DRUG-INDUCED HPA AXIS ALTERATIONS DURING ACUTE CRITICAL ILLNESS:

A MULTIVARIABLE ASSOCIATION STUDY

Short title: Iatrogenic modulation of the HPA axis

Authors: *Bram Peeters[1], Fabian Güiza[1], Eva Boonen[1], Philippe Meersseman[1,2], Lies Langouche[1], Greet Van den Berghe[1,*]*

[1]Clinical Division and Laboratory of Intensive Care Medicine, Department of Cellular and Molecular Medicine, KU Leuven, BE, and [2]Medical Intensive Care Unit, Department of General Internal Medicine, UZ Leuven, Leuven, BE

*Correspondence: Greet Van den Berghe, Intensive Care Medicine, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium. Phone: 32-16-34-40-21; Fax: 32-16-34-40-15; E-mail: greet.vandenberghe@med.kuleuven.be.

Keywords: Critical Illness; HPA axis; Pharmacology; Stress Response.

Acknowledgments: The authors thank the patients and healthy volunteers for participating, the clinical staff for excellent protocol compliance and the research assistants for sample handling and data entry.

Funding: This work was supported by the Research Foundation-Flanders (FWO) [Grant numbers 11W9315N to BP, G.0417.12 to GVdB]; by the Methusalem Program of the Flemish Government [METH/08/07 and METH/14/06 to GVdB via KU Leuven]; by an European Research Council Advanced Grant [AdvG-2012-321670 to GVdB] from the Ideas Program of the European Union 7th framework program.

Conflict of interest: There is no conflict of interest.

Word count: 4061

Abstract

Objective Critical illness is hallmarked by low plasma ACTH in the face of high plasma cortisol. We hypothesized that frequently used drugs could play a role by affecting the hypothalamic-pituitary-adrenal axis.

Design Observational association study.

Patients 156 medical-surgical critically ill patients.

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

Results Upon ICU-admission, compared with healthy subjects, patients revealed low mean±SEM plasma ACTH concentrations (2.68±0.6 pmol/l vs. 9.0±1.6 pmol/l, P<0.0001) in the face of unaltered total plasma cortisol (336.7±30.4 nmol/l vs. 300.8±16.6 nmol/l, P=0.3) and elevated free plasma cortisol concentrations (41.4±5.5 nmol/l vs. 5.5±0.8 nmol/l, P=0.04). Plasma ACTH concentrations remained low (P<0.001) until day 3 whereas plasma (free)cortisol concentrations steeply increased and remained high (P<0.001). No independent correlations with plasma ACTH were found. In contrast, the total admission plasma cortisol concentration was independently and negatively associated with the cumulative opioid (P=0.001) and propofol (P=0.02) dose, the use of etomidate (P=0.03), and positively with the cumulative dobutamine dose (P=0.0007).

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

Introduction

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

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

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

acute phase of critical illness prior to ICU admission may explain the acute 'ACTH-cortisol dissociation', as observed already upon admission to the ICU. To test this hypothesis, we used a multivariable linear regression analysis to investigate any independent associations between these drugs and the concentrations of ACTH and cortisol in plasma collected upon ICU admission from a mixed set of surgical and medical ICU patients.[17]

Methods

Patients and documentation of the administered drugs prior to ICU admission

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

All documented intravenous, subcutaneous or inhaled drugs were grouped into 5 relevant drug categories, of which a potential impact on the HPA axis has been suggested in the literature. Drugs that were given to fewer than 5 patients were not taken into account to exclude findings by chance (Table 2). Equipotent

drug doses were calculated for opioids and for volatile anesthetics, taking into account the relative potency of each individual drug (Table 2). For dobutamine, cumulative doses were expressed as folds of 4200 μg, corresponding to an infusion rate of 1 μg kg^{-1} min^{-1} for a 70 kg individual during 1 hour.

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

Quantification of plasma ACTH and (free) cortisol concentrations

Admission (IQR 14:14PM – 19:29PM) and daily morning (6:00AM) blood samples were collected in pre-chilled ethylenediaminetetraacetic acid (EDTA) tubes and immediately placed on ice, centrifuged at 4°C and then stored at -80°C until assay. Total plasma cortisol concentrations (Immunotech, Prague, Czech Republic) and plasma cortisol-binding-globulin (CBG) concentrations (Riazen, Louvain-La-Neuve, Belgium) were quantified with use of radioimmunoassay. Plasma ACTH concentrations were measured with a double-monoclonal immunoradiometric assay (Brahms Diagnostics, Berlin, Germany). Plasma albumin was quantified by the bromocresol green method with a Colorimetric assay (BioAssay Systems, Hayvard, CA). Plasma free cortisol was calculated with use of the previously validated Coolens' formula adapted for individual albumin and CBG concentrations.[18]

Statistical analyses

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

The primary study aim was to assess, in a multivariable linear regression analysis, the presence of an independent association between the cumulative doses of the drugs given during the 24h prior to ICU

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

As a secondary aim, the time courses of plasma ACTH and total and free cortisol concentrations during the first 3 consecutive days in ICU were plotted for those drugs that were identified, in the multivariable linear regression analysis, as independently associated with admission plasma ACTH or cortisol concentrations. For this, the cumulative doses of the drugs given 24h prior to ICU admission were divided in the identified categories as explained above, or, for those drugs with a (log)linear association with the outcomes, in two groups, at or below versus above the median cumulative dose. The presence of any potential subsequent rebound effect on plasma cortisol or ACTH concentrations was investigated in these time series with use of repeated measures ANOVA. For those drugs that were identified as independently

associated with admission plasma ACTH or cortisol concentrations, patient files were screened to assess whether or not and in which dose range this drug administration was continued.

Statistical analyses were performed with use of JMP® version 11.0.0 (SAS Institute, Inc, Cary, NC) and SPSS software, version 22 (IBM, Armonk, NY). Two-sided P-values of 0.05 or less were considered to indicate statistical significance. No corrections for multiple comparisons were done.

Results

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

All 156 patients remained in the ICU for at least 3 days. Upon admission to the ICU, patients had much lower mean±SEM plasma ACTH concentrations than healthy subjects with comparable demographics (2.68±0.6 pmol/l vs. 9.0±1.6 pmol/l, P<0.0001) and plasma ACTH concentrations remained below normal throughout the 3 first days in ICU (P<0.001) (Fig. 1, panel A). Upon ICU admission, mean±SEM total plasma cortisol concentrations in patients were not different from those in healthy subjects (336.7±30.4 nmol/l vs. 300.8±16.6 nmol/l, P=0.3) but plasma mean±SEM free cortisol concentrations were 7-fold elevated (41.4±5.5 nmol/l vs. 5.5±0.8 nmol/l, P=0.04) (Fig. 1, panel B-C). The latter can be explained by a decrease in mean±SEM plasma CBG and albumin levels compared to healthy controls (621.4±11.5 nmol/l vs. 801.7±36.4 nmol/l (P=0.001) and 35.9±0.8 g/l vs. 47.3±1.3 g/l (P<0.0001), respectively) from ICU admission onwards. From the morning after ICU admission, total and free cortisol plasma concentrations were significantly higher than those upon ICU admission (total cortisol: 502.3±22.1 nmol/l vs. 336.7±30.4 nmol/l, P<0.0001; free cortisol: 58.0±5.5 nmol/l vs. 41.4±5.5 nmol/l, P<0.0001) and remained high until day 3 in ICU (P<0.001).

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

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

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

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

The multivariable linear regression analysis, adjusted for baseline risk factors and admission plasma ACTH concentrations, revealed that the cumulative doses of several drugs, administered within 24h prior to ICU admission, were independent determinants for total plasma cortisol concentrations upon ICU admission (total model R^2=0.56, P<0.0001) (Table 4). Indeed, total plasma cortisol concentration upon ICU admission was independently and negatively associated with the cumulative opioid dose [a decrease

of 8.6 (95% CI -13.6 to -3.6) nmol/l in total plasma cortisol for every 10 mg morphine-equivalent given; P=0.001], the cumulative propofol dose [a decrease of 7.2 (95% CI -.13.4 to -1.0) nmol/l in total plasma cortisol for every 100 mg of propofol given; P=0.02] and the use of etomidate [a decrease of 65.6 (95% CI .125.6 to -5.7) nmol/l in total plasma cortisol when given; P=0.03], and positively with the cumulative dobutamine dose [an increase of 18.7 (95% CI 8.2 to 29.2) nmol/l plasma cortisol for every 4200 μg given (equal to 1 $\mu g\ kg^{-1}\ min^{-1}$ for a 70 kg individual for one hour); P=0.0007]. Similar independent associations between plasma free cortisol and the cumulative doses of opioids and dobutamine were found (data not shown).

Using an additional forward-backward stepwise regression sensitivity analysis, with a probability to enter the model (F-to-enter) of 0.05 and a probability to leave the model (F-to-remove) of 0.05, the independent associations of etomidate, opioids, propofol and dobutamine with total plasma cortisol concentrations were confirmed (data not shown). The assessment of multicollinearity had identified potential collinearity for enoxaparin and age. Repeating the multivariable linear regression analyses after taking enoxaparin and age out of the model did not affect any of the results. In the subset of surgical ICU patients, adjusted for the same baseline risk factors and admission plasma ACTH concentrations, the independent associations of etomidate, opioids, propofol and dobutamine with total plasma cortisol concentrations were confirmed (data not shown).

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

Of all patients, 142 received at least 1 of the 4 drugs that were associated with admission total cortisol levels. 21 patients received all 4 drugs. Patients who had received etomidate in the 24h prior to ICU admission, revealed significantly lower total and free plasma cortisol concentrations upon ICU admission than did patients who had not received etomidate, but equally low plasma ACTH concentrations. On the

following 3 days in the ICU, the plasma cortisol levels of patients who had received etomidate prior to ICU admission showed a rebound rise reaching levels that were higher than those of patients not having received etomidate, while in this latter group plasma ACTH levels declined more quickly (Figure 2A). On the following 3 days in ICU, patients of both groups did not receive etomidate. A similar rebound rise in plasma cortisol levels was apparent for patients who had received a cumulative opioid dose higher than the median (180 mg morphine equivalent dose) in the 24h prior to ICU admission as compared with patients who had received less opioids (Figure 2B). On the following 3 days in ICU, the majority of patients of both groups (83% of patients on day 1, 83% on day 2, and 63% on day 3) still received opioids, in a dose that was much lower than the cumulative median dose upon admission of 180 mg (median dose of 26.1 mg on day 1, 17.9 mg on day 2, and 6.5 mg on day 3). In patients receiving more than the median opioid dose, plasma ACTH concentrations were lower upon ICU admission and further decreased more slowly in comparison with patients receiving opioids in a lower dose. Patients who had received a cumulative dose of propofol higher than the median of 227.5 mg in the 24h prior to ICU admission displayed lower ICU admission total and free plasma cortisol concentrations than patients who had received propofol at a lower dose, after which the two groups became comparable (Figure 2C). Propofol administration increased during the first day in ICU, after which it declined (81% of patients on day 1, 47% on day 2, and 27% on day 3) with a median dose of 1248 mg on day 1, 0 mg on day 2, and 0 mg on day 3). No rebound rise in plasma cortisol levels was apparent. Plasma ACTH concentrations were equally low in both groups. In contrast, the stimulatory effect of dobutamine, shown by the results of the multivariable analysis, could not be illustrated in the univariate plots, and also no effect on plasma ACTH concentrations was observed (Figure 2D). On the following 3 days in ICU, dobutamine administration declined (29% of patients on day 1, 23% on day 2, and 15% on day 3), with a median dose of 0 mg on day 1, 2, and 3.

Discussion

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

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

sympathetic nervous system synergistically interact with each other in the complex microenvironment of the adrenal gland and that they are functionally interdependent.[25] As sedative and analgesic drugs have been shown to evoke a sustained suppression of sympathetic activity[4, 9, 10, 13], this may abolish such an effect.

In contrast with ACTH, plasma cortisol was significantly and independently associated with the cumulative doses of etomidate, propofol, opioids and dobutamine. A suppressive effect of opioids, etomidate and propofol and a stimulatory effect of dobutamine on plasma cortisol was suggested. The effect size of opioids was large and dose-dependent. Acute opioid administration to healthy individuals, prolonged opioid administration to patients suffering from chronic pain and intraoperative intravenous opioid administration in surgical patients have shown to result in suppressed plasma ACTH and/or cortisol concentrations.[8-10, 26-30] An effect on the hypothalamus and the pituitary are assumed to mediate such an effect of opioids on the HPA axis[27] most likely via the κ opioid receptor.[16] However, also the adrenal gland expresses specific opioid binding sites, which, after binding with opioids, could mediate a direct inhibition of cortisol secretion.[31] This could explain why only cortisol and not ACTH appeared affected by opioids in the here studied critically ill patients.

The results of this study also confirmed a suppressive effect of etomidate on plasma cortisol, even after one single induction dose. Etomidate is a known suppressor of adrenocortical cortisol production by inhibiting 11-beta-hydroxylase, a key enzyme for cortisol synthesis.[32] Prolonged etomidate infusion during critical illness has been shown to be associated with an increased mortality and was therefore abandoned as sedative for patients in ICUs.[33] However, a recent Cochrane review concluded that a single induction dose of etomidate was not related with an increase in mortality in critical ill patients, although random plasma cortisol concentrations were lowered.[6]

The current study also identified propofol as a possible suppressor of plasma cortisol. A previous study concluded that continuous intravenous infusion of propofol for up to 24h in critically ill patients did not

impair adrenal steroidogenesis.[11] However, in patients admitted to the ICU after cardiopulmonary bypass surgery, continuous infusion of propofol has been associated with an attenuated rise of plasma cortisol, when compared with an anesthetic regimen based on sufentanil and midazolam.[4] It is well known that propofol rapidly binds to GABA(gamma-aminobutyric acid)-A-receptors, which are ubiquitous in the central nervous system. However, no effect on plasma ACTH could be demonstrated in the current study, which is not in favour of a central effect of propofol. However, GABA-A-receptor sites have been reported in rat adrenal chromaffin cells[34] which modulate catecholamine secretion[35] whereby indirectly cortisol secretion could be affected in this complex microenvironment. The GABA-A-receptor has also been described in bovine glomerulosa cells of the adrenal cortex, which mediate an inhibition of aldosterone secretion.[36] Therefore, rather than via a central inhibitory effect, propofol might also directly inhibit cortisol secretion by binding to the GABA-A-receptor in the adrenal gland. This hypothesis is supported by the rise in corticosterone secretion which has been shown in rats immediately after discontinuation of propofol, in the absence of an effect on ACTH secretion.[14]

The results of the multivariable analysis suggested that dobutamine prior to ICU admission may increase plasma cortisol levels without an effect on plasma ACTH. As in univariate analysis, this effect was negligible, these data suggest that any stimulatory effect of dobutamine is likely small and context dependent. Catecholamines are known to stimulate the HPA axis by an activation of α1-adrenergic receptors and to inhibit the HPA axis by an activation of α2-adrenergic receptors in the central nervous system.[37] Dobutamine predominantly activates β1-adrenergic receptors, but also β2- and α1-adrenergic receptors are activated at doses used clinically.[38] Although dobutamine has been shown to increase plasma ACTH levels in a study of freely moving non-anesthetized rats, no results on corticosterone were given.[39] As the current evidence suggests that catecholamines activate the HPA axis via receptors in the central nervous system, the absence of an association with plasma ACTH in the current study does not support such an effect. Again, the complex interaction between the adrenal medulla and cortex suggests that a direct effect of catecholamines on the adrenal cortex is possible. In our study, the stimulatory effect

on plasma cortisol levels could not be shown in a univariate analysis, suggesting that these effects are a result of the interaction of dobutamine with the HPA axis, only in the presence of the other administered drugs.

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

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

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

References

1 Vermes, I., Beishuizen, A., Hampsink, R.M., et al. (1995) Dissociation of plasma adrenocorticotropin and cortisol levels in critically ill patients: possible role of endothelin and atrial natriuretic hormone. *J Clin Endocrinol Metab*, 80, 1238-1242.

2 Boonen, E., Vervenne, H., Meersseman, P., et al. (2013) Reduced cortisol metabolism during critical illness. *N Engl J Med*, 368, 1477-1488.

3 Lindgren, C., Dahlqvist, P., Lindvall, P., et al. (2013) Cortisol levels are influenced by sedation in the acute phase after subarachnoid haemorrhage. *Acta Anaesthesiol Scand*, 57, 452-460.

4 Plunkett, J.J., Reeves, J.D., Ngo, L., et al. (1997) 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.

5 Brorsson, C., Dahlqvist, P., Nilsson, L., et al. (2014) Adrenal response after trauma is affected by time after trauma and sedative/analgesic drugs. *Injury*, 45, 1149-1155.

6 Bruder, E.A., Ball, I.M., Ridi, S., et al. (2015) Single induction dose of etomidate versus other induction agents for endotracheal intubation in critically ill patients. *Cochrane Database Syst Rev*, 1, CD010225.

7 Molenaar, N., Bijkerk, R.M., Beishuizen, A., et al. (2012) Steroidogenesis in the adrenal dysfunction of critical illness: impact of etomidate. *Crit Care*, 16, R121.

8 Raff, H., Flemma, R.J. & Findling, J.W. (1988) Fast cortisol-induced inhibition of the adrenocorticotropin response to surgery in humans. *J Clin Endocrinol Metab*, 67, 1146-1148.

9 Shinoda, T., Murakami, W., Takamichi, Y., et al. (2013) Effect of remifentanil infusion rate on stress response in orthopedic surgery using a tourniquet application. *BMC Anesthesiol*, 13, 14.

10 Watanabe, K., Kashiwagi, K., Kamiyama, T., et al. (2014) High-dose remifentanil suppresses stress response associated with pneumoperitoneum during laparoscopic colectomy. *J Anesth*, 28, 334-340.

11 Aitkenhead, A.R., Pepperman, M.L., Willatts, S.M., et al. (1989) Comparison of propofol and midazolam for sedation in critically ill patients. *Lancet*, 2, 704-709.

12 Skoglund, K., Enblad, P., Hillered, L., et al. (2012) The neurological wake-up test increases stress hormone levels in patients with severe traumatic brain injury. *Crit Care Med*, 40, 216-222.

13 Marty, J., Gauzit, R., Lefevre, P., et al. (1986) Effects of diazepam and midazolam on baroreflex control of heart rate and on sympathetic activity in humans. *Anesth Analg*, 65, 113-119.

14 Dispersyn, G., Sage, D., Challet, E., et al. (2009) Plasma corticosterone in rats is specifically increased at recovery from propofol anesthesia without concomitant rise of plasma ACTH. *Chronobiol Int*, 26, 697-708.

15 Kostopanagiotou, G., Kalimeris, K., Christodoulaki, K., et al. (2010) The differential impact of volatile and intravenous anaesthetics on stress response in the swine. *Hormones (Athens)*, 9, 67-75.

16 Pascoe, J.E., Williams, K.L., Mukhopadhyay, P., et al. (2008) Effects of mu, kappa, and delta opioid receptor agonists on the function of hypothalamic-pituitary-adrenal axis in monkeys. *Psychoneuroendocrinology*, 33, 478-486.

17 Meersseman, P., Boonen, E., Peeters, B., et al. (2015) Effect of Early Parenteral Nutrition on the Hpa Axis and on Treatment with Corticosteroids in Icu Patients. *J Clin Endocrinol Metab*, jc20151846.

18 Boonen, E., Meersseman, P., Vervenne, H., et al. (2014) Reduced nocturnal ACTH-driven cortisol secretion during critical illness. *Am J Physiol Endocrinol Metab*, 306, E883-892.

19 Bone, R.C., Balk, R.A., Cerra, F.B., et al. (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*, 101, 1644-1655.

20 Midi, H., Sarkar, S.K. & Rana, S. (2010) Collinearity diagnostics of binary logistic regression model. *Journal of Interdisciplinary Mathematics*, 13, 253-267.

21 Roth-Isigkeit, A.K. & Schmucker, P. (1997) Postoperative dissociation of blood levels of cortisol and adrenocorticotropin after coronary artery bypass grafting surgery. *Steroids*, 62, 695-699.

22 Gibbison, B., Angelini, G.D. & Lightman, S.L. (2013) Dynamic output and control of the hypothalamic-pituitary-adrenal axis in critical illness and major surgery. *Br.J.Anaesth.*, 111, 347-360.

23 Bornstein, S.R., Engeland, W.C., Ehrhart-Bornstein, M., et al. (2008) Dissociation of ACTH and glucocorticoids. *Trends Endocrinol.Metab*, 19, 175-180.

24 Deutschman, C.S., Raj, N.R., McGuire, E.O., et al. (2013) Orexinergic activity modulates altered vital signs and pituitary hormone secretion in experimental sepsis. *Crit Care Med.*, 41, e368-e375.

25 Ehrhart-Bornstein, M., Hinson, J.P., Bornstein, S.R., et al. (1998) Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr.Rev.*, 19, 101-143.

26 Taylor, T., Dluhy, R.G. & Williams, G.H. (1983) beta-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J Clin Endocrinol Metab*, 57, 592-596.

27 Rittmaster, R.S., Cutler, G.B., Jr., Sobel, D.O., et al. (1985) Morphine inhibits the pituitary-adrenal response to ovine corticotropin-releasing hormone in normal subjects. *J Clin Endocrinol Metab*, 60, 891-895.

28 Aloisi, A.M., Aurilio, C., Bachiocco, V., et al. (2009) Endocrine consequences of opioid therapy. *Psychoneuroendocrinology*, 34 Suppl 1, S162-168.

29 Abs, R., Verhelst, J., Maeyaert, J., et al. (2000) Endocrine consequences of long-term intrathecal administration of opioids. *J Clin Endocrinol Metab*, 85, 2215-2222.

30 Taniguchi, H., Sasaki, T., Fujita, H., et al. (2013) 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.

31 Vuong, C., Van Uum, S.H., O'Dell, L.E., et al. (2010) The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev*, **31**, 98-132.

32 Hildreth, A.N., Mejia, V.A., Maxwell, R.A., et al. (2008) Adrenal suppression following a single dose of etomidate for rapid sequence induction: a prospective randomized study. *J Trauma*, **65**, 573-579.

33 Watt, I. & Ledingham, I.M. (1984) Mortality amongst multiple trauma patients admitted to an intensive therapy unit. *Anaesthesia*, **39**, 973-981.

34 Kato, K., Nakagawa, C., Murabayashi, H., et al. (2014) Expression and distribution of GABA and GABAB-receptor in the rat adrenal gland. *J Anat*, **224**, 207-215.

35 Amenta, F., Collier, W.L., Erdo, S.L., et al. (1988) GABAA receptor sites modulating catecholamine secretion in the rat adrenal gland: evidence from 3H-muscimol autoradiography and in vivo functional studies. *Pharmacology*, **37**, 394-402.

36 Kenyon, C.J., Thomson, I. & Fraser, R. (1999) Stimulation of aldosterone secretion by benzodiazepines in bovine adrenocortical cells. *Fundam Clin Pharmacol*, **13**, 213-219.

37 al-Damluji, S. (1993) Adrenergic control of the secretion of anterior pituitary hormones. *Baillieres Clin Endocrinol Metab*, **7**, 355-392.

38 Ruffolo, R.R., Jr. (1987) The pharmacology of dobutamine. *Am J Med Sci*, **294**, 244-248.

39 Seo, D.O., Lee, S. & Rivier, C. (2003) Role of specific adrenergic receptors in mediating the adrenocorticotropic hormone response to increased nitric oxide levels. *J Neuroendocrinol*, **15**, 530-537.

TABLES

Table 1: Characteristics of the patients and healthy volunteers.

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

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

admission category ‘Complicated surgery / Trauma’ comprises complicated abdominal or pelvic surgery, complicated pulmonary or esophageal surgery, complicated neurosurgery, complicated vascular surgery, trauma, burns or reconstructive surgery. The diagnostic admission category ‘Medical’ comprises respiratory disease, gastroenterologic or hepatic disease, hematological or oncological disease, neurological presentation of medical disease, and other.

Table 2: Drug categories.

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

Equipotent drug doses were calculated for volatile anesthetics (Minimum Alveolar Concentration (MAC) in O_2 at 37°C P_B760 (%) for sevoflurane = 1.8, for desflurane = 6.6) and for opioids (relative to morphine (=1): alfentanil = 30, fentanyl = 120, remifentanil = 120, sufentanil = 1200, tramadol = 0.1, piritramide = 0.75).

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

Variables	*Estimated difference (95% CI) in plasma ACTH concentration (pmol/l)*				*P-value*
Gender (male vs. female)	-1,34	(-2,74	-	0,06)	0.06
BMI dichotomized (25>BMI≤40 vs. other)	-0,26	(-1,64	-	1,12)	0.7
Diabetes (present vs. not present)	1,56	(-0,19	-	3,31)	0.08
Malignancy (present vs. not present)	-0,22	(-1,97	-	1,54)	0.8
Pre-admission dialysis (present vs. not present)	0,32	(-5,69	-	6,33)	0.9
Sepsis upon admission (present vs. not present)	0,31	(-1,46	-	2,09)	0.7
APACHE II score on admission (per unit added)	-0,07	(-0,27	-	0,13)	0.5
NRS dichotomized (NRS ≥5 vs. <5)	-0,94	(-2,82	-	0,94)	0.3
eGFR (per mL min-1 1.73 m^{-2})	-0,01	(-0,06	-	0,04)	0.7
Plasma total bilirubin (per mg/dL)	-0,22	(-0,98	-	0,54)	0.6
Elective vs. emergency admission	-1,64	(-4,32	-	1,05)	0.2
Randomization to early PN vs. late PN	-0,64	(-1,93	-	0,64)	0.3
Diagnostic Category - as compared with Medical					
Cardiac surgery	0,70	(-3,33	-	4,72)	0.7
Complicated surgery/Trauma)	-1,15	(-3,96	-	1,65)	0.4
Free Cortisol (per nmol/l)	0,20	(-0,36	-	0,76)	0.5
Propofol (per 100 mg given)	0,00	(-0,18	-	0,17)	0.9
Etomidate (given vs. not given)	-0,21	(-1,88	-	1,46)	0.8
Midazolam (per 1 mg given)	-0,04	(-0,15	-	0,08)	0.5
Opioids (per 10 mg morphine-equivalent given)	-0,03	(-0,17	-	0,12)	0.7
Acetaminophen (given vs. not given)	-0,43	(-2,83	-	1,97)	0.7
Volatile Anesthetics (per % min equipotent dose given)	0,00	(-0,01	-	0,02)	0.4
Norepinephrine - as compared with not given					
When given >0. <2277 µg	-1,64	(-4,59	-	1,31)	0.9
When given >=2277 µg	-0,05	(-2,50	-	2,39)	0.3
Dobutamine (per 4200 µg given)	0,14	(-0,16	-	0,44)	0.4
Enoximone (given vs. not given)	-0,88	(-2,99	-	1,23)	0.4
Enoxaparin (given vs. not given)	0,17	(-2,96	-	3,31)	0.9
Heparin (per 1 IU given)	0,00	(0,00	-	0,00)	0.4
Desmopressin (given vs. not given)	-0,43	(-4,18	-	3,32)	0.8
Vasopressin (given vs. not given)	0,65	(-2,08	-	3,38)	0.6

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

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

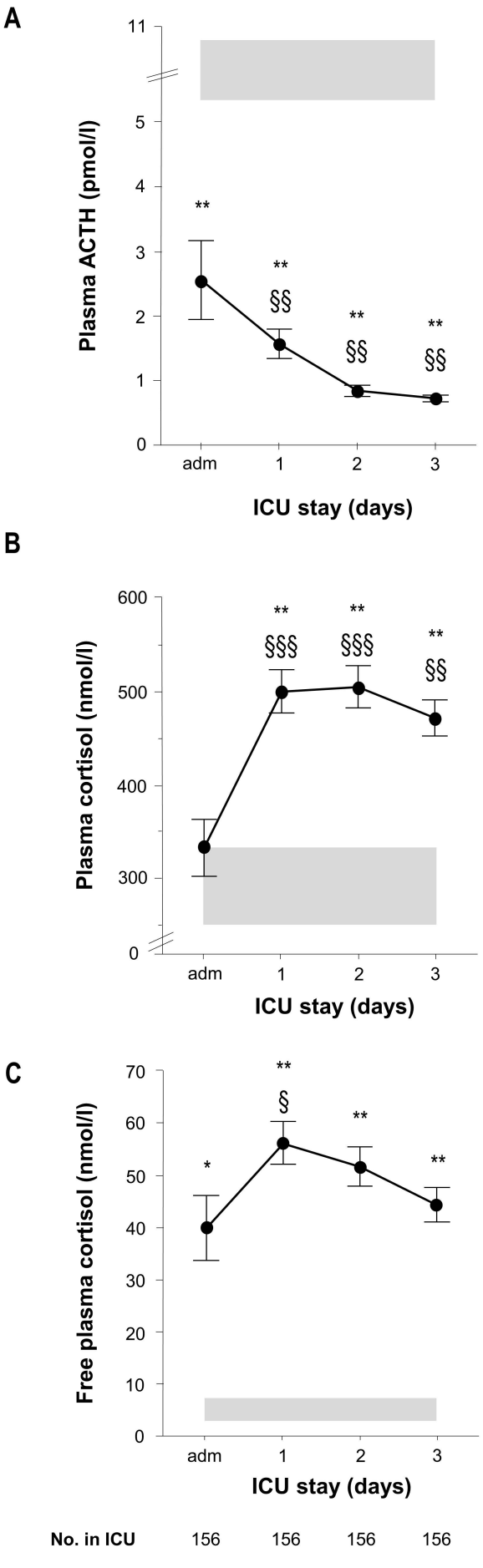
Variables	*Estimated difference (95% CI) in plasma cortisol concentration (nmol/l)*	*P-value*
Gender (male vs. female)	-44,7 (-96,3 - 6,8)	0.09
BMI dichotomized (25>BMI≤40 vs. other)	-42,6 (-91,5 - 6,2)	0.09
Diabetes (present vs. not present)	19,3 (-44,4 - 83,0)	0.6
Malignancy (present vs. not present)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

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

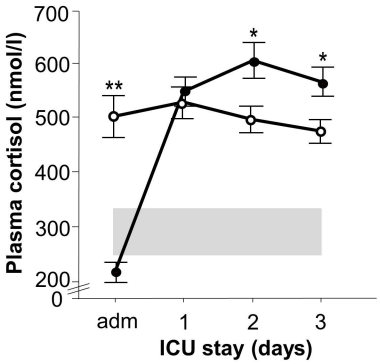
FIGURE LEGENDS

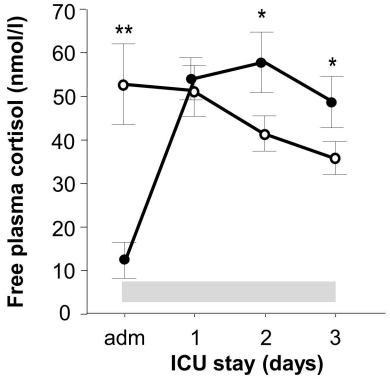
Figure 1: Plasma ACTH, cortisol and free cortisol time course from ICU admission throughout the first 3 days of critical illness. Mean values and standard errors for plasma ACTH (Panel A), total cortisol (Panel B), and free cortisol (Panel C) in patients from admission onwards until day 3 of ICU stay. The shaded area represents the interquartile range of morning values in healthy control subjects. * P≤0.05, ** P<0.001, for the comparison with controls. § P≤0.05, §§ P<0.01, §§§ P<0.0001, for the comparison of paired values of the consecutive days with the admission sample. For each day, the number of patients still in ICU is displayed below the figure. ICU denotes intensive care unit, adm denotes admission.

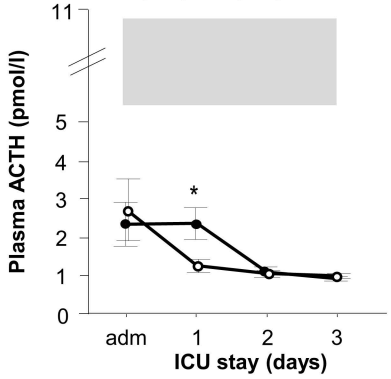
Figure 2: Univariate time course of total/free cortisol and ACTH plasma concentrations for those drugs that were identified by multivariable analysis as independently associated with cortisol. Mean values and standard errors for plasma total and free cortisol in patient groups from admission onwards until day 3 of ICU stay, divided by the received/not received etomidate (panel A), median cumulative opioid dose (panel B), median cumulative propofol dose (panel C), and median cumulative dobutamine dose (panel D), during the 24h prior to ICU admission. The presence of a subsequent rebound effect on plasma total/free cortisol concentrations was investigated. * P≤0.05, ** P<0.001, comparing mean values of both groups. The shaded area represents the interquartile range of morning values in healthy control subjects. For each consecutive day, the number of patients who received the drug (panel A), or who received more than the cumulative median dose upon admission (panel B, C, D), is displayed below the figure. ICU denotes intensive care unit, adm denotes admission.


A
Plasma ACTH (pmol/l)
11
5
4
3
2
1
0
**
**
§§
**
§§
**
§§
adm
1
2
3
ICU stay (days)
B
Plasma cortisol (nmol/l)
600
500
400
300
0
**
§§§
**
§§§
**
§§
adm
1
2
3
ICU stay (days)
C
Free plasma cortisol (nmol/l)
70
60
50
40
30
20
10
0
*
**
§
**
**
adm
1
2
3
ICU stay (days)
No. in ICU
156
156
156
156

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

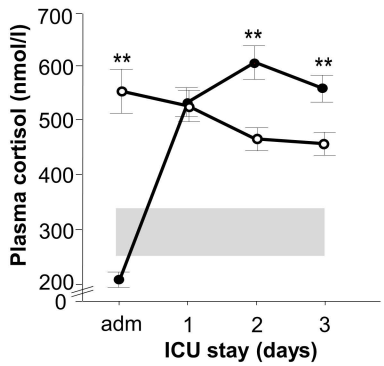


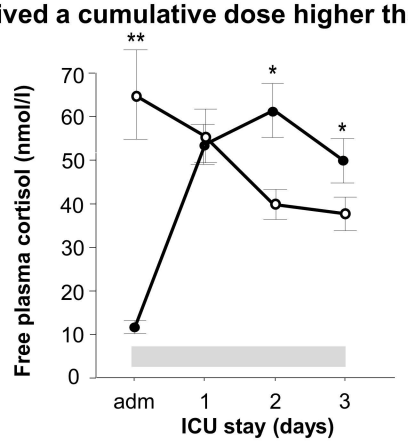


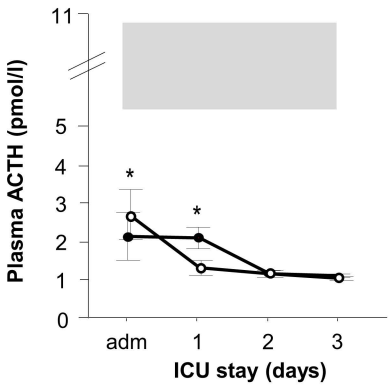


	adm	1	2	3
(-●-):	62/62	0/62	0/62	0/62
(-○-):	0/94	0/94	0/94	0/94

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

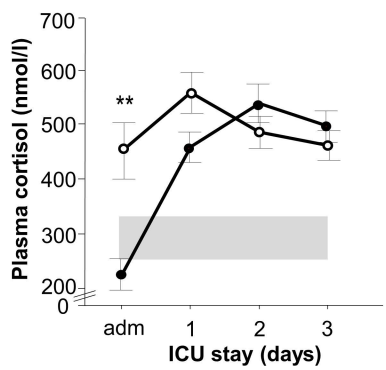


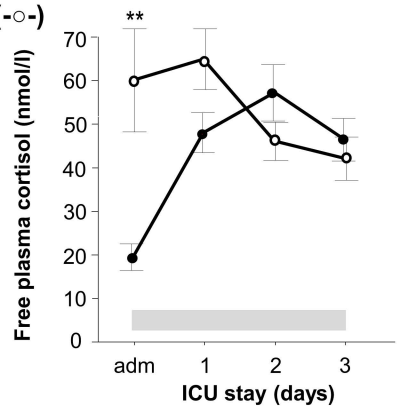


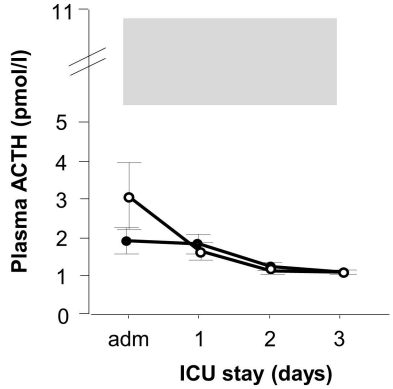


	adm	1	2	3
(-●-):	75/75	0/75	0/75	0/75
(-○-):	0/81	0/81	0/81	1/81

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

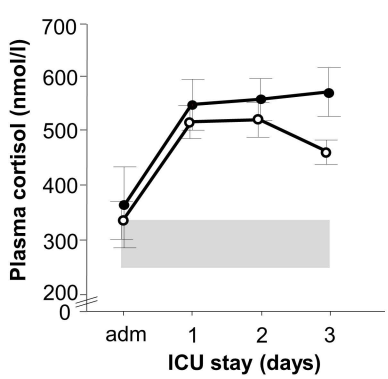


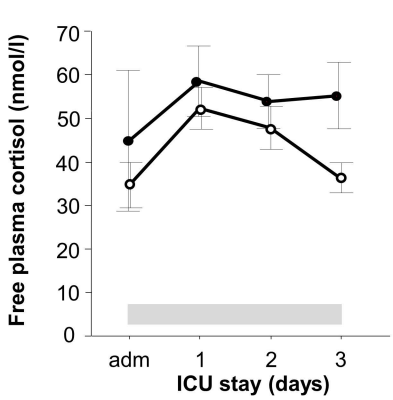


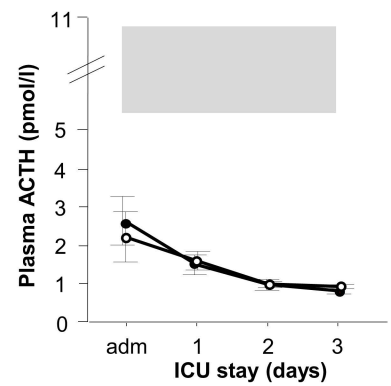


	adm	1	2	3
(-●-):	78/78	71/78	39/78	20/78
(-○-):	0/78	50/78	25/78	18/78

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







	adm	1	2	3
(-●-):	50/50	37/50	31/50	21/50
(-○-):	0/106	8/106	5/106	3/106