# ON THE CONTROVERSY OF ADRENAL (DYS)FUNCTION DURING CRITICAL ILLNESS: NEW INSIGHTS

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ABSTRACT (200 words)

Critical illness represents a life-threatening condition necessitating recruitment of defense mechanisms for survival. Herein, the hypothalamus-pituitary-adrenal axis is essential. However, the relevance of a 'relative' insufficiency of the hypothalamus-pituitary-adrenal axis in critical illness, currently diagnosed by a suppressed cortisol response to exogenous ACTH irrespective of the plasma cortisol level, remains controversial.

Several recent studies provided insights that clarify at least part of the controversy. Rather than an activated hypothalamus-pituitary-adrenal axis, ACTH-independent regulators were found to contribute to increased cortisol availability during critical illness. One of these is reduced cortisol breakdown, mediated by suppressed expression and activity of cortisol metabolizing enzymes in liver and kidney. This downstream mechanism elevates plasma cortisol concentrations but the ensuing feedback-inhibited ACTH release, when sustained beyond one week, was shown to negatively affect adrenocortical integrity/function. Reduced adrenocortical ACTH signaling could explain reduced cortisol responses to exogenous ACTH. Whether such reduced cortisol responses in the presence of elevated plasma (free)cortisol always identify adrenal failure requiring treatment is less likely. Also, reduced cortisol breakdown affects the optimal dosing of hydrocortisone treatment during critical illness.

In conclusion, identification of patients with an insufficient hypothalamus-pituitary-adrenal axis response and the optimal treatment for this disorder clearly require more well-designed (pre)clinical studies.

## INTRODUCTION

The human organism is constantly exposed to variable levels of stress exerted by external and internal stimuli.[1] Critical illness is defined as any life-threatening condition that requires support of vital organ function without which death would be imminent. It thus represents physical stress of such a severity and magnitude that it imposes a major challenge to the human body. Coping with such severe stress is mediated by complex endocrine responses. Herein, the hypothalamus-pituitary-adrenal axis (HPA) plays a key role as increased exposure to cortisol is essential to acutely provide energy, to retain fluid, increase cardiac output and blood pressure and to induce an appropriate immune response while protecting against excessive inflammation.[2,3] Failing of this stress response can have rapid and lethal consequences, as is the case for patients with pre-existing adrenal failure who develop an Addisonian crisis when undergoing surgery for example without adequate coverage with hydrocortisone. Absolute and 'relative' adrenal failure also occur during critical illness, most often reported in patients suffering from sepsis.[4] However, the relevance of such a 'relative' adrenal insufficiency during critical illness, currently defined as a suppressed cortisol response to an ACTH injection irrespective of the level of plasma cortisol,[4] remains highly controversial.

Several recent studies provided new insights that clarify part of this controversy. These studies indicated that the HPA-axis stress response to critical illness may differ in several aspects from that to less severe stressors, which may have major consequences for the function of the adrenal glands. In the light of previous understandings, these novel insights will be reviewed. In addition, new research questions will be formulated to redirect future research and to investigate novel treatments for improving outcome of this life-threatening condition.

## SEARCH STRATEGY AND SELECTION CRITERIA

For this review a search was performed in the pubmed database where the terms "HPA-axis", "adrenal gland" "ACTH" and "cortisol" were used in combination with "critical illness" or "sepsis" or "trauma".

The focus was on papers published in the last 5 years. However, since recent papers challenged a classical dogma, a more extensive search of earlier literature was also performed.

Human studies were preferred to animal studies, the latter only included to strengthen human observations or to speculate on future experiments. The reference lists of articles identified by this search strategy were also evaluated for selection of other papers when considered relevant. Review articles and book chapters are cited to guide readers to more detailed information than this review can contain.

## HPA-AXIS ACTIVATION TO ACUTE AND CHRONIC STRESS CONDITIONS: TRADITIONAL CONCEPT

The adrenal gland, a key organ to cope with stress, unites steroid-producing adrenocortical cells and catecholamine-producing chromaffin cells. Stress-induced activation of the HPA-axis starts by the release of corticotrophin-releasing hormone (CRH) from the hypothalamus which, via the hypophyseal portal system, reaches the anterior pituitary corticotrophs to induce ACTH secretion (Panel 1 – background information). ACTH is the main controller of adrenal glucocorticoid production, stimulating steroidogenesis by binding to the melanocortin 2 receptor (MC2R) (Figure 1). The responses within the different components of the HPA axis to several stressors have been studied in the context of major surgery, acute infections, but also chronic infections, autoimmune diseases, the metabolic syndrome and affective and mood disorders. A few older studies measured plasma ACTH concentrations during and shortly after surgery and indeed observed a transiently elevated plasma ACTH concentration that normalized on the first post-operative day.[5, 6] Elevation of the circulating levels of ACTH is thus the typical clinically measurable response to such stressors, which within the adrenal cortex dose-dependently activates MC2R-mediated post-receptor effects.(Figure 1) The adrenal gland is characterized by a remarkable capacity to adapt to acute or chronic stressors. Beside an immediate effect on glucocorticoid production, ACTH also increases the longer-term steroidogenic capacity of the adrenal cells by upregulating proteins

important for steroidogenesis. Furthermore, *in vitro* and *in vivo* animal studies observed structural changes in the adrenal gland such as hyper-vascularization of the already highly vascularized adrenal glands and cellular adrenocortical hypertrophy/hyperplasia.[7] This may in part explain increased vascular vulnerability of the adrenal tissue during acute stress conditions which, when extreme such as with meningococcal sepsis, can evoke adrenal hemorrhage and failure.[8] In addition, as evidenced by CRH injection in rats, HPA axis activation evokes important ultra-structural changes in adrenocortical cells with an increased number of mitochondria and expansion of the smooth endoplasmic reticulum as well as filopodia, and decreased liposomes that are known to store cholesterol, the substrate for glucocorticoid biosynthesis.[9] Furthermore, the adrenal gland has the highest anti-oxidative capacity of all tissues in the human body which appears necessary to cope with the increased production of ROS due to steroidogenesis since mutations in antioxidant defense genes lead to glucocorticoid deficiency.[10]

In conditions of chronic stress, a continued stimulation of the adrenal gland and hereby adrenal hypertrophy can be considered to be an adaptive response essential for the continued provision of cortisol in proportion to the sustained higher requirements of glucocorticoid effects. Inferentially, hyperplasia and nodular transformation of adrenal cortical tissue could result from such chronic hyperstimulation of the adrenal cortex. In this context, it is of interest that patients who suffer from the metabolic syndrome, in particular the form with inflammatory vascular complications, and also patients suffering from depression were shown to have hyperplastic adrenal glands.[11] Patients with metabolic syndrome also present with an increased incidence of adrenal nodules or incidentally discovered adrenal masses.[11] In turn, such adrenal hyperplasia and/or adenomas with manifest or subclinical production of excess steroids, especially cortisol and aldosterone, can contribute to the symptoms and complications of the metabolic syndrome, diabetes, obesity and depression.[11]

In analogy, sustained elevated plasma ACTH and cortisol concentrations could inferrentially result in adrenal hypertrophy and hyperplasia in other conditions of sustained and severe stress, such as prolonged critical illness.

## HPA-AXIS RESPONSE TO CRITICAL ILLNESS : ACTIVATED OR NOT?

As critical illness indeed is an extreme example of sustained and severe physicial stress, one would expect that the elevated plasma concentrations of cortisol are also accompanied by high plasma ACTH concentrations that mediate several-fold increased adrenocortical cortisol production. However, there are only few studies that report plasma concentrations of ACTH during critical illnes. Most of these studies only reported ACTH measured at one single time point, which holds limited information given the pulsatile secretory pattern and the circadian rhythm of the hormone.[4,12-14] Vermes *et al.* reported daily plasma ACTH and cortisol concentrations measured during the first week of critical illness in patients suffering from trauma or sepsis. Acutely elevated plasma ACTH and cortisol concentrations were documented, followed by a steep fall in plasma ACTH after 3 days of critical illness whereas plasma cortisol concentrations remained high.[15] More recently it was shown in a more heterogeneous critically ill patient population that plasma ACTH concentrations were uniformly low, much lower than normal, already from the first day of ICU stay onward, and remained below the lower limit of normality throughout the first week of critical illness.[16,17] This "ACTH-cortisol dissociation" of critical illness is not what one would expect in the context of an activated HPA axis in response to such a high and sustained level of physical stress. Also, it remains unclear what is driving the low ACTH levels in the face of high plasma cortisol. Interestingly, a small study by Polito *et al.* reported reduced *ACTH mRNA* levels in 9 human pituitary glands harvested postmortem from patients who died after septic shock as compared with patients who died suddenly from other diseases, in the absence of a compensatory rise in the expression of CRH or vasopressin in the hypothalamus.[18] Also in experimental models of sepsis, it was recently shown that pituitary ACTH expression levels were suppressed in the more chronic phase of critical illness,[19] which could be evoked by nitric oxide or by suppressed orexin.[18, 19] However, if such sepsis-induced suppression of pituitary ACTH expression were a primary manifestation of organ damage due to shock, this would inferrentially cause abnormally low plasma cortisol concentrations, which in

patients is usually not the case. Another possible explanation could be increased adrenocortical sensitivity to ACTH.[20] However, the adrenocortical cortisol secretory response to any given endogenous plasma ACTH level is normal during critical illness,[21] and in response to exogenous ACTH often low as illustrated by the ACTH stimulation test. Furthermore, a recent study of human adrenal glands showed that ACTH signalling was unaltered during the first week of critical illness, but was severely suppressed in the prolonged phase.[22] Another explanation for the observed high plasma cortisol concentrations, the concomitantly low plasma ACTH levels and subsequently low ACTH-regulated gene expression in the adrenal cortex during critical illness could be negative feedback inhibition exerted by elevated plasma cortisol, in turn evoked by alternative, non-ACTH driven, pathways.

## ALTERNATIVE ACTIVATORS OF THE ADRENAL CORTEX DURING CRITICAL ILLNESS

The dissociation of plasma ACTH and cortisol levels may indeed suggest other ACTH-independent activators of adrenal cortisol production, comprising the sympatico-adrenergic system, the immune system and adipokines.[21,23,24]

The adrenal gland provides a complex microenvironment of close cellular interactions between the two endocrine stress systems, the sympatho-adrenomedullary system and the adrenal cortex.[7] Furthermore, the splanchnic nervous system can directly activate the neuro-adrenocortical axis. In addition, there is a close interaction between adrenocortical cells and resident macrophages, blood immune cells and the vasculature.(Figure 2) It has been previously shown that CRH can activate the sympatho-adrenomedullary system explaining its ability to prevent adrenocortical atrophy in animals with hypophysectomy.[25] The chromaffin cells may play a key role as it was shown in co-culture systems that the addition of chromaffin cells to adrenocortical cells increase the release of cortisol up to 10-times.[26, 27] Vice versa, intra-adrenal glucocorticoids are known to induce expression of catecholaminergic enzymes, particularly phenylethanolamine N-methyltransferase, and to stimulate catecholamine release from chromaffin cells.[7] Exogenous glucocorticoids can induce adrenal atrophy through negative feedback

inhibition of ACTH, inducing low levels of intra-adrenal cortisol which, in turn, can lead to a decline in adrenal catecholamine release. This is further supported by patients with Addison's disease or congenital adrenal hyperplasia, who present with low circulating adrenaline. Adrenomedullary dysfunction in these patients was shown to correlate with cardiovascular instability and hypoglycemia.[28, 29] This close functional interdependence of the two endocrine systems within the adrenal gland is further corroborated by the *in vivo* phenotype of knock-out animals with specific defects in either system and of patients with defects either in the function of the adrenal cortex or medulla.[29,30] In patients with mental diseases such as depression, who present with hypercortisolism, it was recently shown that classical feed-forward overdrive and impaired feedback theories of hypercortisolemia may not apply and that the hypercortisolemia of depression may result from alternative mechanisms involving irregular basal hypersecretion of cortisol possibly driven by splanchnic sympathetic activation.[31,32]

In models of inflammatory bowel disease and viral infection, immune mediators such as interleukin-6 and other ACTH-independent immune-adrenal pathways have been identified to account for a chronic hyperstimulation of the adrenal cortex in the absence of an elevated pituitary ACTH secretion. Hence, numerous studies have reported a "dissociation" between ACTH and cortisol release, both in physiological stress responses and in pathophysiology. These include fetal and early postnatal life stress, aging, inflammation and infection, mental disorders, Alzheimer's disease, chronic pulmonary disease, bone fractures, alcoholism and metabolic diseases.[24] It was further shown recently that not only cytokines and immune mediators released from macrophages, monocytes or other immune cells may directly stimulate (or block) cortisol release from human adrenocortical cells, but that also a direct interaction exists between viral or bacterial pathogens and the adrenocortical cells.[33] Adrenocortical cells express toll-like receptors (TLR) which can directly respond to the presence of gram-negative or gram-positive bacterial pathogens. However, a detailed analysis of the mechanisms of hypothalamic-pituitary and immune-dependent adrenal regulation during systemic inflammation in genetically modified mice models suggested that the primary activation of the HPA axis in such conditions seems to occur via immune cells.[34] Indeed, it was

shown that TLR signaling in immune cells, but not in adrenocortical cells, mediates LPS-induced adrenal inflammation and HPA axis stimulation.[35]

Finally, adipokines released from adipose tissue as well as neuropeptides and immune mediators secreted from endothelial cells, including the production of local morphogens, such as sonic hedgehog and WNT, have been implicated in an ACTH-independent activation of adrenal cortisol regulation.[35-37] Whatever the driver during life-threatening critical illnesses in patients, cortisol production is inferred to be substantially increased.

## CORTISOL PRODUCTION AND METABOLISM DURING CRITICAL ILLNESS

Although it is indeed generally accepted that cortisol production rate is at least several-fold increased to generate and maintain hypercortisolemia during critical illness, it was never quantified in patients until recently. Boonen *et al.* documented that morning cortisol production rate, quantified by the stable isotope infusion technique, was only moderately increased, less than doubled (Figure 3-A), in critically ill patients suffering from the systemic inflammation response syndrome (SIRS) and unchanged in critically ill patients without SIRS as compared with the cortisol production rates of healthy matched control subjects, in the face of several-fold higher plasma total and free cortisol levels in all patients.[16] This finding was quite unexpected. The stable isotope technique also allowed to quantify cortisol plasma clearance, which was found to be suppressed to less than half in all patients, irrespective of the inflammation status (Figure 3-B). With 3 subsequent studies this finding was further explored.[16] Cortisol half-life and plasma clearance during critical illness was also quantified after the administration of a 100 mg bolus of hydrocortisone. Similar results were obtained: plasma cortisol clearance reduced to 40% of that in matched control subjects, and a cortisol half-life that was a median 5-fold longer in patients. This reduced cortisol breakdown was explained by reduced expression and activity of the cortisol metabolizing enzymes, predominantly the A-ring reductases in liver and 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) in kidney, as suggested by urinary steroid ratios, tracer kinetics and assessment of human liver-biopsy

samples. Importantly, the reduced cortisol clearance was robustly observed in all tested critically ill patients, irrespective of type and severity of illness and irrespective of ICU stay and prognosis.[16] This suggested that a pronounced suppression of cortisol breakdown may be a key mechanism which, together with the ongoing normal or slightly elevated cortisol production, contributes to increased plasma cortisol in the sustained severe stress condition of critical illness. Interestingly, a reduced cortisol breakdown was also described in patients with anorexia nervosa, posttraumatic stress disorders and depression,[38-40] suggesting that it could be a more fundamental part of the general stress response.

With the knowledge that cortisol half-life and plasma clearance is uniformly reduced during critical illness, further study of ACTH and cortisol secretion rates and the interaction between ACTH concentrations and cortisol secretion was performed by constructing time series of plasma concentrations measured every 10 minutes over 9 nocturnal hours in critically ill patients and in healthy control subjects.[21] Such plasma concentration time series can be transformed into hormonal secretion profiles with use of deconvolution analysis, that takes into account elimination half-life of the hormone, and which allows to quantify pulsatile and non-pulsatile (basal) secretion of the hormone.[41] Two older studies had previously evaluated repeated blood samples of ACTH and cortisol to assess pulsatile secretion during surgery and critical illness, but these did not apply deconvolution analysis and did not take into account the fact that cortisol half-life is much longer than normal.[6,42]

The recent study revealed that both nocturnal ACTH and cortisol pulsatile and total secretion rates were reduced in critically ill patients, explained by reduction of the hormonal pulse masses while pulse frequencies were unaltered.[21] Interestingly, the dose-response between a given ACTH concentration and cortisol secretory response was preserved normal, which suggested that the term 'ACTH-cortisol dissociation' may not be entirely correct. Indeed, the cortisol secretion was still "connected" to the amount of circulating ACTH, but *both* were suppressed, not increased, in the critically ill patients in the presence of high total and free plasma cortisol concentrations. Hence, beyond the very acute phase of critical illness, high nocturnal plasma cortisol concentrations seem to be predominantly maintained by reduced

cortisol breakdown, a conclusion that was further strengthened by the increased markers of irregularity for both ACTH and cortisol time series in this study. Together, the results of the nocturnal deconvolved ACTH and cortisol secretion study and the results from the stable isotope study, which had shown that daytime cortisol secretion is ACTH-independent and not even double that of healthy subjects, it appears that overall 24h cortisol production rates during critical illness may not be, or at best only moderately, higher than during health.

## CORTISOL EFFECT AT TISSUE LEVEL DURING CRITICAL ILLNESS

Elevated plasma cortisol concentrations during critical illness do not necessarily mean that there is increased cortisol receptor activation at the level of the many tissues that express these receptors. Indeed, 90% of the total cortisol concentration in circulation is bound to corticosteroid binding globulin (CBG). Therefore, changes in binding of cortisol to CBG may influence availability of free cortisol, the form that is responsible for the biological and clinical effect of the endogenous hormone. In previous work it has been demonstrated that CBG levels are significantly decreased in patients in the early stage of septic shock and multiple trauma.[43] This results in much higher free than total circulating cortisol levels and suggests that CBG plays an important role in the regulation of cortisol availability to the target tissues during the severe stress of critical illness.[43] Furthermore, CBG in plasma occurs in two forms, the intact CBG and CBG which is cleaved by neutrophil elastase. The latter has a lower affinity for cortisol. Cleavage by neutrophil elastase increases the concentration of free cortisol at the site of neutrophil action, which may target an increased cortisol bioavailability to sites of interest during critical illness.[44,45] Furthermore, CBG binding affinity is decreased by high body temperature.[46]

Free cortisol has been proposed to be a better parameter for assessing hypercortisolemia in critical illness, particularly in patients with systemic infection, since it provides a better correlation with the severity of disease.[47] Salivary cortisol levels have been proposed as a possible surrogate of the circulating free cortisol levels in patients with septic shock.[48] However, difficulties to adequately sample enough saliva

without contamination via the local conversion of cortisol to cortisone through expression of 11β-HSD2 in the salivary gland, limit the use of this technique.

The real meaning of any form of measurable cortisol in the circulation during critical illness should be evaluated in the light of regulated glucocorticoid receptor (GR) expression and signaling. Increasing evidence from both animal and human experiments suggests that alternative splicing of the GR mRNA, GR expression, GR affinity and GR translocation are regulated and could be tissue specific during critical illness.[49-56] Children with critical illness due to sepsis and traumatic brain injury have shown to exhibit lower total and cytoplasmatic GR levels in white blood cells than healthy controls.[51] Other suggested mechanisms of corticosteroid resistance during critical illness involve an increased expression of glucocorticoid receptor β, the dominant negative isoform of the receptor [51] and by downregulation of glucocorticoid receptor α, mediated by micro RNA124.[52] Also reduced translocation to the nucleus or the presence of less functional polymorphisms may play a role. However, the expression of GR in different tissues warrants more research in critically ill patients and the clinical relevance of these cellular changes remains to be further elucidated.

## WHEN THE ADRENAL GLAND FAILS TO RESPOND ADEQUATELY TO CRITICAL ILLNESS

### *Absolute Adrenal failure during critical illness*

During critical illness, "absolute" adrenal failure can be present for 2 reasons: 1) known primary or secondary adrenocortical insufficiency for example due to autoimmune Addison's disease, pituitary tumors or trauma and 2) critical illness-associated acquired loss of adrenal function. For patients suffering from either of these conditions, appropriate and immediate diagnosis and treatment is essential to prevent life threatening shock.

The acquired loss of adrenal function during critical illness may have several causes. It may be due to hemorrhage within the adrenal gland, to adrenocortical ischemia or apoptosis, or to the effect of certain

drugs that interfere with and impair cortisol production. Alternatively, the ACTH suppression observed beyond the very acute phase of critical illness appears to have important negative consequences on integrity and function of the adrenal gland of critically ill patients, predominantly in the prolonged phase of illness where these ACTH-effects within the adrenal cortex were found to be reduced.[22] Furthermore, increasing evidence emphasizes the importance of hormonal pulsatility in maintaining normal cellular function in both the adrenal gland and the target tissues of cortisol, by preventing desensitization of transcriptional responses. Both pulsatile ACTH secretion and cortisol secretion are shown to be reduced in critical illness.[21] One could speculate that this may further contribute to loss of the trophic ACTH impact on the adrenal gland and to tissue specific cortisol resistance. This should be further investigated. It is equally essential to look for other predisposing factors for adrenal dysfunction in intensive care patients.[57] There is a growing number of patients who may have underlying disorders or use medication that cause a subclinical form of adrenocortical impairment that can become clinically relevant during the severe stress of critical illness. For example, the number of individuals in the aging population who are receiving some forms of chronic exogenous glucocorticoid treatment is rising, which may lead to hypotrophy of the adrenal cortex. In addition there are congenital abnormalities, polyglandular autoimmune disorders, trauma, infectious diseases, coagulation disorders, liver diseases, mental disorders, certain non-steroid medications and addictions that need to be considered in relation to potential predisposing factors for adrenal insufficiency in patients with critical illness.[58,59] Clinicians should thus be aware of these underlying conditions or disorders and predisposing factors to rapidly identify patients at risk for developing life-threatening adrenal failure during critical illness.

*Relative adrenal failure during critical illness*

Already in 1946, Hans Selye suggested that ‘exhaustion’ of the adrenal cortex may occur in certain stress conditions. ”Relative adrenal failure” is a term that was proposed to describe such a state during critical illness, in which plasma cortisol concentrations, although still higher than during health, are insufficiently high to cope with the stress level of the disease.[60] In this concept, the adrenal gland is functionally normal

and maximally activated which still does not suffice to face the challenge. More recently the term 'critical illness-related corticosteroid insufficiency' (CIRCI) [61] was introduced to comprise 'relative failure' which may occur at any level of the HPA-axis.[61,62]

Despite the extensive literature on this topic, the presence of this condition and the underlying mechanisms of such failure still remain debated. Pro-inflammatory cytokines are suggested to play an important role by inducing tissue resistance or competing with ACTH at the receptor level. Target tissue resistance during critical illness can also be explained by decreased glucocorticoid delivery or decreased glucocorticoid action due to an altered function of CBG or to altered glucocorticoid receptor levels and affinity. Furthermore, impaired blood supply to the pituitary can induce subtle levels of pituitary ischemia, which is followed by the accumulation of nitric oxide or central neuropeptides, leading to decreased hormone secretion.[63] Additionally, since every adrenal cell is in direct contact with an endothelial cell, the adrenal cortex is susceptible to hemorrhage during severe stress or sepsis, which can result in full blown Addisonian like crisis, but also more subtle changes that could cause a "relatively" impaired cortisol production. Finally, different neuropeptides, oxidative stress, substrate deficiency due to low circulating cholesterol [64] or interfering medications are also suggested to play a role in reducing the ability to produce cortisol.[57]

*Diagnostic criteria*

Given the controversy about the underlying mechanisms of this 'relative' adrenal insufficiency, the appropriate diagnostic criteria and treatment have also not been settled. Suggested diagnostic criteria were based on findings from a landmark study by Annane *et al.* who identified a plasma cortisol incremental response of <9 μg/dl after injection of 250 μg ACTH and a high baseline cortisol level (>34 μg/dl) as most discriminative to identify patients at high risk of death.[60] Hence, relative adrenal failure during critical illness was from then on diagnosed by a subnormal plasma cortisol incremental response to exogenous ACTH, irrespective of the level of plasma cortisol.[4] However, other investigators have not all been able to replicate the original observations by Annane and thus there is currently no consensus

on how to diagnose adrenal failure in the ICU. Intriguingly, the non-ACTH drivers of cortisol production and alterations in cortisol breakdown could explain reduced cortisol responses to ACTH injection, but do not support the exclusive interpretation of adrenal failure, as long as plasma (free) cortisol is several-fold higher than normal.[16] This is in line with recent guidelines on the topic, which no longer advise to use the ACTH test to guide treatment with hydrocortisone.[65] Also, a dose of 250 μg of ACTH leads to supra-physiologic ACTH levels and could therefore overcome any ACTH resistance. As an alternative, a 1 μg stimulation dose was suggested, but has not been extensively studied in critically ill patients and results have been conflicting. A random total cortisol of <10 μg/dl during critical illness has also been suggested for the diagnosis of CIRCI.[61] However, total plasma cortisol concentration is the net result of adrenal production and secretion, distribution, binding and elimination of cortisol. Also, as cortisol is secreted in a pulsatile manner[20] it could be problematic to judge the adequacy of the adrenal cortisol production in response to critical illness merely by a single measurement of total plasma cortisol. Furthermore, as mentioned, total plasma cortisol concentrations do not quite reflect glucocorticoid signaling. Taken together, and given that all the changes that occur during critical illness could in part be adaptive or instead maladaptive, it remains difficult to conclude on 'adequacy' of cortisol availability during illness based on any of these tests.

*Therapeutic consequences*

Patients with an established diagnosis of primary or secondary adrenal failure or patients on chronic treatment with systemic glucocorticoids prior to critical illness should receive additional coverage to cope with the acute stress.[66,67] Today, such patients in the ICU receive quite high doses of glucocorticoids, based on the assumption that cortisol production is several-fold increased in critical illness. This assumption may not be correct. The current treatment strategy consists of the administration of a bolus of 100 mg of hydrocortisone followed by 50 to 100 mg every 6 hours on the first day, 50 mg every 6 hours on the second day, and 25 mg every 6 hours on the third day, tapering to a maintenance dose by the

fourth to fifth day.[66,67] This dosing regimen could be too high, in the light of the now documented reduced cortisol breakdown during critical illness.[16]

Whether or not 'relative' adrenal failure should be treated with exogenous glucocorticoid 'substitution' therapy and in that case, with which doses, remains even less clear. In current practice, some intensivists may use hydrocortisone in septic patients who fail to respond adequately to vasopressors and/or volume loading.[65] However, a recent systematic review that could only withhold six high quality RCTs concluded that hydrocortisone therapy does not reduce mortality of severe sepsis.[68] This is mainly because the two largest randomized controlled studies generated conflicting results.[69,70] Currently, another well-powered study is recruiting patients and aims to investigate the effect on 90-day mortality of 200 mg hydrocortisone therapy per day for maximum 7days in 3800 patients.[71]

However, since it is now known that cortisol production is at most only moderately increased in critically ill patients with a well-functioning HPA axis, and as cortisol breakdown is substantially and robustly reduced in those patients, the therapeutic doses of 200 mg hitherto used for these trials may have been too high, which could have induced side effects that could abrogate any potential benefit. Therefore, future studies should evaluate whether lower doses can be used to raise plasma cortisol to levels that are sufficient to obtain the targeted effects while minimizing adverse effects of excessive doses. A dose of approximately 60 mg of hydrocortisone, equivalent to about a doubling of the normal daily cortisol production as quantified with stable isotopes might be an interesting alternative for further investigation. Furthermore, the novel insight that cortisol half-life is so much longer during critical illness than during health also has implications for treatment of ICU-patients with steroids for other indications. In general a tapering down as soon as possible should be advised to limit the adverse effects of excessive amounts of glucocorticoids during critical illness.

## FUTURE RESEARCH

More research is clearly needed to investigate appropriate diagnosis and optimal substitution therapy for adrenal failure based on the recent novel insights. The impact of sustained high cortisol concentrations, endogenous or iatrogenically induced, at the level of hypothalamus, pituitary and cortical brain functions, both in relation to acute delirium and to long-term sequellae of critical illness, warrants further investigation. Furthermore, the HPA axis response to sepsis and inflammation will have to be studied in appropriate preclinical models allowing a tissue-specific dissection of the role of the immune system, vasculature and endocrine cells. Also identifying the underlying mechanisms of the reduced expression and activity of the cortisol metabolizing enzymes in liver and kidney is of high priority.

## CONCLUSION

Recent new evidence suggests that, beyond the first hours after onset of critical illness, an activated HPA axis is not the main driver of the essential increase in cortisol availability (Figure 4, Panel 2 – key messages). Although non-ACTH drivers of cortisol production may be involved, increased cortisol exposure during critical illness does not appear to be regulated primarily by cortisol production. Instead, reduced cortisol metabolism during critical illness substantially contributes to elevated cortisol availability. Elevated plasma cortisol via reduced cortisol breakdown is comparable to the condition of exogenous treatment with (high dose) hydrocortisone. In both these conditions, the suppressed endogenous ACTH signaling could explain reduced cortisol responses to exogenous ACTH. These reduced cortisol responses to ACTH thus do not necessarily indicate (relative) adrenal failure requiring treatment, as long as plasma (free) cortisol is several-fold higher than normal. The reduced cortisol breakdown should also be taken into account for adequate dosing of hydrocortisone for any indication during critical illness.

More well-designed (pre)clinical studies are needed to better identify patients with critical illness-induced adrenal failure and to define the optimal treatment for this disorder.

Panel 1 - ADDITIONAL BACKGROUND

Whenever stress occurs, the stress signal activates the hypothalamus to release corticotropin releasing hormone (CRH). CRH reaches via the hypophyseal portal system the anterior pituitary corticotrophs to induce secretion of ACTH. Consequently, the release of ACTH causes cortisol production in the adrenal gland. ACTH is the main controller of adrenal glucocorticoid production and release; it stimulates steroidogenesis by binding to its receptor, the melanocortin 2 receptor (MC2R) present in the cell membrane of the adrenocortical cells and which, when activated, stimulates adenylate cyclase. ACTH upregulates the expression of its own receptor, mediates the release of cholesterol from the lipid droplets while increasing the expression of genes encoding the proteins for cholesterol uptake [such as the LDL-receptor (LDLR) and scavenger-receptor class B member 1 (SCARB1)] and for cholesterol synthesis [via 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)]. ACTH also increases expression of genes encoding key steroidogenic enzymes, such as steroidogenic acute regulatory protein (STAR) and cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1). Besides this feed-forward activation of cortisol secretion, feed-back inhibition of CRH and ACTH by cortisol regulates its own release. This negative feedback inhibition occurs both at the pituitary and the hypothalamic level and involves fast and delayed forms of inhibition circuits. Under healthy resting conditions, ACTH and cortisol are released in a tightly coupled pulsatile fashion following a characteristic circadian rhythm. The normal patterns in the early morning and afternoon hours display quite some inter-individual variation, which is further modified by sleep, any shift in light-dark responses, feeding, and by physical as well as mental stress or illnesses.

In the circulation, cortisol is predominantly transported bound to cortisol binding globulin. Only free cortisol can enter the cell. In certains cells, cortisol can be inactivated into cortisone via 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), which can be again activated via 11β-HSD1 into cortisol. Cortisol and cortisone are metabolized via A-ring reductases predominantly in the liver.

Only cortisol can bind to the glucocorticoid receptor (GR) or the mineralocorticoid receptor (MR) to exert its function by binding in the nucleus to DNA or exert non-genomics effects. The molecular basis of cell-specific glucocorticoid responsiveness is not fully understood, but involves a differential expression of the receptor isoforms, co-receptor proteins functioning as co-activators and co-repressors of transcription.

Panel 2 - KEY MESSAGES

- Instead of hypercortisolism driven by elevated ACTH release, several ACTH-independent regulators have been identified to contribute to increased cortisol availability during critical illness.
- The amount of cortisol that is produced during critical illness was shown to be much less that previously assumed: often less than normal or normal and at the most twice that of healthy subjects.
- Cortisol breakdown was shown to immediately and substantially suppressed during critical illness, mediated by reduced expression and activity of the cortisol metabolizing enzymes in liver and kidney. These altered pharmacokinetics have implications for dosing of corticosteroid treatment during critical illness.
- Elevated plasma cortisol driven by reduced breakdown suppresses plasma ACTH levels through feedback-inhibition. Such low plasma ACTH levels have shown to persist for weeks in the ICU, which was associated with abnormal adrenal structure and with impaired adrenocortical ACTH signaling and reduced expression of key steroidogenetic enzymes.
- Reduced adrenocortical ACTH signaling could mediate reduced adrenocortical cortisol production as well as explain reduced cortisol responses to exogenous ACTH injection. However, in the presence of elevated plasma cortisol and suppressed cortisol breakdown, such reduced cortisol responses to exogenous ACTH may be adaptive. When plasma cortisol concentration is not elevated or low during prolonged critical illness, low cortisol responses to ACTH could be indicative of adrenal failure requiring treatment.

- More well-designed (pre)clinical studies are needed to better identify patients with a failing hypothalamus-pituitary-adrenal axis response and to refine optimal treatment modalities

## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

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

FIGURE LEGENDS

## Figure 1 - Overview of the ACTH effect on the adrenal gland

ACTH is the main controller of adrenal glucocorticoid production and release; it stimulates steroidogenesis by binding to its receptor, the melanocortin 2 receptor (MC2R) present in the cell membrane of the adrenocortical cells and which stimulates adenylate cyclase. ACTH upregulates the expression of its own receptor, mediates the release of cholesterol from the lipid droplets while increasing the expression of genes encoding the proteins for cholesterol uptake [such as the LDL-receptor (LDLR) and scavenger-receptor class B member 1 (SCARB1)] and for cholesterol synthesis [via 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)]. ACTH also increases expression of genes encoding key steroidogenic enzymes, such as steroidogenic acute regulatory protein (STAR) and cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1).[72-77]

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

Anti-inflammatory as well as pro-inflammatory cytokines derived from immune cells differentially regulate adrenal cortisol secretion during critical illness especially critical illness induced by sepsis. Bacterial and viral toxins mediated by toll-like receptors modify pituitary-adrenal hormone synthesis and glucocorticoid tissue sensitivity and activation of peripheral cortisol metabolism indirectly via the immune system and directly through an action on the adrenocortical cell itself. Adipokines such as leptin, IL6, TNF$\alpha$ released from subcutaneous, visceral or peri- and intra-adrenal adipocytes, modify directly adrenal steroidogenesis. Similarly HPA-axis function is influenced by blood flow, endothelial derived factors, neurotransmitters and neuropeptides.

## Figure 3 – Cortisol production in critically ill patients

Panel A depicts cortisol production calculated via a continuous infusion of deuterated cortisol tracer in the morning (from 10:00 -13:00) in 11 patients and 9 healthy control subjects.[16] Bar charts represent means and standard errors. Panel B depicts pulsatile cortisol secretion calculated from time concentration profile series, sampled every 10 minutes from 21:00-06:00 in 40 patients and 8 healthy control subjects, by deconvolution analysis.[21] Bar charts represent means and standard errors. Together, these data indicate that the 24h cortisol production rate is not different from that in healthy matched control subjects.

Figure 4 – Overview of the HPA-axis regulation in health and during critical illness

During health, CRH controls the pulsatile and tonic release of ACTH and cortisol, which both follow a diurnal pattern. During critical illness, ACTH release is only briefly increased in the very acute plase, after which it is suppressed. Beyond the very acute phase of critical illness, elevated plasma concentrations of cortisol are predominantly brought about via reduced plasma clearance of cortisol. [16, 21] With time, the low plasma ACTH concentrations may negatively affect adrenocortical structure and function and hereby contribute to the increased risk of adrenal failure observed in prolonged critically ill patients.[22]

Figure 1

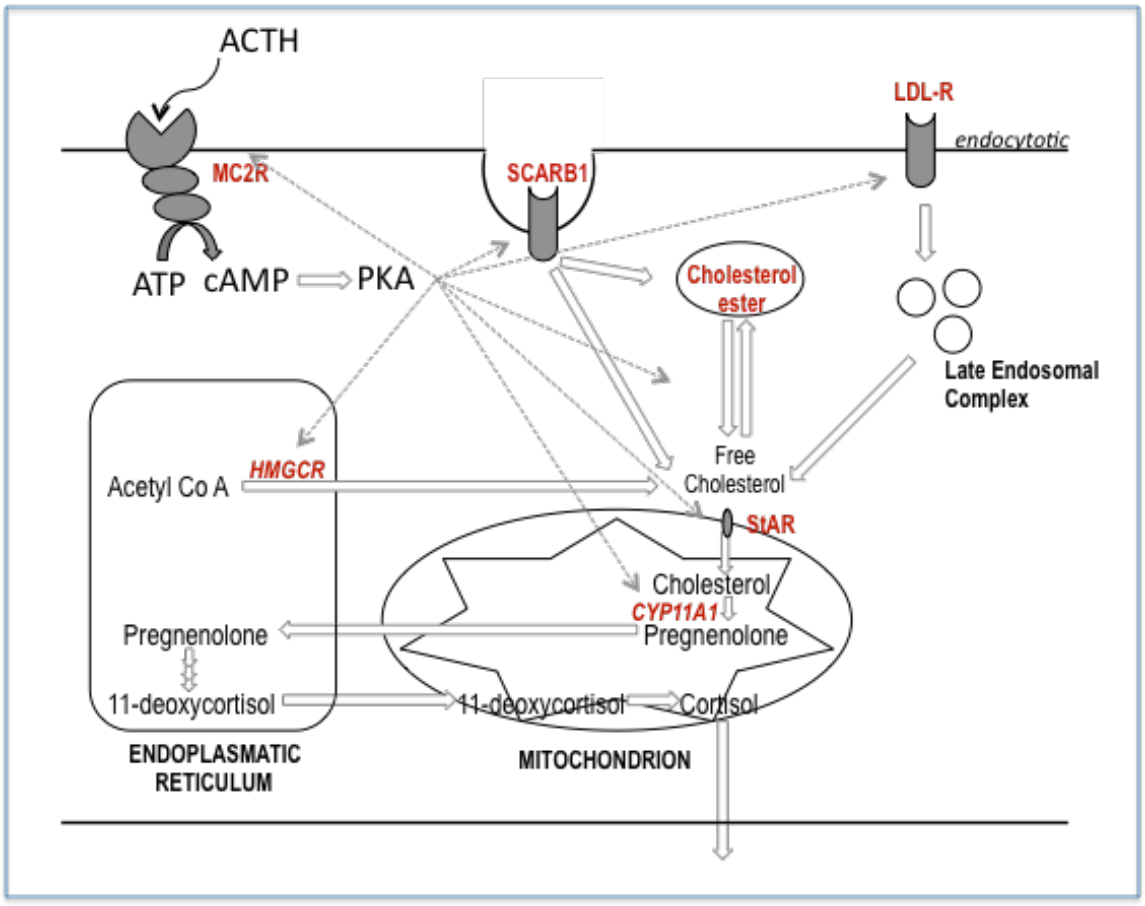

ACTH
MC2R
SCARB1
LDL-R
endocytotic
ATP cAMP PKA
Cholesterol ester
Late Endosomal Complex
Acetyl Co A
HMGCR
Free Cholesterol
StAR
Cholesterol
CYP11A1
Pregnenolone
Pregnenolone
11-deoxycortisol
11-deoxycortisol
Cortisol
ENDOPLASMATIC RETICULUM
MITOCHONDRION

Figure 2

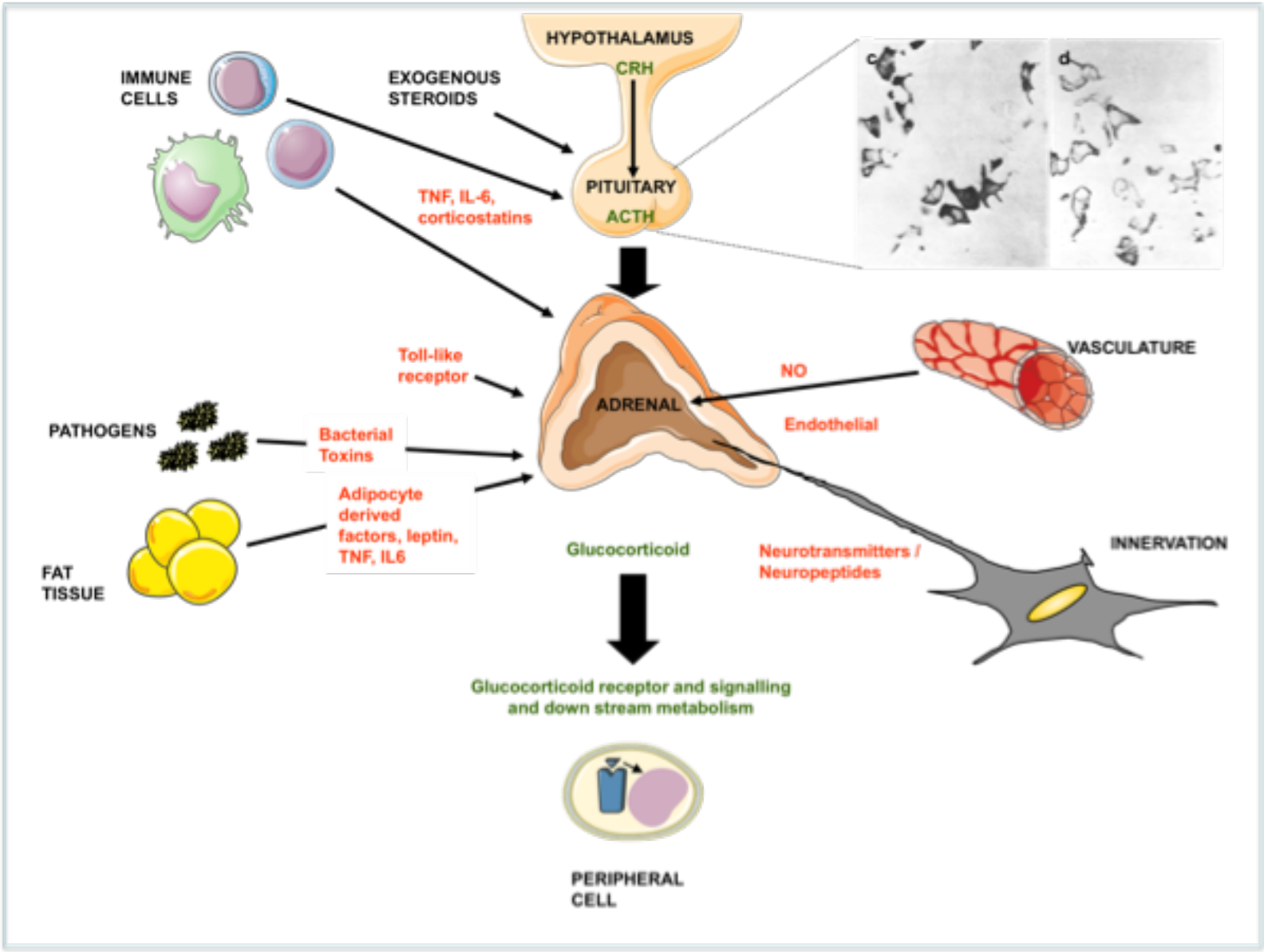

HYPOTHALAMUS
CRH
IMMUNE CELLS
EXOGENOUS STEROIDS
PITUITARY
ACTH
TNF, IL-6, corticostatins
VASCULATURE
NO
Endothelial
Toll-like receptor
ADRENAL
PATHOGENS
Bacterial Toxins
Adipocyte derived factors, leptin, TNF, IL6
FAT TISSUE
Glucocorticoid
Neurotransmitters / Neuropeptides
INNERVATION
Glucocorticoid receptor and signalling and down stream metabolism
PERIPHERAL CELL

Figure 3

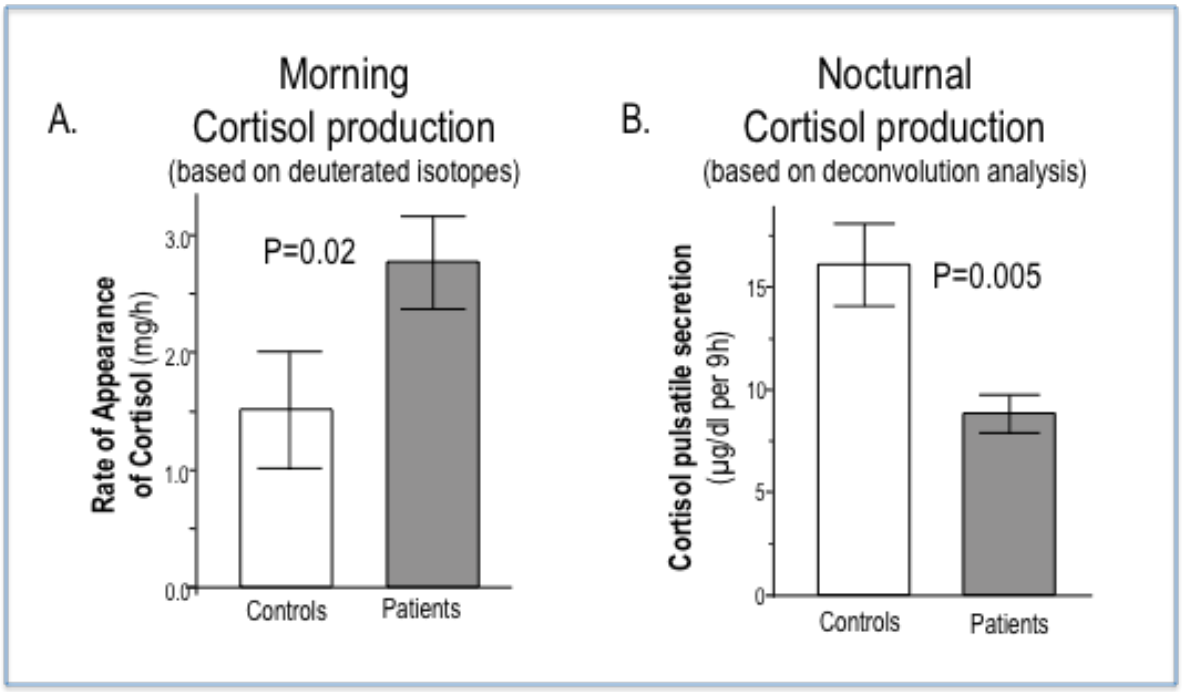
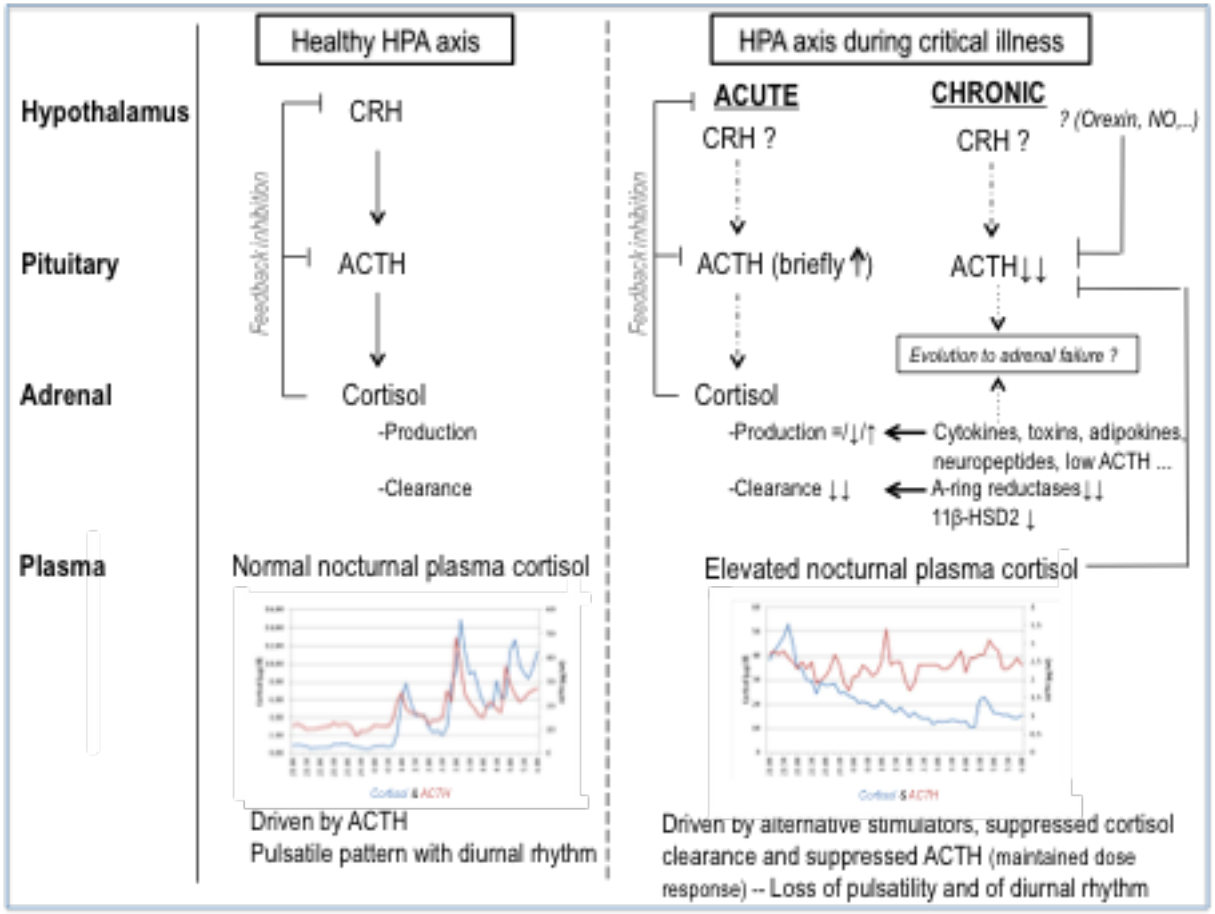

A.
Morning
Cortisol production
(based on deuterated isotopes)
Rate of Appearance of Cortisol (mg/h)
3.0
2.0
1.0
0.0
P=0.02
Controls
Patients
B.
Nocturnal
Cortisol production
(based on deconvolution analysis)
Cortisol pulsatile secretion (µg/dl per 9h)
15
10
5
0
P=0.005
Controls
Patients

Figure 4


Healthy HPA axis
HPA axis during critical illness
Hypothalamus
Pituitary
Adrenal
Plasma
CRH
ACTH
Cortisol
Feedback inhibition
-Production
-Clearance
ACUTE
CHRONIC
CRH ?
CRH ?
? (Orexin, NO,...)
ACTH (briefly ↑)
ACTH↓↓
Evolution to adrenal failure ?
Cortisol
-Production =/↓/↑
Cytokines, toxins, adipokines, neuropeptides, low ACTH ...
-Clearance ↓↓
A-ring reductases↓↓
11β-HSD2 ↓
Normal nocturnal plasma cortisol
Elevated nocturnal plasma cortisol
Driven by ACTH
Pulsatile pattern with diurnal rhythm
Driven by alternative stimulators, suppressed cortisol clearance and suppressed ACTH (maintained dose response) -- Loss of pulsatility and of diurnal rhythm

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