisol secretion are reduced in critical illness.³² This reduction could further contribute to the loss of the trophic effects of ACTH on the adrenal gland and to tissue-specific cortisol resistance, but needs to be further investigated. Equally, it is necessary to look for other predisposing factors for adrenal dysfunction in patients in intensive care.¹³ A growing number of patients might have underlying disorders or have a history of medication

use that cause a subclinical form of adrenocortical impairment that can become clinically relevant during the severe stress of critical illness. For example, the number of individuals in the ageing population who are receiving some form of chronic exogenous glucocorticoid treatment is rising, which could lead to hypotrophy of the adrenal cortex. Additionally, congenital abnormalities, polyglandular autoimmune disorders, trauma, infectious diseases, coagulation disorders, liver diseases, mental disorders, specific non-steroid drugs, and addictions need to be considered in relation to potential predisposing factors for adrenal insufficiency in patients with critical illness.^{69,70} Clinicians should thus be aware of these underlying conditions or disorders and predisposing factors to rapidly identify patients at risk of developing life-threatening adrenal failure during critical illness.

Relative adrenal failure

In 1946, Hans Selye⁷¹ suggested that so-called exhaustion of the adrenal cortex might occur in some situations of stress. Relative adrenal failure is a term that was proposed to describe such a state during critical illness, in which concentrations of plasma cortisol, although still higher than during good health, are not high enough to cope with the level of stress caused by the condition.⁷² In this concept, the adrenal gland is functionally normal and maximally activated, but is still insufficient when facing challenge. In 2008, the term critical illness-related corticosteroid insufficiency⁷³ was introduced to define relative failure of the adrenal gland, which could happen at any level of the HPA axis.^{73,74}

Despite the extensive published work about this topic, the presence of this disorder and underlying mechanisms of such failure are still debated. Pro-inflammatory

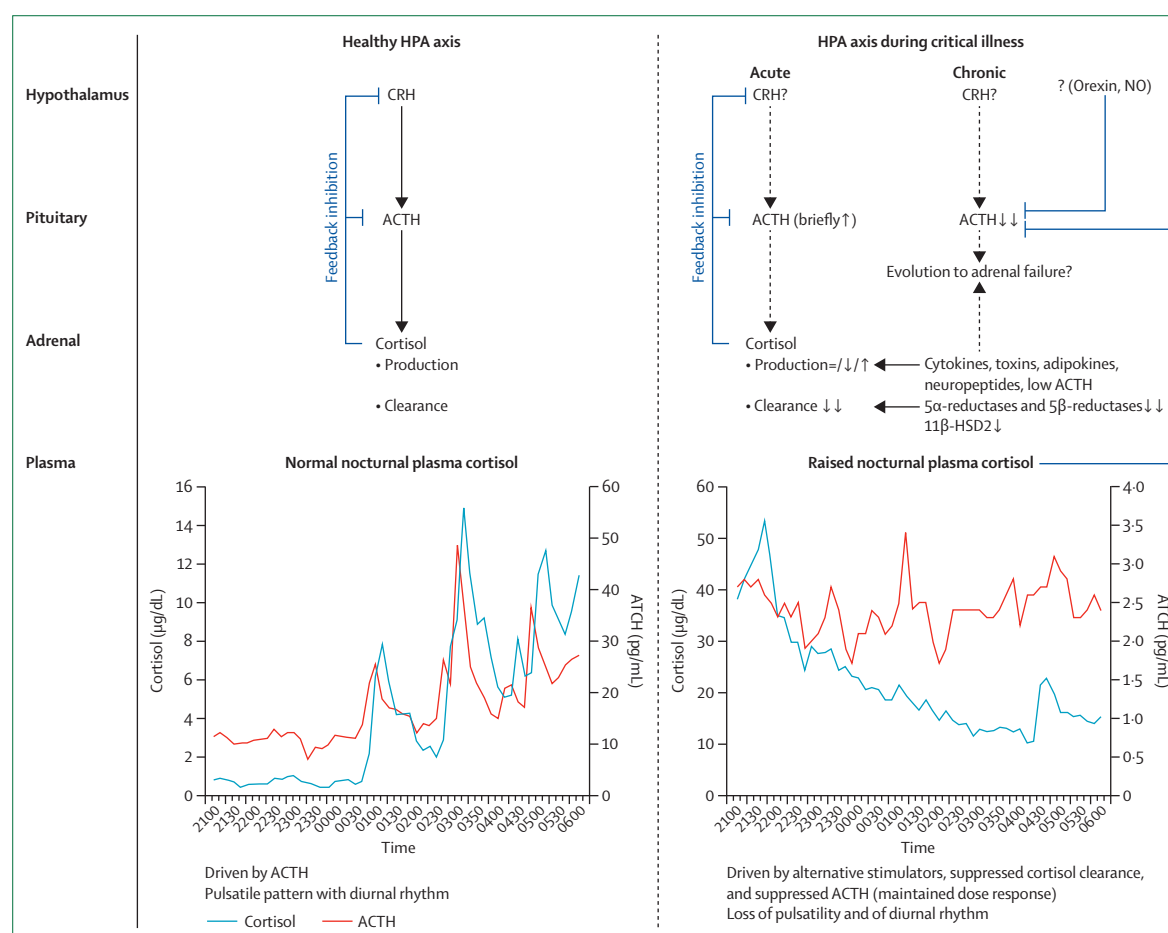


Figure 4: Overview of the hypothalamic-pituitary-adrenal-axis regulation in health and during critical illness

During health, CRH controls the pulsatile and tonic basal release of both ACTH and cortisol, which both follow a diurnal pattern. During critical illness, ACTH release is only briefly increased in the very acute phase, after which it is suppressed. Beyond the very acute phase of critical illness, raised plasma concentrations of cortisol are predominantly brought about via reduced plasma clearance of cortisol.^{27,32} With time, the low plasma ACTH concentrations might negatively affect adrenocortical structure and function and hereby contribute to the increased risk of adrenal failure noted in patients who have long lasting critical illness.³³ Solid lines represent proven regulation. Dotted lines represent regulation that is still speculated and needing additional research. Question marks represent an unknown part of the pathway. ACTH=adrenocorticotrophic hormone. CRH=corticotropin-releasing hormone. HPA=hypothalamus-pituitary-adrenal. NO=nitrous oxide. 11 β -HSD2=11 β -hydroxysteroid dehydrogenase 2. \uparrow =raised. \downarrow =low. $\downarrow\downarrow$ =very low. $\neq/\downarrow/\uparrow$ =can be normal, low, or raised.

cytokines might have an important role in this phenomenon by inducing tissue resistance to ACTH or competing with ACTH at the receptor level. Resistance of target tissues to cortisol during critical illness can also be attributed to decreased glucocorticoid delivery or decreased glucocorticoid action due to an altered function of CBG or to changes in glucocorticoid receptor concentrations and affinity. Furthermore, impaired blood supply to the pituitary gland can induce subtle levels of pituitary ischaemia, which is followed by the accumulation of nitric oxide or central neuropeptides, leading to decreased hormonal secretion of ACTH.⁷⁵ Additionally, because every adrenal cell is in direct contact with an endothelial cell, the adrenal cortex is

susceptible to haemorrhage during severe stress or sepsis, which can result in the full onset of an Addisonian-like crisis, and also more subtle changes that could cause a relative impairment to cortisol production. Finally, different neuropeptides, oxidative stress, substrate deficiency due to low circulating cholesterol,⁷⁶ or interfering drugs are also suggested to play a part in reducing the ability to produce cortisol.¹³

Diagnostic criteria

In view of the controversy about the underlying mechanisms of this relative adrenal insufficiency, the appropriate diagnostic criteria and treatment have also not been agreed upon. Suggested diagnostic criteria were based on findings from a landmark study by Annane and colleagues⁷² who identified an incremental response of plasma cortisol of less than 9 µg/dL after injection of 250 µg ACTH and a high baseline cortisol concentration (>34 µg/dL) as most discriminative to identify patients at high risk of death. Hence, relative adrenal failure during critical illness was, from then on, diagnosed by a subnormal plasma cortisol incremental response to exogenous ACTH, irrespective of the concentration of plasma cortisol.⁴ However, some investigators have not been able to replicate the original findings by Annane and colleagues⁷² and thus no consensus exists on how to diagnose adrenal failure in the ICU. The non-ACTH drivers of cortisol production and changes in cortisol breakdown could explain reduced cortisol responses to ACTH injection, but do not support the exclusive interpretation of adrenal failure, as long as plasma (free) cortisol is several times higher than normal concentrations.²⁷ This theory is in accordance with the 2008 guidelines on the topic, which no longer advise the use of the ACTH test to guide treatment with hydrocortisone.⁷⁷ Additionally, a dose of 250 µg of ACTH leads to supra-physiological concentrations of ACTH and could, therefore, overcome any ACTH resistance. As an alternative, a 1 µg stimulation dose has been suggested, but this dose has not been extensively studied in patients who are critically ill and results have been conflicting. A random total cortisol of less than 10 µg/dL during critical illness has been suggested for the diagnosis of corticosteroid insufficiency related to critical illness.⁷³ However, total plasma cortisol concentration is the net result of adrenal production and secretion, distribution, binding, and elimination of cortisol. Furthermore, because cortisol is secreted in a pulsatile manner,³¹ adequacy of adrenal cortisol production in response to critical illness could be problematic to judge merely by one measurement of total plasma cortisol. In addition, total plasma cortisol concentrations do not entirely represent glucocorticoid signalling. Taken together, and in view of all the changes that occur during critical illness that could, in part, be adaptive or maladaptive, difficulty remains about how to conclude for adequacy of cortisol availability during illness on the basis of any of these tests.

Panel 2: Key messages

- Instead of hypercortisolism driven by raised adrenocorticotropic hormone (ACTH) release, several ACTH-independent regulators contribute to increased availability of cortisol during critical illness
- The amount of cortisol produced during critical illness was shown to be much less than previously assumed: often less than normal or normal and at most, double that of healthy participants
- Cortisol breakdown is immediately and substantially suppressed during critical illness, mediated by reduced expression and activity of cortisol metabolising enzymes in the liver and kidneys; these altered pharmacokinetics have implications for dosing of corticosteroid treatment during critical illness
- Raised plasma cortisol, driven by its reduced breakdown, suppresses plasma concentrations of ACTH through feedback inhibition; such low plasma concentrations of ACTH can persist for weeks in patients in the intensive care unit, and is associated with abnormal adrenal structure, impaired adrenocortical ACTH signalling, and reduced expression of key steroidogenic enzymes
- Reduced adrenocortical ACTH signalling could mediate reduced adrenocortical cortisol production and explain reduced cortisol responses to exogenous ACTH injection; however, in the presence of increased plasma cortisol and suppressed cortisol breakdown, reduced cortisol responses to exogenous ACTH could be adaptive. When plasma cortisol concentration is normal or low during long-lasting critical illness, low cortisol responses to ACTH could be indicative of adrenal failure and might need treatment
- Well designed preclinical and clinical studies are needed to better identify patients with a failing hypothalamic-pituitary-adrenal axis response and to refine optimum treatment modalities

Search strategy and selection criteria

We searched the PubMed database using the terms “HPA-axis”, “adrenal gland”, “ACTH”, and “cortisol” in combination with “critical illness”, “sepsis”, or “trauma”. We selected reports published between Jan 1, 2009, and Dec 31, 2014. However, since recent reports challenged a classic dogma, we also did a more extensive search of earlier scientific literature because landmark papers needed to be reviewed but were published before our search date. Therefore, we focused on previous dogma knowledge.

Human studies were preferred to animal studies; we only included animal studies to strengthen human observations or to speculate on future experiments. We assessed the reference lists of articles identified in this search for selection of other reports when deemed relevant. Review articles and book chapters are cited to guide readers to more detailed information than this Series paper contains.

Therapeutic implications

Patients with an established diagnosis of primary or secondary adrenal failure or patients on chronic treatment with systemic glucocorticoids before critical illness should receive additional glucocorticoid coverage to cope with the acute stress.^{78,79} At present, such patients in the ICU receive quite high doses of glucocorticoids based on the assumption that cortisol production is increased by several times in critical illness. However, this assumption might not be correct. The present treatment strategy consists of the administration of a bolus of 100 mg of hydrocortisone and then 50–100 mg every 6 h on first day of treatment, followed by 50 mg every 6 h on the second day, and 25 mg every 6 h on the third day, tapering to a maintenance dose by the fourth to fifth day.^{78,79} This dosing regimen could be too high, especially in view of the now documented reduced cortisol breakdown during critical illness.²⁷

Whether or not relative adrenal failure should be treated with exogenous glucocorticoid substitution therapy and, in that case, with which doses, is not clear. In practice, some intensivists might use hydrocortisone in patients with sepsis who do not adequately respond to vasopressors, volume loading, or both.⁷⁷ However, findings from a systematic review⁸⁰ of six high-quality, randomised controlled trials (published in 2012) showed that hydrocortisone therapy does not reduce mortality from severe sepsis.⁸⁰ This result is mainly because the two largest randomised controlled studies generated conflicting results.^{81,82} Another well powered study is recruiting patients (NCT01448109) and aims to investigate the effect on 90 day mortality from treatment of 200 mg hydrocortisone per day for a maximum of 7 days in 3800 patients.⁸³

However, cortisol production is now known to be, at most, only moderately increased in patients who are critically ill with a well functioning HPA axis. Additionally, cortisol breakdown is substantially and robustly reduced in these patients; therefore, therapeutic doses of 200 mg hydrocortisone used for these trials might have been too high, which could have induced side-effects that could have abrogated any potential benefit. As a result, future studies should assess whether lower doses can be used to raise plasma cortisol to sufficient concentrations to obtain the targeted effects while minimising adverse effects of excessive doses. A dose of about 60 mg of hydrocortisone, equivalent to roughly double the normal daily production of cortisol, as quantified with stable isotopes, might be an alternative for further investigation. Furthermore, the novel insight that cortisol half-life is much longer during critical illness than during health also has implications for treatment of patients in the ICU with steroids for other indications. Generally, a tapering down of dose as soon as possible should be advised to restrict the adverse effects of excessive amounts of glucocorticoids during critical illness.

Future research

Further research is clearly needed to investigate appropriate diagnosis and optimum substitution treatments for adrenal failure on the basis of the latest novel insights. Effects of sustained high concentrations of cortisol, endogenous or iatrogenically induced (at the level of hypothalamus, pituitary, and cortical brain functions), both in relation to acute delirium and to long-term sequelae of critical illness, warrants further investigation. Furthermore, the response of the HPA axis to sepsis and inflammation needs to be studied in appropriate preclinical models to allow a tissue-specific dissection of the roles of the immune system, vasculature, and endocrine cells. Additionally, identification of the underlying mechanisms for the reduced expression and activity of cortisol metabolising enzymes in the liver and the kidneys is of high priority.

Conclusions

New evidence suggests that, beyond the first hours after onset of critical illness, an activated HPA axis is not the main driver of the necessary increases in cortisol availability (figure 4; panel 2). Although non-ACTH drivers of cortisol production might be involved, increased cortisol exposure during critical illness does not seem to be regulated mainly by cortisol production. Instead, reduced cortisol metabolism during critical illness substantially contributes to increased cortisol availability. Raised plasma cortisol via reduced cortisol breakdown is comparable with the condition of exogenous treatment with (high-dose) hydrocortisone. In both these states, the suppressed endogenous ACTH signalling could explain reduced cortisol responses to exogenous ACTH. These reduced cortisol responses to ACTH thus do not necessarily suggest (relative) adrenal failure that needs treatment, as long as plasma (free) cortisol concentrations are several times higher than they are in healthy people. The reduced cortisol breakdown should also be accounted for in the adequate dosing of hydrocortisone for any indication during critical illness.

Well designed studies are needed to better identify patients with critical illness-induced adrenal failure and to define the optimum treatment for this disorder.

Contributors

EB, SRB, and GvdB each drafted parts of the Series paper, which were subsequently integrated by EB and edited by GvdB. The final version of the manuscript was corrected where needed and approved by all authors.

Declaration of interests

We declare no competing interests.

Acknowledgments

GvdB is supported by: the Fund for Scientific Research Flanders, Belgium (FWO G.0417.12); by the Methusalem Programme of the Flemish Government via the KU Leuven University (METH/08/07 and METH/14/06); and by an European Research Council Advanced grant (AdvG-2012–321670) from the Ideas Programme of the European Union 7th framework programme. SRB is supported by a grant from the Deutsche Forschungsgemeinschaft (KFO 252).

References

- 1 Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 1992; **267**: 1244–52.
- 2 Bornstein SR, Briegel J. A new role for glucocorticoids in septic shock: balancing the immune response. *Am J Respir Crit Care Med* 2003; **167**: 485–86.
- 3 Galon J, Franchimont D, Hiroi N, et al. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. *FASEB J* 2002; **16**: 61–71.
- 4 Annane D, Maxime V, Ibrahim F, Alvarez JC, Abe E, Boudou P. Diagnosis of adrenal insufficiency in severe sepsis and septic shock. *Am J Respir Crit Care Med* 2006; **174**: 1319–26.
- 5 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. *J Clin Invest* 1994; **93**: 1828–33.
- 6 Lehoux JG, Lefebvre A, Bélisle S, Bellabarba D. Hormonal regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in the rat adrenal gland. *J Steroid Biochem* 1989; **34**: 379–84.
- 7 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.
- 8 Liu J, Heikkilä P, Meng QH, Kahri AI, Tikkanen MJ, Voutilainen R. Expression of low and high density lipoprotein receptor genes in human adrenals. *Eur J Endocrinol* 2000; **142**: 677–82.
- 9 Simpson ER, Waterman MR. Regulation by ACTH of steroid hormone biosynthesis in the adrenal cortex. *Can J Biochem Cell Biol* 1983; **61**: 692–707.
- 10 Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev* 1996; **17**: 221–44.
- 11 Kanczkowska W, Suea M, Zacharowski K, Reincke M, Bornstein SR. The role of adrenal gland microenvironment in the HPA axis function and dysfunction during sepsis. *Mol Cell Endocrinol* 2014; published online Dec 24.
- 12 Chrousos GP. The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. *Int J Obes Relat Metab Disord* 2000; **24** (suppl 2): s50–55.
- 13 Bornstein SR. Predisposing factors for adrenal insufficiency. *N Engl J Med* 2009; **360**: 2328–39.
- 14 Naito Y, Fukata J, Tamai S, et al. Biphasic changes in hypothalamic-pituitary-adrenal function during the early recovery period after major abdominal surgery. *J Clin Endocrinol Metab* 1991; **73**: 111–17.
- 15 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.
- 16 Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev* 1998; **19**: 101–43.
- 17 Waterhouse R. A case of suprarenal apoplexy. *Lancet* 1911; **1**: 577–78.
- 18 Bornstein SR, Ehrhart-Bornstein M, Güse-Behling H, Scherbaum WA. Structure and dynamics of adrenal mitochondria following stimulation with corticotropin releasing hormone. *Anat Rec* 1992; **234**: 255–62.
- 19 Prasad R, Kowalczyk JC, Meimaridou E, Storr HL, Metherell LA. Oxidative stress and adrenocortical insufficiency. *J Endocrinol* 2014; **221**: R63–73.
- 20 Szigethy E, Conwell Y, Forbes NT, Cox C, Caine ED. Adrenal weight and morphology in victims of completed suicide. *Biol Psychiatry* 1994; **36**: 374–80.
- 21 Dinan T. Novel approaches to the treatment of depression by modulating the hypothalamic-pituitary-adrenal axis. *Hum Psychopharmacol* 2001; **16**: 89–93.
- 22 Lamoumier-Zepter V, Ehrhart-Bornstein M, Bornstein SR. Metabolic syndrome and the endocrine stress system. *Horm Metab Res* 2006; **38**: 437–41.
- 23 Drucker D, Shandling M. Variable adrenocortical function in acute medical illness. *Crit Care Med* 1985; **13**: 477–79.
- 24 Michalaki M, Margeli T, Tsekouras A, Gogos CH, Vagenakis AG, Kyriazopoulou V. Hypothalamic-pituitary-adrenal axis response to the severity of illness in non-critically ill patients: does relative corticosteroid insufficiency exist? *Eur J Endocrinol* 2010; **162**: 341–47.
- 25 Roth-Isigkeit AK, Schmucker P. Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids* 1997; **62**: 695–99.
- 26 Vermees 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.
- 27 Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477–88.
- 28 Vassiliadi DA, Dimopoulou I, Tzanela M, et al. Longitudinal assessment of adrenal function in the early and prolonged phases of critical illness in septic patients: relations to cytokine levels and outcome. *J Clin Endocrinol Metab* 2014; **99**: 4471–80.
- 29 Polito A, Sonnevill R, Guidoux C, et al. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoS One* 2011; **6**: e25905.
- 30 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**: e368–75.
- 31 Gibbison B, Angelini GD, Lightman SL. Dynamic output and control of the hypothalamic-pituitary-adrenal axis in critical illness and major surgery. *Br J Anaesth* 2013; **111**: 347–60.
- 32 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.
- 33 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.
- 34 Bornstein SR, Chrousos GP. Clinical review 104: Adrenocorticotropin (ACTH)- and non-ACTH-mediated regulation of the adrenal cortex: neural and immune inputs. *J Clin Endocrinol Metab* 1999; **84**: 1729–36.
- 35 Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends Endocrinol Metab* 2008; **19**: 175–80.
- 36 Bornstein SR, Ehrhart M, Scherbaum WA, Pfeiffer EF. Adrenocortical atrophy of hypophysectomized rats can be reduced by corticotropin-releasing hormone (CRH). *Cell Tissue Res* 1990; **260**: 161–66.
- 37 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**: 900–06.
- 38 Engeland WC, Arnhold MM. Neural circuitry in the regulation of adrenal corticosterone rhythmicity. *Endocrine* 2005; **28**: 325–32.
- 39 Bornstein SR, Breidert M, Ehrhart-Bornstein M, Kloos B, Scherbaum WA. Plasma catecholamines in patients with Addison's disease. *Clin Endocrinol (Oxf)* 1995; **42**: 215–18.
- 40 Merke DP, Chrousos GP, Eisenhofer G, et al. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *N Engl J Med* 2000; **343**: 1362–68.
- 41 Bornstein SR, Tian H, Haidan A, et al. Deletion of tyrosine hydroxylase gene reveals functional interdependence of adrenocortical and chromaffin cell system in vivo. *Proc Natl Acad Sci USA* 2000; **97**: 14742–47.
- 42 Carroll BJ, Iranmanesh A, Keenan DM, Cassidy F, Wilson WH, Veldhuis JD. Pathophysiology of hypercortisolism in depression: pituitary and adrenal responses to low glucocorticoid feedback. *Acta Psychiatr Scand* 2012; **125**: 478–91.
- 43 Petrowski K, Wintermann GB, Kirschbaum C, Bornstein SR. Dissociation between ACTH and cortisol response in DEX-CRH test in patients with panic disorder. *Psychoneuroendocrinology* 2012; **37**: 1199–208.
- 44 Franchimont D, Bouma G, Galon J, et al. Adrenal cortical activation in murine colitis. *Gastroenterology* 2000; **119**: 1560–68.
- 45 Bornstein SR, Zacharowski P, Schumann RR, et al. Impaired adrenal stress response in toll-like receptor 2-deficient mice. *Proc Natl Acad Sci USA* 2004; **101**: 16695–700.
- 46 Kanczkowski W, Alexaki VI, Tran N, et al. Hypothalamic-pituitary and immune-dependent adrenal regulation during systemic inflammation. *Proc Natl Acad Sci USA* 2013; **110**: 14801–06.

- 47 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. *Int J Obes (Lond)* 2007; **31**: 1605–16.
- 48 Ansurudeen I, Willenberg HS, Kopprasch S, Krug AW, Ehrhart-Bornstein M, Bornstein SR. Endothelial factors mediate aldosterone release via PKA-independent pathways. *Mol Cell Endocrinol* 2009; **300**: 66–70.
- 49 Ehrhart-Bornstein M, Lamoumier-Zepter V, Schraven A, et al. Human adipocytes secrete mineralocorticoid-releasing factors. *Proc Natl Acad Sci USA* 2003; **100**: 14211–16.
- 50 Yehuda R. Status of glucocorticoid alterations in post-traumatic stress disorder. *Ann N Y Acad Sci* 2009; **1179**: 56–69.
- 51 Boyar RM, Hellman LD, Roffwarg H, et al. Cortisol secretion and metabolism in anorexia nervosa. *N Engl J Med* 1977; **296**: 190–93.
- 52 Römer B, Lewicka S, Kopf D, et al. Cortisol metabolism in depressed patients and healthy controls. *Neuroendocrinology* 2009; **90**: 301–06.
- 53 Veldhuis JD, Keenan DM, Pincus SM. Motivations and methods for analyzing pulsatile hormone secretion. *Endocr Rev* 2008; **29**: 823–64.
- 54 Voerman HJ, Strack van Schijndel RJ, Groeneveld AB, de Boer H, Nauta JP, Thijs LG. Pulsatile hormone secretion during severe sepsis: accuracy of different blood sampling regimens. *Metabolism* 1992; **41**: 934–40.
- 55 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.
- 56 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. *J Steroid Biochem Mol Biol* 2014; **141**: 16–25.
- 57 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.
- 58 Chan WL, Carrell RW, Zhou A, Read RJ. How changes in affinity of corticosteroid-binding globulin modulate free cortisol concentration. *J Clin Endocrinol Metab* 2013; **98**: 3315–22.
- 59 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.
- 60 Estrada-Y-Martin RM, Orlander PR. Salivary cortisol can replace free serum cortisol measurements in patients with septic shock. *Chest* 2011; **140**: 1216–22.
- 61 Bergquist M, Nurkkala M, Rylander C, Kristiansson E, Hedenstierna G, Lindholm C. Expression of the glucocorticoid receptor is decreased in experimental *Staphylococcus aureus* sepsis. *J Infect* 2013; **67**: 574–83.
- 62 Guerrero J, Gatica HA, Rodríguez 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.
- 63 Indyk JA, Candido-Vitto C, Wolf IM, et al. Reduced glucocorticoid receptor protein expression in children with critical illness. *Horm Res Paediatr* 2013; **79**: 169–78.
- 64 Ledderose C, Möhmler P, Limbeck E, et al. Corticosteroid resistance in sepsis is influenced by microRNA-124-induced downregulation of glucocorticoid receptor- α . *Crit Care Med* 2012; **40**: 2745–53.
- 65 Meduri GU, Muthiah MP, Carratu P, Eltorkey M, Chrousos GP. Nuclear factor-kappaB- and glucocorticoid receptor alpha-mediated mechanisms in the regulation of systemic and pulmonary inflammation during sepsis and acute respiratory distress syndrome. Evidence for inflammation-induced target tissue resistance to glucocorticoids. *Neuroimmunomodulation* 2005; **12**: 321–38.
- 66 Peeters RP, Hagendorf A, Vanhorebeek I, et al. Tissue mRNA expression of the glucocorticoid receptor and its splice variants in fatal critical illness. *Clin Endocrinol (Oxf)* 2009; **71**: 145–53.
- 67 Siebig S, Meinel A, Rogler G, et al. Decreased cytosolic glucocorticoid receptor levels in critically ill patients. *Anaesth Intensive Care* 2010; **38**: 133–40.
- 68 van den Akker EL, Koper JW, Joosten K, et al. Glucocorticoid receptor mRNA levels are selectively decreased in neutrophils of children with sepsis. *Intensive Care Med* 2009; **35**: 1247–54.
- 69 Krone N, Stewart PM. Adrenal function: an overlooked cause of glucocorticoid deficiency? *Nat Rev Endocrinol* 2009; **5**: 362–63.
- 70 Theocharidou E, Krag A, Bendtsen F, Møller S, Burroughs AK. Cardiac dysfunction in cirrhosis—does adrenal function play a role? A hypothesis. *Liver Int* 2012; **32**: 1327–32.
- 71 Selye H. The general adaptation syndrome and the diseases of adaptation. *J Clin Endocrinol Metab* 1946; **6**: 117–230.
- 72 Annane D, Sébille V, Troché G, Raphaël 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.
- 73 Marik PE, Pastores SM, Annane D, et al, and the American College of Critical Care Medicine. 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.
- 74 Marik PE. Critical illness-related corticosteroid insufficiency. *Chest* 2009; **135**: 181–93.
- 75 Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. *Lancet* 2014; **383**: 2152–67.
- 76 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.
- 77 Dellinger RP, Levy MM, Carlet JM, et al, the International Surviving Sepsis Campaign Guidelines Committee, the American Association of Critical-Care Nurses, the American College of Chest Physicians, the American College of Emergency Physicians, the Canadian Critical Care Society, the European Society of Clinical Microbiology and Infectious Diseases, the European Society of Intensive Care Medicine, the European Respiratory Society, the International Sepsis Forum, the Japanese Association for Acute Medicine, the Japanese Society of Intensive Care Medicine, the Society of Critical Care Medicine, the Society of Hospital Medicine, the Surgical Infection Society, and the World Federation of Societies of Intensive and Critical Care Medicine. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008; **36**: 296–327.
- 78 Chung TT, Grossman A, Clark AJL. Adrenal insufficiency. In: Jameson JL, De Groot LJ, eds. *Endocrinology Adult and Pediatric*. 6th edn. St Louis; WB Saunders; 2010: 1853–63.
- 79 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.
- 80 Patel GP, Balk RA. Systemic steroids in severe sepsis and septic shock. *Am J Respir Crit Care Med* 2012; **185**: 133–39.
- 81 Annane D, Sébille 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.
- 82 Sprung CL, Annane D, Keh D, et al, and the CORTICUS Study Group. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111–24.
- 83 Venkatesh B, Myburgh J, Finfer S, et al, and the ANZICS CTG investigators. The ADRENAL study protocol: adjunctive corticosteroid treatment in critically ill patients with septic shock. *Crit Care Resusc* 2013; **15**: 83–88.