Adrenocortical Stress Response during the Course of Critical Illness

Bram Peeters,[1] *Lies Langouche,*[1] *and Greet Van den Berghe*[*1]

ABSTRACT

Critically ill patients have elevated plasma cortisol concentrations, in proportion to illness severity. This was traditionally attributed exclusively to a central activation of the hypothalamus-pituitary axis. However, low rather than high plasma ACTH concentrations have been reported in critically ill patients, 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. Several drugs administered to ICU patients suppress plasma cortisol in a dose-dependent manner.

Sustained low circulating ACTH might contribute to adrenal atrophy and dysfunction in the prolonged phase of critical illness. In the acute phase of sepsis or septic shock, a condition referred to as "relative adrenal insufficiency" has been suggested to ensue from glucocorticoid resistance and insufficiently elevated circulating cortisol to overcome such resistance, with pathological changes possibly occurring at every level of the HPA axis. However, it remains highly controversial whether tissue-specific glucocorticoid resistance is adaptive or maladaptive, how to diagnose "relative" adrenal insufficiency, and how it should be treated. Large RCTs, investigating the effect of 200 mg/d hydrocortisone treatment for sepsis or septic shock have shown conflicting, mainly negative, results. Not taking into account the reduced cortisol breakdown, which increases the risk of overdosing hydrocortisone, might have played a role. Further research on diagnostic, therapeutic and dosing aspects is urgently warranted. *Compr Physiol* 8:283-298, 2018.

Didactic Synopsis

Major teaching points

- Unlike the hypothalamus-pituitary-adrenal axis response to stress outside the context of intensive care, the stress response to critical illness is hallmarked by low rather than high plasma ACTH in the face of high plasma cortisol.
- During critical illness, the diurnal rhythm of ACTH and cortisol secretion is absent.
- A normal or only slightly increased cortisol production and a consistently reduced cortisol breakdown determine the degree of hypercortisolism during critical illness.
- Sustained suppressed circulating ACTH can contribute to risk of adrenal atrophy specifically in prolonged critically ill patients.
- Drugs often given to critically ill patients such as etomidate, opioids and propofol can suppress plasma cortisol. One should consider omitting these before initiating treatment with hydrocortisone for low plasma cortisol.
- It remains controversial whether "relative" adrenal insufficiency is a clinical entity ensuing from glucocorticoid resistance with cortisol availability that is insufficiently elevated to overcome such resistance.

Introduction

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, 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 (137). Both neuronal and endocrine systems are involved, among which the activation of the sympathetic nervous system, the release of catecholamines from the adrenal

[*]Correspondence to greet.vandenberghe@kuleuven.be
[1]Clinical Division and Laboratory of Intensive Care Medicine, Department of Cellular and Molecular Medicine, KU Leuven, Belgium
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medulla, and the activation of the hypothalamus-pituitary-adrenal (HPA) axis to increase the availability of the stress hormone cortisol (32, 144).

Critically ill patients indeed typically present with elevated plasma cortisol concentrations, in proportion to the severity of illness (177). Whereas this elevation in cortisol traditionally has been attributed to a central activation of the HPA axis with an increased ACTH release from the pituitary, low rather than high plasma ACTH concentrations have been reported in critically ill patients (20, 169). Low ACTH together with high cortisol is referred to as "ACTH-cortisol dissociation." ACTH-independent mechanisms, among which direct inflammation-induced adrenocortical cortisol synthesis and release, a reduced cortisol breakdown as well as iatrogenic modulators are considered to explain this dissociation (20, 115). Recent findings have shed new light on the HPA-axis response to critical illness, with potentially important diagnostic and therapeutic implications.

In this article, we will give an overview of the adrenocortical stress response to stress, with a point-by-point discussion on the causes and consequences observed during critical illness. Next, we will discuss potential failure or dysfunction of the HPA axis, and current diagnostic and therapeutic options.

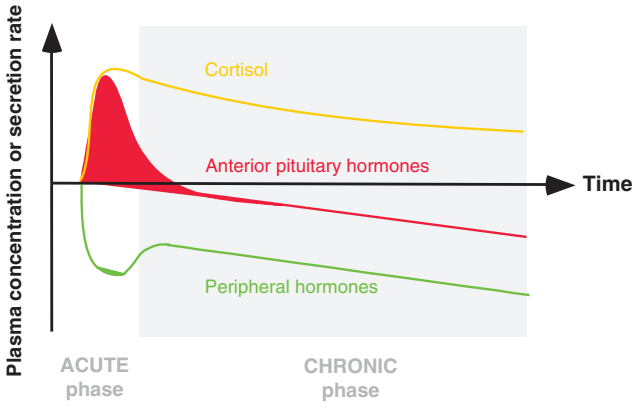

Figure 1 Shown are the biphasic neuroendocrine responses of the anterior pituitary hormones and their peripheral hormones to acute and chronic critical illness. In the acute phase of illness the growth hormone (GH) and thyrotrophin (TSH) secretory activity is amplified (red), and adrenocorticotropic 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 (yellow). 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. *(Figure was reproduced from Van den Berghe (160), with permission from The Journal of Clinical Endocrinology and Metabolism.)*

Acute and Prolonged Critical Illness

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 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 (80). 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 (77).

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 (Fig. 1) (159-163, 166). 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 T3 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 participates in the general wasting syndrome, with persisting hypercatabolism, causing weakness and delayed or non-recovery from intensive care dependency (105, 149).

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 (Fig. 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, 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, which both activate the anterior pituitary gland

to release ACTH. In turn, ACTH release exerts important dose-dependent functions on the cortex of 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 (40). The first line response to stress is thus mediated by the effects of (nor)epinephrine, facilitating immediate physical reactions, such as an increase in heart rate and cardiac output, an improvement of the respiratory function, and an increase in blood glucose and fatty acids. 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 (50).

ACTH-cortisol dissociation during critical illness

The hypothalamic CRH release is the first step in the HPA stress response, but to our knowledge, no data on CRH levels in critically ill patients have been reported. However, CRH is rarely measured in peripheral blood, as these levels do not correlate well with those in the hypothalamic-hypophyseal portal circulation. Also published data on ACTH 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) (96). In burn patients, plasma ACTH levels were found not to be elevated and did not show a correlation with burn size (167). In patients undergoing minor surgical interventions, plasma ACTH increased during surgery and normalized rapidly afterward (99, 100, 158). In a study on more extensive surgery, plasma ACTH was unaltered (174). 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 (59, 130). 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 (169). Also in septic shock patients, baseline plasma ACTH levels were low in comparison with healthy volunteers, independent of the severity of illness (7). In a recent study on a mixed population of 156 critically ill patients, low plasma ACTH concentrations, in comparison with healthy controls, were observed already upon admission to the ICU, with a further lowering from the morning after admission onward (Fig. 2A) (115).

In contrast with ACTH, an increase of its peripheral hormone 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 (177). In burn patients, plasma cortisol concentrations were shown to be elevated in proportion to burn size (167). In patients undergoing surgery, cortisol concentrations also reflect the degree of surgical stress (31, 106, 108, 177). In addition, septic shock induces

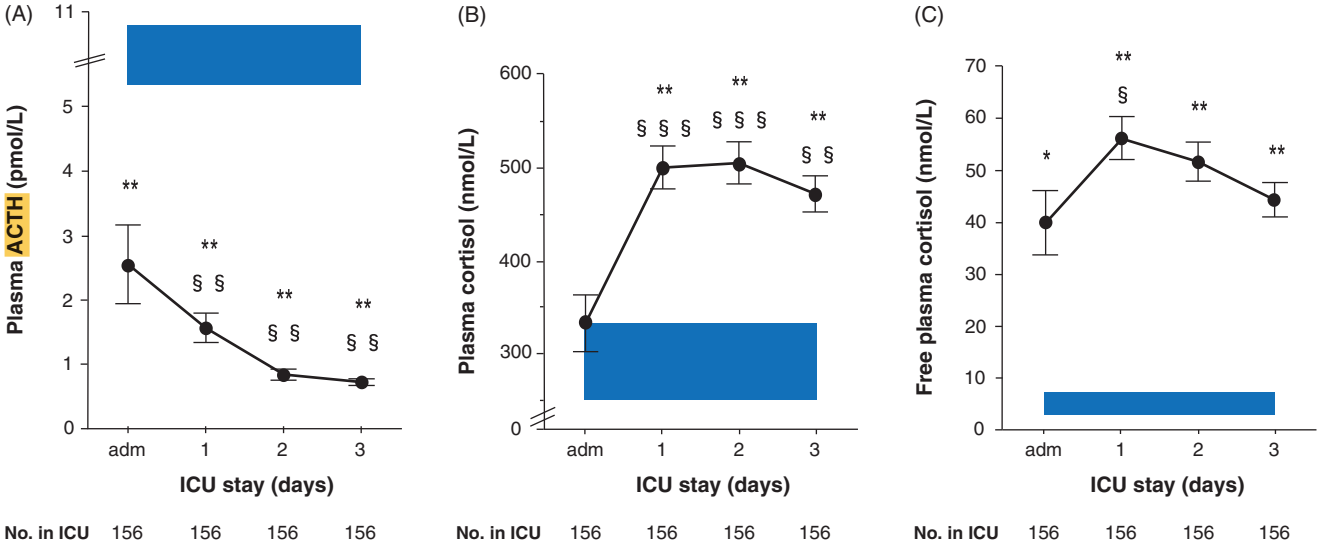

Figure 2 Mean values and standard errors for plasma ACTH (Panel A), total cortisol (Panel B), and free cortisol (Panel C) in ICU patients from admission onward until day 3 of ICU stay. The blue shaded area represents the interquartile range of morning values in healthy control subjects. $^{*}P \leq 0.05$, $^{**}P < 0.001$, for the comparison with controls. $^{\S}P \leq 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.0001$, 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. *(Figure was reproduced from Peeters (115), with permission from Clinical Endocrinology.)*

elevated cortisol levels proportionally to disease severity (9, 38, 104, 131).

The onset of this hypercortisolism is expected to happen instantaneously, facilitating immediate effects. However, the exact timing of the rise of plasma cortisol concentrations during the course of illness is not that clear. In patients undergoing minor or mild 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 (99, 100, 158, 174). 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 (59, 130). In a mixed population of severely ill medical and surgical patients, directly coming from the emergency department, the operating room or from the ward, normal cortisol plasma concentrations upon admission to the ICU were observed, which increased quickly thereafter (Fig. 2B) (115).

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 (20). 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, for example, patients suffering from mild infections or during a COPD exacerbation (36, 37). 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 fivefold 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 (Fig. 3). In the kidney, cortisol can be inactivated to cortisone via 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), and further degraded to tetrahydrocortisone by 5β-reductase. Indeed, Boonen and colleagues showed that expression and activity of the hepatic A-ring reductases

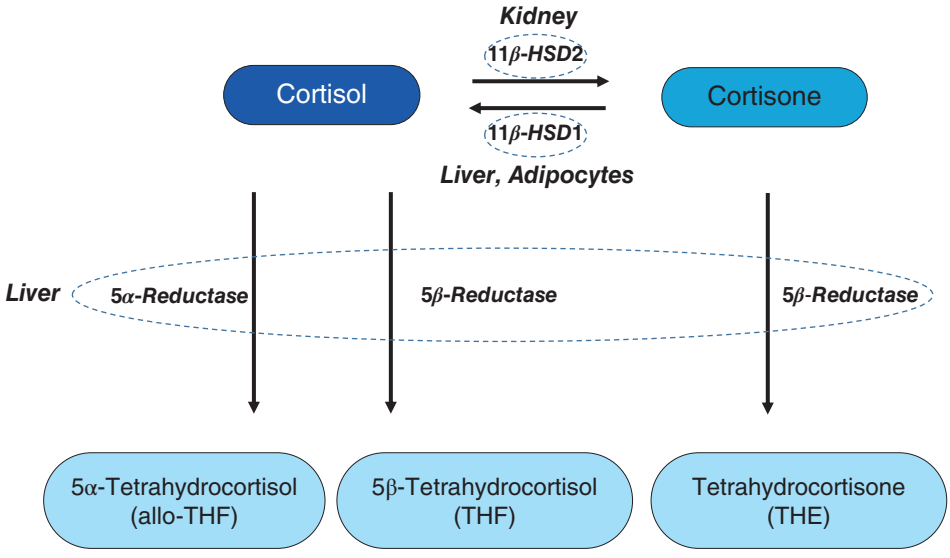

Figure 3 Cortisol metabolism in humans. Cortisol and cortisone are mainly broken down via A-ring reductases, 5α-reductase and 5β-reductase, in the liver to generate 5α- and 5β-tetrahydrocortisol. In the kidney, cortisol is metabolized by 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2, generating cortisone, which can further be broken down to tetra-hydrocortisone (THE) by 5β-reductase. 11β-HSD type 1 can reconvert cortisone to cortisol.

and the 11β-HSD2 activity in kidney were significantly diminished in critically ill patients (20).

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.

Non-ACTH-driven cortisol production?

A dissociation of ACTH and cortisol levels has also been observed in other noncritically ill stress conditions such as depression and anxiety (28, 132). Also in Alzheimer disease patients, high plasma cortisol though normal plasma ACTH levels have been shown (113). In mice, it has been shown that adrenal responsiveness to plasma ACTH is increased with endurance training, chronic stress, or hypoxia (48, 49, 124). Other clinical conditions ranging from metabolic disease, chronic pulmonary disease, and alcoholism, also show a dissociation between plasma ACTH and cortisol (58,126,170). 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 (20, 36, 37) could indeed theoretically be brought about by alternative stimuli, such as cytokines, neuropeptides, or certain adipokines (23). 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 (24). 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 (81). Adipocytes situated in subcutaneous, visceral or periadrenal fat release certain adipokines, which can induce cortisol secretion but also sensitize adrenocortical cells for ACTH (88). 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 (73).

As mentioned above, the sympathetic nervous system and the HPA axis synergistically interact with each other in the complex microenvironment of the adrenal gland (50). 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 (22).

Also an increased ACTH sensitivity, that is, an upregulation of its melanocortin 2 receptor (MC2R), could play a role (23). However, a human study that quantified pulsatile and nonpulsatile 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 (168). 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 (168).

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 nonseptic 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 (121). 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 (117). 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 (69). Thus in patients with systemic infection, free rather than total cortisol correlate with the severity of disease and better reflect biologically active cortisol availability (74). 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 (34).

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 (14). Plasma CBG levels were also transiently decreased following abdominal surgery, with a normalization on postoperative day 2 (46). Also in a mixed population of medical and surgical ICU patients, plasma CBG and albumin were acutely downregulated already upon ICU admission, causing a several fold increase in plasma free cortisol concentrations, in the face of unaltered total plasma cortisol (Fig. 2C) (115). In this set of patients, from the morning after ICU admission, free plasma

concentrations increased further mainly due to the rise in total cortisol levels, and remained high during the following days. In an observational cohort study in patients with sepsis and septic shock, total CBG levels decreased in proportion to disease severity (109). 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 (109). 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 (110). 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 (109).

Cortisol signaling

Local cortisol activity is also further regulated by tissue-specific alterations of glucocorticoid signaling (29, 62). At the levels of the target cells, free cortisol diffuses the cell membrane 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, to exert its effects (165). 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 (62). Other common GR isoforms are GRγ and GR-P. Expression of the GR receptor is normally downregulated by cortisol to maintain homeostasis (43). Cortisol can also bind to the mineralocorticoid receptor (MR) with a 10-fold higher affinity (127). 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 (55).

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 (15, 63, 116, 141). GRβ expression was found to be transiently increased in white blood cells of adult septic patients (63). 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 (141). 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 (75). A suppression of GR expression in white blood cells has also been reported in adult septic patients (63). 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 (116). *In vitro* and animal research indeed suggested increased GR expression in muscle tissue, but decreased GR expression in liver tissue during critical illness (4, 151).

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 (84). Fast feedback exerts negative feedback by inhibiting ACTH and CRH release, and does not influence gene expression or protein synthesis (133). Intermediate feedback inhibits both CRH and ACTH synthesis, and slow feedback involves regulation of pro-opiomelanocortin (POMC) mRNA levels in the pituitary (133). However, the hypothalamic-pituitary feedback regulation appears much more complex than the initially proposed simple closed loop feedback system (176). For example, whereas TSH becomes completely unresponsive to the hypothalamic thyrotropin-releasing hormone when thyroid hormone levels are high (57), CRH can overrule the feedback inhibition on ACTH exerted by high cortisol levels (11, 157). Furthermore, 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 (176).

The sustained high circulating cortisol levels during critical illness could potentially exert negative feedback inhibition at the hypothalamic (CRH) and pituitary (ACTH) level, as such explaining the low plasma ACTH concentrations. This would be similar to the inhibition of ACTH and CRH synthesis and secretion in response to a prolonged exposure (24 h or more) to high doses of exogenous corticosteroids (84, 121). 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 might maintain some degree of ACTH secretion and partially overcome the feedback inhibition (176).

Whether CRH and/or ACTH synthesis and release is suppressed during the various phases of critical illness is however currently unclear. One could speculate that the longer

the feedback inhibition persists, the more CRH and/or 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 (136). 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 neurons and/or its higher regulatory inputs (61). Alternatively, hypoxia or inflammation might also directly damage the pituitary and thereby lower ACTH in the critically ill (21, 120). 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, thereby explaining at least part of the acute "ACTH-cortisol dissociation", as observed already upon admission to the ICU (25, 94, 119). A well-known suppressor of adrenocortical cortisol production, by inhibiting 11-beta-hydroxylase, is etomidate (72). Prolonged etomidate infusion has been shown to be associated with an increased mortality and was therefore abandoned as a sedative from all ICUs (175). A single induction dose of etomidate, however, was not related with an increase in mortality, but still lowered plasma cortisol concentrations (26). Also opioids, frequently used as strong painkillers 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 (1, 5, 123, 129, 140, 153, 154, 174). Furthermore, many other frequently used drugs such as anesthetics and sedatives may have HPA suppressive properties as suggested by animal experiments (47, 86, 112), small interventional studies in surgical and ICU patients (2, 123, 140, 174), and by observational studies in surgical patients (105, 143).

It has been generally assumed that anesthetic drugs may predominantly suppress the stress response via a central inhibition of the HPA axis and of the sympathetic nervous system (25). In a recent study of a mixed population of critically ill patients, however, it was shown that opioids, etomidate, and propofol had a suppressive effect on plasma cortisol, and dobutamine had a stimulatory effect, but none of these drugs independently affected plasma ACTH (115). The associations between these drugs and cortisol levels were independent of the medical or surgical nature of ICU admission, severity of illness, occurrence of sepsis, or other patient characteristics. Other, yet unknown, mechanisms might have suppressed plasma ACTH, which may have hidden any additional central pharmacological suppression on ACTH release, such as negative feedback inhibition exerted by the elevated plasma free cortisol, or inflammation and ischemia at the level of the pituitary or the hypothalamus (23, 44).

From the current available literature, it cannot be concluded whether the suppressive effect of drugs such as opioids, propofol or a single dose of etomidate on plasma cortisol is beneficial or harmful. However, when a patient displays low plasma cortisol during critical illness and treatment with hydrocortisone is considered, it should also be considered if suppressive drugs can be stopped or replaced, in which case plasma cortisol should be reassessed.

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 (68). 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 (149).

Circadian rhythms in physiological processes are ubiquitous in living organisms and rely on a complex system of self-sustained clocks with approximately 24 h periods (111). 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 (173). Moreover, the SCN directly signals the adrenal cortex by a multisynaptic neural pathway (27). 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 (3). 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 (56). 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 (145). This sensitivity is also increased by the SCN, especially during the rising part of the diurnal rhythm, mediated through autonomic pathways (135). 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 (60).

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 assessed 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 (19). 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 (168). 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 (155). Also in adrenalectomized mice, it was shown that mortality strongly increased when sepsis was induced by bacterial endotoxin administration (16).

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) (Fig. 4) (142). 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 (156). 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 (142). STAR is indispensable for cortisol production (93). 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 (150). 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 (150). 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 (91, 92, 95, 142, 150). 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 (53, 139). Finally, ACTH has a direct stimulatory effect on the expression of its own receptor (MC2R) which amplifies the responsiveness to ACTH (89). 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 hypofunction (33, 82). Also in human patients with POMC deficiency, a loss of adrenocortical zonational structure, lipid depletion, reduced ACTH signaling and adrenal atrophy is observed (87). In adrenal glands, harvested postmortem from critically ill patients with an ICU stay of more than 7 days in ICU, 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 (Fig. 5) (18). Normal pulsatile release of ACTH is necessary for transcriptional and behavioral responses (149). 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 (149). Of note, these observations argue against an increased ACTH sensitivity during critical illness. However, the causal relationship between sustained ACTH deprivation, critical illness-associated acquired loss of adrenal function and low plasma cortisol still has to be established.

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 (102). Pro-inflammatory cytokines, such as TNF-α and IL-1, may reduce cortisol synthesis during sepsis by inhibiting stimulatory actions of ACTH (146). Furthermore, ischemia or hemorrhage within the adrenal cortex during severe stress or sepsis can cause changes that impair cortisol production. Also, a decreased blood supply to the pituitary can evoke ischemia, followed by accumulation of nitric oxide and impaired ACTH secretion (30). 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 (164). 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 (35).

Some acute inflammatory conditions, such as sepsis, may become refractory to endogenous hypercortisolemia and exogenous treatment with glucocorticoids, for reasons which are poorly understood (43). It has been suggested that adrenal failure in ICU patients ensues from glucocorticoid resistance and insufficiently elevated circulating cortisol to overcome such resistance (102). Glucocorticoid resistance in peripheral cells could be caused by an increase of the expression

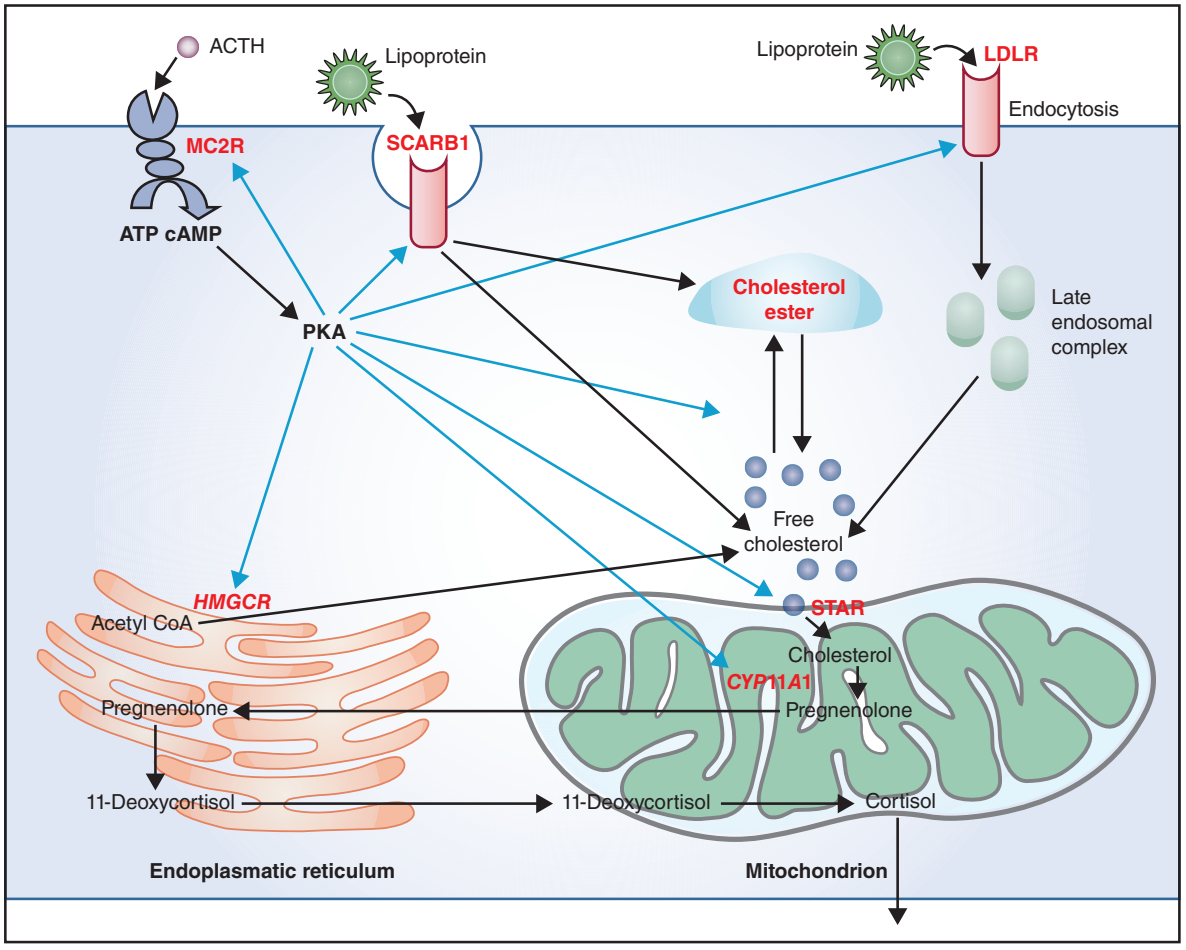

Figure 4 Adrenocorticotropic hormone (ACTH) binds to its receptor, the melanocortin 2 receptor (MC2R), on the membrane of the adrenocortical cells, which increases cyclic AMP (cAMP) and stimulates protein kinase A (PKA). PKA causes the release of cholesterol from the lipid droplets into the cytoplasm 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. *(Figure was reproduced from Boonen (17), with permission from The Lancet Diabetes & Endocrinology.)*

of GRβ, and/or by downregulation of GRα, which could be mediated by micro RNA124 (75, 90). 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 (85). 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, insufficient to cope with the level of stress of septic shock in particular (138). 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 (9). However, the term "relative" adrenal insufficiency is currently quite controversial and many experts now challenge its existence (97).

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 (103). 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 <80 nmol/L), although this can be highly variable (45, 65). Therefore, plasma cortisol concentrations <18 μg/dL or <500 nmol/L upon stimulation with

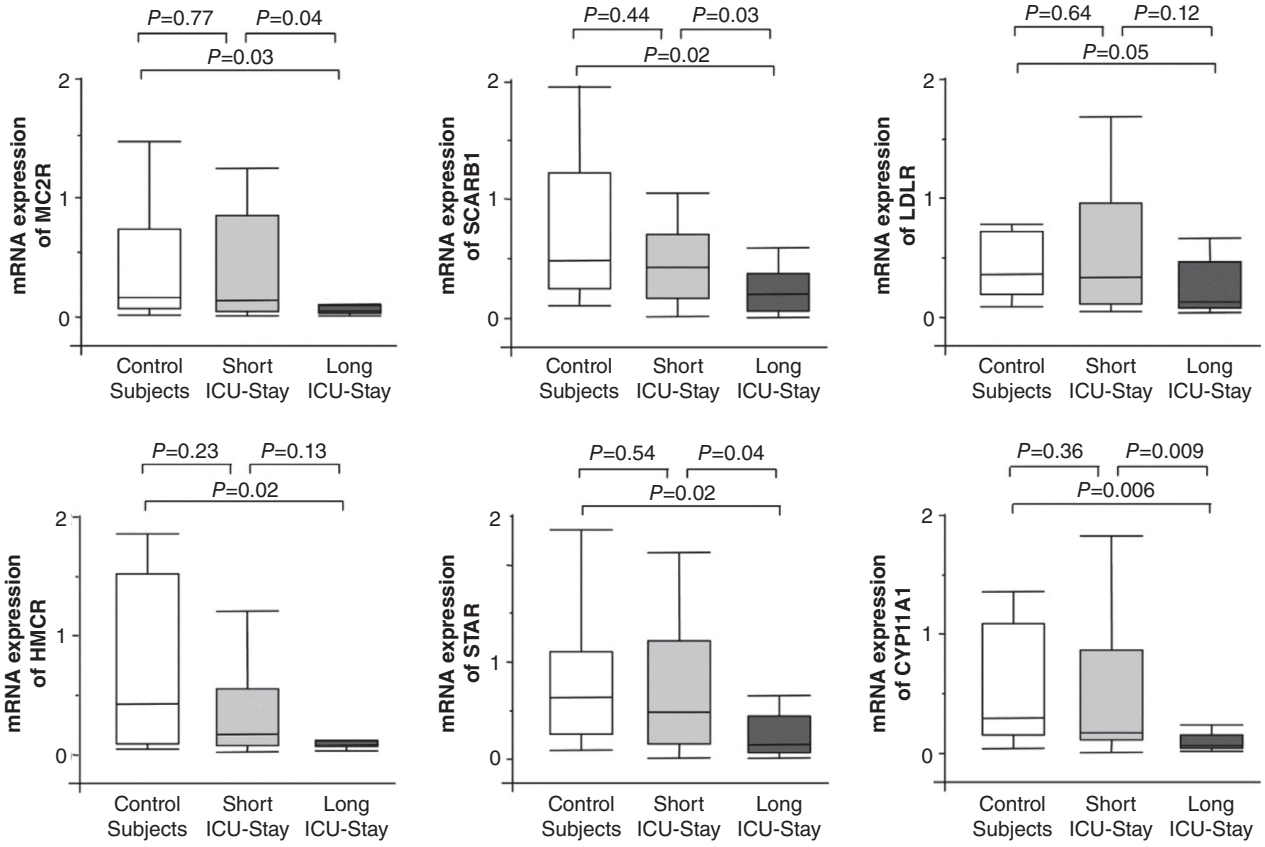

Figure 5 mRNA expression of ACTH-regulated proteins in adrenal glands, harvested from individuals dying suddenly out of hospital (control subjects), from patients dying after short critical illness and from patients after prolonged critical illness. The mRNA data are expressed, normalized to RNA18S as 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. *(Figure was reproduced from Boonen (18), with permission from The Journal of Clinical Endocrinology and Metabolism.)*

250 µg of synthetic ACTH(1-24) (short ACTH stimulation test, Synacthen), are more indicative of adrenal failure (125).

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 (66). 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 Synacthen, indicating adrenal insufficiency, while free plasma cortisol levels were consistently elevated and several times higher than in healthy volunteers (67). Therefore, the free cortisol response to Synacthen 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 (98). 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 (97). 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 (78,79,147). 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 (10, 171). 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 (13, 42, 118). 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 (13, 42). 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 (10, 52, 64, 121). 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 (101). But ACTH-stimulated free plasma and salivary cortisol concentrations increased in parallel in both adult and

pediatric critically ill patients (10, 12). However, also opposing results between free and salivary cortisol were measured in patients with severe sepsis (12, 51) Interestingly, in a study of 28 acutely ill patients with a clinical suspicion of adrenal insufficiency, 13 patients had a similar response to ACTH in peak serum total and salivary cortisol, whereas 15 patients displayed a subnormal serum total cortisol response, but a normal salivary cortisol response (122). 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 an ACTH stimulation test, irrespective of the baseline plasma cortisol. A cortisol increase of less than 9 μg/dL or 240 nmol/L after stimulation with 250 μg of synthetic ACTH(1-24), 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 (9). A low cortisol response to exogenous ACTH was also associated with a higher baseline cortisol and ACTH and with more severe disease and presence of sepsis and septic shock (39, 54). However, in critically ill patients, a low rise in plasma cortisol in response to an ACTH stimulation test was associated both with a low cortisol production rate (but still equal to healthy individuals), and, more importantly, with low clearance of plasma cortisol (20). This suggested that a suppression of cortisol breakdown may explain a reduced cortisol response to ACTH, and actually may reflect the degree of negative feedback inhibition exerted by supranormal cortisol availability. This mechanism is also observed in patients treated with exogenous glucocorticoids, with a lower response to an ACTH stimulation test (134). Therefore, in the presence of increased plasma cortisol and suppressed cortisol metabolism, a reduced cortisol response to ACTH may not necessarily point to an insufficient cortisol availability. Most recent practice guidelines therefore do not advise to use the ACTH stimulation test to guide treatment, in line with the lack of consensus on how to diagnose "relative" adrenal failure in the ICU (128). Clearly, more research on this topic is needed.

Treatment

Evidently, ICU patients suffering from adrenal failure should receive coverage to cope with the stress (41). Currently, it is recommended to treat adrenal failure during critical illness with a bolus of 100 mg hydrocortisone, followed by 50 mg every 6 hours on the first and second day, followed by 25 mg every 6 h on day 3, tapering to a maintenance dose by days 4 to 5. As such, this dose is the equivalent of a several-fold increased daily cortisol production, which is normally around 25 to 30 mg per day.

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 (128). 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 (66). 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 (97, 148). Empiric treatment with hydrocortisone, which results in hemodynamic improvement in some patients, does not assume a previous diagnosis of "relative" adrenal failure. 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 (8). This pioneer study showed reduction in mortality with this treatment only in patients who did not have an incremental cortisol response to synacthen above 9 μg/dl (8). In contrast, a subsequent large European RCT failed to show any mortality benefit with use of hydrocortisone (148). Other smaller RCTs and systematic reviews also generated conflicting results (6, 76, 83, 172). 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 (107, 152). 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 (20), doses of 200 mg hydrocortisone could be too high, and induce side effects such as myopathy, muscle wasting, whereby extending the intensive care dependency (70,71). It is reasonable to assume that lower doses of hydrocortisone, for any indication during critical illness, might actually be sufficient (114). 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 (20). 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 (Fig. 6). Besides total plasma cortisol levels, the dynamics of increased biologically active free plasma

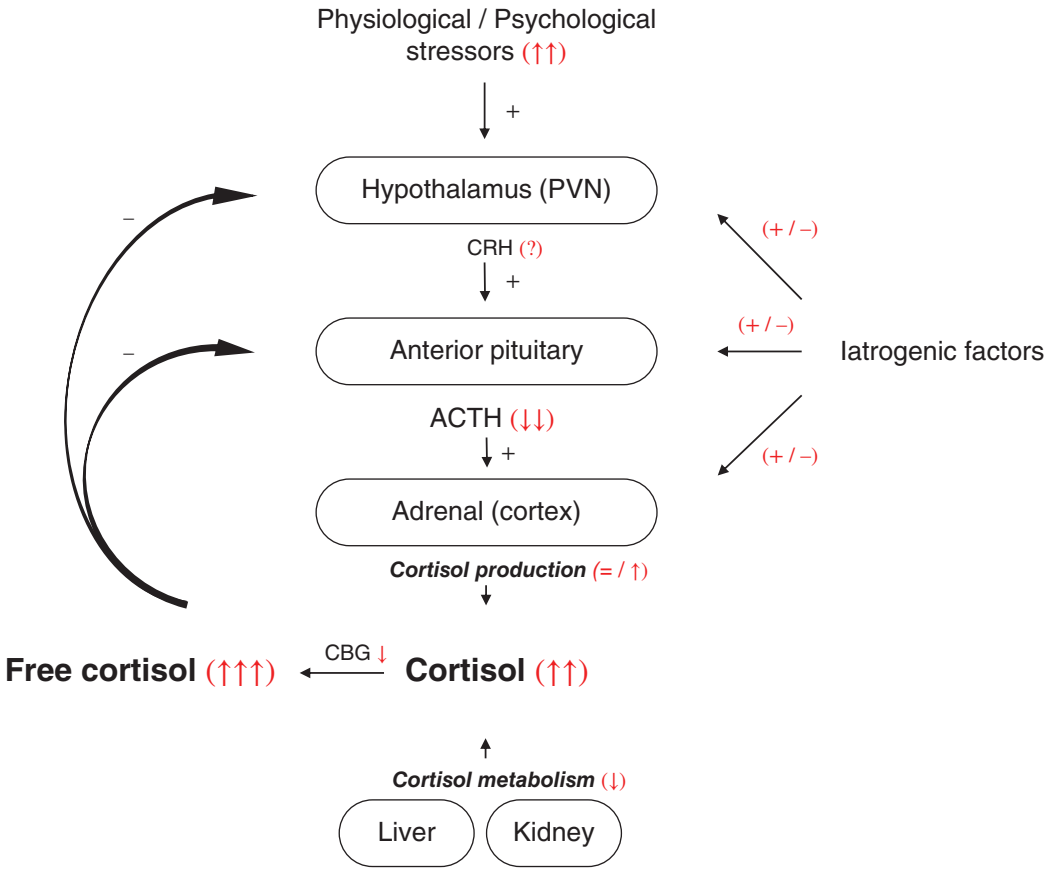

Figure 6 Overview of the regulation of hypercortisolism during critical illness. ↑, elevated plasma concentrations; ↓, decreased plasma concentrations; ?, no univocal data available; +, stimulates; –, inhibits; PVN, paraventricular nucleus; ACTH, adrenocorticotropic hormone; CBG, corticosteroid-binding globulin.

cortisol, and tissue-specific alterations of glucocorticoid signaling, further characterize these changes. Clinicians should be aware that several drugs that are often administered to ICU patients, such as opioids, etomidate, and propofol, suppress cortisol in a dose-dependent manner. 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.

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References

1. Abs R, Verhelst J, Maeyaert J, Van Buyten JP, Opsomer F, Adriaensen H, Verlooy J, Van Havenbergh T, Smet M, Van Acker K. Endocrine consequences of long-term intrathecal administration of opioids. *J Clin Endocrinol Metabol* 85: 2215-2222, 2000.
2. Aitkenhead AR, Pepperman ML, Willatts SM, Coates PD, Park GR, Bodenham AR, Collins CH, Smith MB, Ledingham IM, Wallace PG. Comparison of propofol and midazolam for sedation in critically ill patients. *Lancet* 2: 704-709, 1989.
3. Albrecht U. Timing to perfection: The biology of central and peripheral circadian clocks. *Neuron* 74: 246-260, 2012.
4. Ali M, Allen HR, Vedeckis WV, Lang CH. Depletion of rat liver glucocorticoid receptor hormone-binding and its mRNA in sepsis. *Life Sci* 48: 603-611, 1991.
5. Aloisi AM, Aurilio C, Bachiocco V, Biasi G, Fiorenzani P, Pace MC, Paci V, Pari G, Passavanti G, Ravaioli L, Sindaco G, Vellucci R, Ceccarelli I. Endocrine consequences of opioid therapy. *Psychoneuroendocrinology* 34(Suppl 1): S162-168, 2009.
6. Annane D, Bellissant E, Bollaert PE, Briegel J, Confalonieri M, De Gaudio R, Keh D, Kupfer Y, Oppert M, Meduri GU. Corticosteroids in the treatment of severe sepsis and septic shock in adults: A systematic review. *JAMA* 301: 2362-2375, 2009.
7. 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* 174: 1319-1326, 2006.

8. Annane D, Sebille V, Charpentier C, Bollaert PE, Francois B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troche G, Chaumet-Riffaud P, Bellissant E. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 288: 862-871, 2002.
9. 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* 283: 1038-1045, 2000.
10. 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 Metabol* 92: 2965-2971, 2007.
11. Axelrod J, Reisine TD. Stress hormones: Their interaction and regulation. *Science* 224: 452-459, 1984.
12. Balbao VM, Costa MM, Castro M, Carlotti AP. Evaluation of adrenal function in critically ill children. *Clin Endocrinol* 81: 559-565, 2014.
13. Bartanusz V, Corneille MG, Sordo S, Gildea M, Michalek JE, Nair PV, Stewart RM, Jezova D. Diurnal salivary cortisol measurement in the neurosurgical-surgical intensive care unit in critically ill acute trauma patients. *J Clin Neurosci* 21: 2150-2154, 2014.
14. Beishuizen A, Thijs LG, Vermes I. Patterns of corticosteroid-binding globulin and the free cortisol index during septic shock and multitrauma. *Intensive Care Med* 27: 1584-1591, 2001.
15. 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* 67: 574-583, 2013.
16. Bertini R, Bianchi M, Ghezzi P. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *J Exp Med* 167: 1708-1712, 1988.
17. Boonen E, Bornstein SR, Van den Berghe G. New insights into the controversy of adrenal function during critical illness. *Lancet Diabetes Endocrinol* 3: 805-815, 2015.
18. Boonen E, Langouche L, Janssens T, Meersseman P, Vervenne H, De Samblanx E, Pironet Z, Van Dijck L, Vander Perre S, Derese I, Van den Berghe G. Impact of duration of critical illness on the adrenal glands of human intensive care patients. *J Clin Endocrinol Metabol* 99: 4214-4222, 2014.
19. Boonen E, Meersseman P, Vervenne H, Meyfroidt G, Guiza F, Wouters PJ, Veldhuis JD, Van den Berghe G. Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab* 306: E883-E892, 2014.
20. Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden YM, Spriet I, Wouters PJ, Vander PS, Langouche L, Vanhorebeek I, Walker BR, Van den Berghe G. Reduced cortisol metabolism during critical illness. *N Engl J Med* 368: 1477-1488, 2013.
21. Bornstein SR. Predisposing factors for adrenal insufficiency. *N Engl J Med* 360: 2328-2339, 2009.
22. 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* 127: 900-906, 1990.
23. Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends Endocrinol Metab* 19: 175-180, 2008.
24. Bornstein SR, Zacharowski P, Schumann RR, Barthel A, Tran N, Papewalis C, Rettori V, McCann SM, Schulze-Osthoff K, Scherbaum WA, Tarnow J, Zacharowski K. Impaired adrenal stress response in Toll-like receptor 2-deficient mice. *Proc Natl Acad Sci U S A* 101: 16695-16700, 2004.
25. 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* 45: 1149-1155, 2014.
26. 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* 1: CD010225, 2015.
27. Buijs RM, Wortel J, Van Heerikhuize JJ, Feenstra MG, Ter Horst GJ, Romijn HJ, Kalsbeek A. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. *Eur J Neurosci* 11: 1535-1544, 1999.
28. Carroll BJ, Cassidy F, Naftolowitz D, Tatham NE, Wilson WH, Iranmanesh A, Liu PY, Veldhuis JD. Pathophysiology of hypercortisolism in depression. *Acta Psychiatr Scand Suppl* 115: 90-103, 2007.
29. Chapman K, Holmes M, Seckl J. 11beta-hydroxysteroid dehydrogenases: Intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev* 93: 1139-1206, 2013.
30. Charmandari E, Nicolaides NC, Chrousos GP. Adrenal insufficiency. *Lancet* 383: 2152-2167, 2014.
31. Chernow B, Alexander HR, Smallridge RC, Thompson WR, Cook D, Beardsley D, Fink MP, Lake CR, Fletcher JR. Hormonal responses to graded surgical stress. *Arch Intern Med* 147: 1273-1278, 1987.
32. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol* 5: 374-381, 2009.
33. Coll AP, Challis BG, Yeo GS, Snell K, Piper SJ, Halsall D, Thresher RR, O'Rahilly S. The effects of proopiomelanocortin deficiency on murine adrenal development and responsiveness to adrenocorticotropin. *Endocrinology* 145: 4721-4727, 2004.
34. 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* 26: 197-202, 1987.
35. Cooper MS, Stewart PM. Corticosteroid insufficiency in acutely ill patients. *N Engl J Med* 348: 727-734, 2003.
36. Cornil A, Copinschi G, Leclercq R, Franckson JR. Cortisol secretion during acute bacterial infections in man. *Acta Endocrinol* 58: 1-5, 1968.
37. Cornil A, Glinoer D, Leclerco R, Copinschi G. Adrenocortical and somatotrophic secretions in acute and chronic respiratory insufficiency. *Am Rev Respir Dis* 112: 77-81, 1975.
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* 24: 362-368, 2012.
39. 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* 19: 1, 2015.
40. de Kloet ER, Karst H, Joels M. Corticosteroid hormones in the central stress response: Quick-and-slow. *Front Neuroendocrinol* 29: 268-272, 2008.
41. Debaveye Y, J V, G. VdB. Endocrine emergencies. In: Tubaro M, Danchin N, Filippatos G, Goldstein P, Vranckx P, and Zahger D, editors. *The ESC Textbook of Intensive and Acute Cardiac Care*. New York: Oxford University Press, 2011, pp. 709-717.
42. Dennesen P, van der Ven A, Vlasveld M, Lokker L, Ramsay G, Kessels A, van den Keijbus P, van Nieuw Amerongen A, Veerman E. Inadequate salivary flow and poor oral mucosal status in intubated intensive care unit patients. *Crit Care Med* 31: 781-786, 2003.
43. Desmet SJ, De Bosscher K. Glucocorticoid receptors: Finding the middle ground. *J Clin Invest* 127: 1136-1145, 2017.
44. Deutschman CS, Raj NR, McGuire EO, Kelz MB. Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med* 41: e368-e375, 2013.
45. Dickstein G, Shechner C, Nicholson WE, Rosner I, Shen-Orr Z, Adawi F, Lahav M. Adrenocorticotropin stimulation test: Effects of basal cortisol level, time of day, and suggested new sensitive low dose test. *J Clin Endocrinol Metabol* 72: 773-778, 1991.
46. Dimopoulou I, Tzanela M, Vassiliadi D, Mavrou I, Kopterides P, Orfanos S, Kotanidou A, Kontogiannopoulou S, Vasdekis S, Tsangaris I, Armaganidis A, Macheras A, Ilias I, Kostopanagiotou G, Tsagarakis S. Pituitary-adrenal responses following major abdominal surgery. *Hormones (Athens)* 7: 237-242, 2008.
47. 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* 26: 697-708, 2009.
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* 86: 26-37, 2007.
49. 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* 18: 915-925, 2006.
50. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *EndocrRev* 19: 101-143, 1998.
51. Elbuken G, Karaca Z, Tanriverdi F, Unluhizarci K, Sungur M, Doganay M, Kelestimur F. Comparison of total, salivary and calculated free cortisol levels in patients with severe sepsis. *J Intensive Care* 4: 3, 2016.
52. Estrada YMR, Orlander PR. Salivary cortisol can replace free serum cortisol measurements in patients with septic shock. *Chest* 140: 1216-1222, 2011.
53. 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* 192: 647-658, 2007.
54. Festti J, Grion CM, Festti L, Mazzuco TL, Lima-Valassi HP, Brito VN, Barbosa DS, Carrilho AJ. Adrenocorticotropic hormone but not high-density lipoprotein cholesterol or salivary cortisol was a predictor of adrenal insufficiency in patients with septic shock. *Shock* 42: 16-21, 2014.
55. Fiebeler A, Muller DN, Shagdarsuren E, Luft FC. Aldosterone, mineralocorticoid receptors, and vascular inflammation. *Curr Opin Nephrol Hypertens* 16: 134-142, 2007.
56. Garaulet M, Madrid JA. Chronobiological aspects of nutrition, metabolic syndrome and obesity. *Adv Drug Deliv Rev* 62: 967-978, 2010.

57. Gemsenjager E, Staub JJ, Girard J. Pituitary thyroid recovery following surgery in TRH-unresponsive patients with uni- and multinodular goiter. *Horm Res* 8: 139-147, 1977.
58. 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* 181: 437-444, 2005.
59. Gibbison B, Spiga F, Walker JJ, Russell GM, Stevenson K, Kershaw Y, Zhao Z, Henley D, Angelini GD, Lightman SL. Dynamic pituitary-adrenal interactions in response to cardiac surgery. *Crit Care Med* 43: 791-800, 2015.
60. 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* 296: E888-E897, 2009.
61. 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 Metabol* 77: 173-177, 1993.
62. Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. *Mol Cell Endocrinol* 300: 7-16, 2009.
63. 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* 17: R107, 2013.
64. Gunnala V, Guo R, Minutti C, Durazo-Arvizu R, Laporte C, Mathews H, Kliethermis S, Bhatia R. Measurement of salivary cortisol level for the diagnosis of critical illness-related corticosteroid insufficiency in children. *Pediatr Crit Care Med* 16: e101-e106, 2015.
65. Hagg E, Asplund K, Lithner F. Value of basal plasma cortisol assays in the assessment of pituitary-adrenal insufficiency. *Clin Endocrinol* 26: 221-226, 1987.
66. Hamrahian AH, Fleseriu M, Committee AAS. Evaluation and management of adrenal insufficiency in critically ill patients' disease state review. *Endocr Pract* 23: 716-725, 2017.
67. Hamrahian AH, Oseni TS, Arafah BM. Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 350: 1629-1638, 2004.
68. Hastings M, O'Neill JS, Maywood ES. Circadian clocks: Regulators of endocrine and metabolic rhythms. *J Endocrinol* 195: 187-198, 2007.
69. Henley DE, Lightman SL. New insights into corticosteroid-binding globulin and glucocorticoid delivery. *Neuroscience* 180: 1-8, 2011.
70. Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G. Clinical review: Critical illness polyneuropathy and myopathy. *Crit Care* 12: 238, 2008.
71. Hermans G, Wilmer A, Meersseman W, Milants I, Wouters PJ, Bobbaers H, Bruyninckx F, Van den Berghe G. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. *Am J Respir Crit Care Med* 175: 480-489, 2007.
72. 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* 65: 573-579, 2008.
73. 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* 128: 275-280, 1991.
74. Ho JT, Al-Musalhi H, Chapman MJ, Quach T, Thomas PD, Bagley CJ, Lewis JG, Torpy DJ. Septic shock and sepsis: A comparison of total and free plasma cortisol levels. *J Clin Endocrinol Metabol* 91: 105-114, 2006.
75. Indyk JA, Candido-Vitto C, Wolf IM, Venkataraman S, Munoz R, Saladino RA, Witchel SF, Defranco DB. Reduced glucocorticoid receptor protein expression in children with critical illness. *Horm Res Paediatr* 79: 169-178, 2013.
76. Investigators CS, Annane D, Cariou A, Maxime V, Azoulay E, D'Honneur G, Timsit JF, Cohen Y, Wolf M, Fartoukh M, Adrie C, Santre C, Bollaert PE, Mathonet A, Amathieu R, Tabah A, Clec'h C, Mayaux J, Lejeune J, Chevret S. Corticosteroid treatment and intensive insulin therapy for septic shock in adults: A randomized controlled trial. *JAMA* 303: 341-348, 2010.
77. Iwashyna TJ, Hodgson CL, Pilcher D, Bailey M, van Lint A, Chavan S, Bellomo R. Timing of onset and burden of persistent critical illness in Australia and New Zealand: A retrospective, population-based, observational study. *Lancet Respir Med* 4: 566-573, 2016.
78. Jarek MJ, Legare EJ, McDermott MT, Merenich JA, Kollef MH. Endocrine profiles for outcome prediction from the intensive care unit. *Crit Care Med* 21: 543-550, 1993.
79. 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* 92: 292-295, 1987.
80. Kahn JM, Le T, Angus DC, Cox CE, Hough CL, White DB, Yende S, Carson SS, ProVent Study Group I. The epidemiology of chronic critical illness in the United States*. *Crit Care Med* 43: 282-287, 2015.
81. Kanczkowski W, Alexaki VI, Tran N, Grossklaus S, Zacharowski K, Martinez A, Popovics P, Block NL, Chavakis T, Schally AV, Bornstein SR. Hypothalamo-pituitary and immune-dependent adrenal regulation during systemic inflammation. *Proc Natl Acad Sci U S A* 110: 14801-14806, 2013.
82. 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* 295: E446-E455, 2008.
83. Keh D, Trips E, Marx G, Wirtz SP, Abduljawwad E, Bercker S, Bogatsch H, Briegel J, Engel C, Gerlach H, Goldmann A, Kuhn SO, Huter L, Meier-Hellmann A, Nierhaus A, Kluge S, Lehmke J, Loeffler M, Oppert M, Resener K, Schadler D, Schuerholz T, Simon P, Weiler N, Weyland A, Reinhart K, Brunkhorst FM, SepNet-Critical Care Trials G. Effect of hydrocortisone on development of shock among patients with severe sepsis: The HYPRESS Randomized Clinical Trial. *JAMA* 316: 1775-1785, 2016.
84. Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev* 5: 1-24, 1984.
85. Kleiman A, Hubner S, Rodriguez Parkitna JM, Neumann A, Hofer S, Weigand MA, Bauer M, Schmid W, Schutz G, Libert C, Reichardt HM, Tuckermann JP. Glucocorticoid receptor dimerization is required for survival in septic shock via suppression of interleukin-1 in macrophages. *FASEB J* 26: 722-729, 2012.
86. Kostopanagiotou G, Kalimeris K, Christodoulaki K, Nastos C, Papoutsidakis N, Dima C, Chrelias C, Pandazi A, Mourouzis I, Pantos C. The differential impact of volatile and intravenous anaesthetics on stress response in the swine. *Hormones (Athens)* 9: 67-75, 2010.
87. Krude H, Gruters A. Implications of proopiomelanocortin (POMC) mutations in humans: The POMC deficiency syndrome. *Trends Endocrinol Metab* 11: 15-22, 2000.
88. Krug AW, Vleugels K, Schinner S, Lamounier-Zepter V, Ziegler CG, Bornstein SR, Ehrhart-Bornstein M. 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)* 31: 1605-1616, 2007.
89. 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* 93: 1828-1833, 1994.
90. Ledderose C, Mohnle P, Limbeck E, Schutz S, Weis F, Rink J, Briegel J, Kreth S. Corticosteroid resistance in sepsis is influenced by microRNA-124–induced downregulation of glucocorticoid receptor-alpha. *Crit Care Med* 40: 2745-2753, 2012.
91. 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* 139: 3913-3922, 1998.
92. Lehoux JG, Lefebvre A, Belisle S, Bellabarba D. Hormonal regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in the rat adrenal gland. *J Steroid Biochem* 34: 379-384, 1989.
93. Lin D, Sugawara T, Strauss JF, 3rd, Clark BJ, Stocco DM, Saenger P, Rogol A, Miller WL. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science* 267: 1828-1831, 1995.
94. 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* 57: 452-460, 2013.
95. Liu J, Heikkila P, Meng QH, Kahri AI, Tikkanen MJ, Voutilainen R. Expression of low and high density lipoprotein receptor genes in human adrenals. *Eur J Endocrinol* 142: 677-682, 2000.
96. Livesey JH, Dolamore B. Stability of plasma adrenocorticotrophic hormone (ACTH): Influence of hemolysis, rapid chilling, time, and the addition of a maleimide. *Clin Biochem* 43: 1478-1480, 2010.
97. Loriaux DL, Fleseriu M. Relative adrenal insufficiency. *Curr Opin Endocrinol Diabetes Obes* 16: 392-400, 2009.
98. Loriaux L. Glucocorticoid therapy in the intensive care unit. *N Engl J Med* 350: 1601-1602, 2004.
99. Marana E, Annetta MG, Meo F, Parpaglioni R, Galeone M, Maussier ML, Marana R. Sevoflurane improves the neuroendocrine stress response during laparoscopic pelvic surgery. *Can J Anaesth* 50: 348-354, 2003.
100. 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* 22: 250-255, 2010.
101. Marcus-Perlman Y, Tordjman K, Greenman Y, Limor R, Shenkerman G, Osher E, Stern N. Low-dose ACTH (1 microg) salivary test: A potential alternative to the classical blood test. *Clin Endocrinol* 64: 215-218, 2006.

102. Marik PE. Critical illness-related corticosteroid insufficiency. *Chest* 135: 181-193, 2009.
103. Marik PE, Pastores SM, Annane D, Meduri GU, Sprung CL, Arlt W, Keh D, Briegel J, Beishuizen A, Dimopoulou I, Tsagarakis S, Singer M, Chrousos GP, Zaloga G, Bokhari F, Vogeser M. 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* 36: 1937-1949, 2008.
104. Marik PE, Zaloga GP. Adrenal insufficiency in the critically ill: A new look at an old problem. *Chest* 122: 1784-1796, 2002.
105. Marty J, Gauzit R, Lefevre P, Couderc E, Farinotti R, Henzel C, Desmonts JM. Effects of diazepam and midazolam on baroreflex control of heart rate and on sympathetic activity in humans. *Anesth Analg* 65: 113-119, 1986.
106. McIntosh TK, Lothrop DA, Lee A, Jackson BT, Nabseth D, Egdahl RH. Circadian rhythm of cortisol is altered in postsurgical patients. *J Clin Endocrinol Metabol* 53: 117-122, 1981.
107. 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* 42: 829-840, 2016.
108. Mohler JL, Michael KA, Freedman AM, Griffen WO, Jr., McRoberts JW. The serum and urinary cortisol response to operative trauma. *Surg Gynecol Obstet* 161: 445-449, 1985.
109. Nenke MA, Rankin W, Chapman MJ, Stevens NE, Diener KR, Hayball JD, Lewis JG, Torpy DJ. Depletion of high-affinity corticosteroid-binding globulin corresponds to illness severity in sepsis and septic shock; clinical implications. *Clin Endocrinol* 82: 801-807, 2015.
110. 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 BiochemMol-Biol* 141: 16-25, 2014.
111. Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, Hoffmann MW, Eichele G. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab* 4: 163-173, 2006.
112. 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* 33: 478-486, 2008.
113. 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* 48: 247-254, 2000.
114. Patel GP, Balk RA. Systemic steroids in severe sepsis and septic shock. *Am J Respir Crit Care Med* 185: 133-139, 2012.
115. 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* 86: 26-36, 2017.
116. Peeters RP, Hagendorf A, Vanhorebeek I, Visser TJ, Klootwijk W, Mesotten D, Wouters PJ, Koper JW, de Jong FH, Feelders RA, Lamberts SW, Van den Berghe G. Tissue mRNA expression of the glucocorticoid receptor and its splice variants in fatal critical illness. *Clin Endocrinol (Oxf)* 71: 145-153, 2009.
117. Perogamvros I, Aarons L, Miller AG, Trainer PJ, Ray DW. Corticosteroid-binding globulin regulates cortisol pharmacokinetics. *Clin Endocrinol* 74: 30-36, 2011.
118. Perogamvros I, Keevil BG, Ray DW, Trainer PJ. Salivary cortisone is a potential biomarker for serum free cortisol. *J Clin Endocrinol Metabol* 95: 4951-4958, 2010.
119. Plunkett JJ, Reeves JD, Ngo L, Bellows W, Shafer SL, Roach G, Howse J, Herskowitz A, Mangano DT. 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* 86: 785-796, 1997.
120. Polito A, Sonneville R, Guidoux C, Barrett L, Viltart O, Mattot V, Siami S, Lorindl G, Chretien F, Singer M, Gray F, Annane D, Brouland JP, Sharshar T. Changes in CRH and ACTH synthesis during experimental and human septic shock. *PLoS One* 6: e25905, 2011.
121. Raff H, Biru N, Reisinger N, Kramer DJ. Dissociation of ACTH and cortisol in septic and non-septic ICU patients. *Endocrine* 55: 307-310, 2017.
122. Raff H, Brock S, Findling JW. Cosyntropin-stimulated salivary cortisol in hospitalized patients with hypoproteinemia. *Endocrine* 34: 68-74, 2008.
123. Raff H, Flemma RJ, Findling JW. Fast cortisol-induced inhibition of the adrenocorticotropin response to surgery in humans. *J Clin Endocrinol Metabol* 67: 1146-1148, 1988.
124. 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* 145: 79-86, 2004.
125. 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* 4: 739-769, 2014.
126. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, Walker BR. Tissue-specific dysregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metabol* 86: 1418-1421, 2001.
127. Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P, Schutz G. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* 93: 531-541, 1998.
128. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving sepsis campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive Care Med* 43: 304-377, 2017.
129. Rittmaster RS, Cutler GB, Jr., Sobel DO, Goldstein DS, Koppelman MC, Loriaux DL, Chrousos GP. Morphine inhibits the pituitary-adrenal response to ovine corticotropin-releasing hormone in normal subjects. *J Clin Endocrinol Metabol* 60: 891-895, 1985.
130. Roth-Isigkeit AK, Schmucker P. Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids* 62: 695-699, 1997.
131. Rothwell PM, Udwadia ZF, Lawler PG. Cortisol response to corticotropin and survival in septic shock. *Lancet* 337: 582-583, 1991.
132. 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* 143: 43-50, 2006.
133. Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, Woltersdorf WW, Peeters BW, Ruigt GS, White A, Veldhuis JD, Lightman SL. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci* 30: 6106-6115, 2010.
134. Sacre K, Dehoux M, Chauveheid MP, Chauchard M, Lidove O, Roussel R, Papo T. Pituitary-adrenal function after prolonged glucocorticoid therapy for systemic inflammatory disorders: an observational study. *J Clin Endocrinol Metabol* 98: 3199-3205, 2013.
135. Sage D, Maurel D, Bosler O. Involvement of the suprachiasmatic nucleus in diurnal ACTH and corticosterone responsiveness to stress. *Am J Physiol Endocrinol Metab* 280: E260-E269, 2001.
136. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocr Rev* 7: 284-301, 1986.
137. Selye H. A syndrome produced by diverse nocuous agents. *Nature* 138: 32, 1936.
138. Selye H. The general adaptation syndrome and the diseases of adaptation. *J Clin Endocrinol Metabol* 6: 117-230, 1946.
139. 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 Metabol* 83: 1342-1347, 1998.
140. 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* 13: 14, 2013.
141. Siebig S, Meinel A, Rogler G, Klebl E, Wrede CE, Gelbmann C, Froh S, Rockmann F, Bruennler T, Schoelmerich J, Langgartner J. Decreased cytosolic glucocorticoid receptor levels in critically ill patients. *Anaesth Intensive Care* 38: 133-140, 2010.
142. Simpson ER, Waterman MR. Regulation by ACTH of steroid hormone biosynthesis in the adrenal cortex. *Can J Biochem Cell Biol* 61: 692-707, 1983.
143. 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* 40: 216-222, 2012.
144. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci* 8: 383-395, 2006.
145. Son GH, Chung S, Kim K. The adrenal peripheral clock: Glucocorticoid and the circadian timing system. *Front Neuroendocrinol* 32: 451-465, 2011.

146. Soni A, Pepper GM, Wyrwinski PM, Ramirez NE, Simon R, Pina T, Gruenspan H, Vaca CE. Adrenal insufficiency occurring during septic shock: Incidence, outcome, and relationship to peripheral cytokine levels. *Am J Med* 98: 266-271, 1995.
147. Span LF, Hermus AR, Bartelink AK, Hoitsma AJ, Gimbrere JS, Smals AG, Kloppenborg PW. Adrenocortical function: An indicator of severity of disease and survival in chronic critically ill patients. *Intensive Care Med* 18: 93-96, 1992.
148. Sprung CL, Annane D, Keh D, Moreno R, Singer M, Freivogel K, Weiss YG, Benbenishty J, Kalenka A, Forst H, Laterre PF, Reinhart K, Cuthbertson BH, Payen D, Briegel J. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 358: 111-124, 2008.
149. Stavreva DA, Wiench M, John S, Conway-Campbell BL, McKenna MA, Pooley JR, Johnson TA, Voss TC, Lightman SL, Hager GL. Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. *Nat Cell Biol* 11: 1093-1102, 2009.
150. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev* 17: 221-244, 1996.
151. Sun X, Mammen JM, Tian X. Sepsis induces the transcription of the glucocorticoid receptor in skeletal muscle cells. *Clin Sci (Lond)* 105: 383-391, 2003.
152. 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* 45: 463-472, 2015.
153. Taniguchi H, Sasaki T, Fujita H, Takano O, Hayashi T, Cho H, Yoshikawa T, Tsuburaya A. 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* 10: 1099-1107, 2013.
154. Taylor T, Dluhy RG, Williams GH. Beta-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J Clin Endocrinol Metabol* 57: 592-596, 1983.
155. Ten S, New M, Maclaren N. Clinical review 130: Addison's disease 2001. *J Clin Endocrinol Metabol* 86: 2909-2922, 2001.
156. Toth IE, Szabo D, Bruckner GG. Lipoproteins, lipid droplets, lysosomes, and adrenocortical steroid hormone synthesis: Morphological studies. *Microsc Res Tech* 36: 480-492, 1997.
157. Uchoa ET, Aguilera G, Herman JP, Fiedler JL, Deak T, de Sousa MB. Novel aspects of glucocorticoid actions. *J Neuroendocrinol* 26: 557-572, 2014.
158. Udelsman R, Norton JA, Jelenich SE, Goldstein DS, Linehan WM, Loriaux DL, Chrousos GP. Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. *J Clin Endocrinol Metabol* 64: 986-994, 1987.
159. Van den Berghe G, de Zegher F, Baxter RC, Veldhuis JD, Wouters P, Schetz M, Verwaest C, Van der Vorst E, Lauwers P, Bouillon R, Bowers CY. Neuroendocrinology of prolonged critical illness: Effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone secretagogues. *J Clin Endocrinol Metabol* 83: 309-319, 1998.
160. Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. *J Clin Endocrinol Metabol* 83: 1827-1834, 1998.
161. Van den Berghe G, de Zegher F, Veldhuis JD, Wouters P, Awouters M, Verbruggen W, Schetz M, Verwaest C, Lauwers P, Bouillon R, Bowers CY. The somatotropic axis in critical illness: Effect of continuous growth hormone (GH)-releasing hormone and GH-releasing peptide-2 infusion. *J Clin Endocrinol Metabol* 82: 590-599, 1997.
162. Van den Berghe G, de Zegher F, Veldhuis JD, Wouters P, Gouwy S, Stockman W, Weekers F, Schetz M, Lauwers P, Bouillon R, Bowers CY. Thyrotrophin and prolactin release in prolonged critical illness: Dynamics of spontaneous secretion and effects of growth hormone-secretagogues. *Clin Endocrinol* 47: 599-612, 1997.
163. Van den Berghe G, Wouters P, Weekers F, Mohan S, Baxter RC, Veldhuis JD, Bowers CY, Bouillon R. 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 Metabol* 84: 1311-1323, 1999.
164. 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* 29: 2199-2203, 2003.
165. Vandevyver S, Dejager L, Libert C. Comprehensive overview of the structure and regulation of the glucocorticoid receptor. *Endocr Rev* 35: 671-693, 2014.
166. Vanhorebeek I, Langouche L, Van den Berghe G. Endocrine aspects of acute and prolonged critical illness. *Nat Clin Pract Endocrinol Metab* 2: 20-31, 2006.
167. Vaughan GM, Becker RA, Allen JP, Goodwin CW, Jr., Pruitt BA, Jr., Mason AD, Jr. Cortisol and corticotrophin in burned patients. *J Trauma* 22: 263-273, 1982.
168. Veldhuis JD, Keenan DM, Pincus SM. Motivations and methods for analyzing pulsatile hormone secretion. *Endocr Rev* 29: 823-864, 2008.
169. 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 Metabol* 80: 1238-1242, 1995.
170. Vierhapper H, Nowotny P, Waldhausl W. Production rates of cortisol in obesity. *Obes Res* 12: 1421-1425, 2004.
171. Vining RF, McGinley RA. The measurement of hormones in saliva: Possibilities and pitfalls. *J Steroid Biochem* 27: 81-94, 1987.
172. Volbeda M, Wetterslev J, Gluud C, Zijlstra JG, van der Horst IC, Keus F. Glucocorticosteroids for sepsis: Systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med* 41: 1220-1234, 2015.
173. Walker JJ, Terry JR, Lightman SL. Origin of ultradian pulsatility in the hypothalamic-pituitary-adrenal axis. *Proc Biol Sci* 277: 1627-1633, 2010.
174. Watanabe K, Kashiwagi K, Kamiyama T, Yamamoto M, Fukunaga M, Inada E, Kamiyama Y. High-dose remifentanil suppresses stress response associated with pneumoperitoneum during laparoscopic colectomy. *J Anesth* 28: 334-340, 2014.
175. Watt I, Ledingham IM. Mortality amongst multiple trauma patients admitted to an intensive therapy unit. *Anaesthesia* 39: 973-981, 1984.
176. Watts AG. Glucocorticoid regulation of peptide genes in neuroendocrine CRH neurons: A complexity beyond negative feedback. *Front Neuroendocrinol* 26: 109-130, 2005.
177. Widmer IE, Puder JJ, Konig C, Pargger H, Zerkowski HR, Girard J, Muller B. Cortisol response in relation to the severity of stress and illness. *J Clin Endocrinol Metabol* 90: 4579-4586, 2005.