Protocol Full Title:
The Dynamics of the ACTH and cortisol response to CRH stimulation during critical illness: A Randomized, double-blind, placebo-controlled crossover study

Protocol Acronym/short title:
DACAR

Version and date of final protocol:
Version 1.3, 05-07-2016

Sponsor:
Name: KU Leuven / UZ Leuven
Address: Herestraat 49, 3000 Leuven

Principal Investigator:
Name: ***Prof. Dr. Greet Van den Berghe***
Address: Intensive Care Medicine, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium
Telephone: **44013**
Email: **greet.vandenberghe@med.kuleuven.be**

Sub-investigator:
Name: ***Dr. Bram Peeters***
Telephone: **42889**
Email: **bram.peeters@kuleuven.be**

Sub-investigator:
Name: ***Dr. Philippe Meersseman***
Telephone: **41335**
Email: **philippe.meersseman@uzleuven.be**

Sub-investigator:
Name: ***Prof. Dr. Lies Langouche***
Telephone: **30524**
Email: **lies.langouche@med.kuleuven.be**

Signatures

Principal Investigator
Print Name: Greet Van den Berghe

Date: 18/1/2016

Sub-investigator
Print Name: Bram Peeters

Date: 18/01/2016

Sub-investigator
Print Name: Philippe Meersseman

Date: 19/1/2016

Sub-investigator
Print Name: Lies Langouche

Date: 18/1/2016

Table of Contents

1. Study Synopsis

Title of clinical study	***The Dynamics of the ACTH and cortisol response to CRH stimulation during critical illness: A Randomized, double-blind, placebo-controlled crossover study***
Protocol Short Title/Acronym	***DACAR***
Sponsor name	***UZ Leuven***
Principal Investigator	***Prof. Dr. Greet Van den Berghe***
Medical condition or disease under investigation	***Critically ill patients***
Purpose of clinical study	***To elucidate the central cause of the low circulating ACTH concentrations in the face of elevated circulating cortisol during acute, intermediate and prolonged critical illness***
Primary objective	***To document the ACTH and cortisol responses to exogenous CRH in patients with acute and prolonged critical illness***
Secondary objective(s)	***To describe the clinical causes/consequences of these changes***
Study Design	***Randomized, double-blind, placebo-controlled crossover study***
Primary endpoints	***The responses of plasma ACTH and cortisol to CRH and the changes hereof over time in ICU in relation to patient characteristics and outcome parameters***
Sample Size	***At least 120 patients - patient recruitment will continue until preset sample numbers are reached in 3 time-in-ICU-cohorts (40 patients per cohort) with a comparable distribution of ICU admission categories – and 20 healthy volunteers (controls who never stayed in the ICU)***
Summary of eligibility criteria	***Critically ill patients in the acute phase (ICU day 3-6), the intermediate phase (ICU day 7-16) and prolonged phase (ICU day 17-28) of critical illness***
Maximum duration of treatment of a Subject	***Not applicable***
Version and date of final protocol	***1.3; 05-07-2016***
Version and date of protocol amendments	***-***

2. Background and rationale

Cortisol, ACTH, and CRH are essential components of the 'fight and flight' response to the stress of illness and trauma. When the brain of a healthy subject senses a stressful event, the hypothalamic-pituitary-adrenal (HPA) axis is activated, releasing the hypothalamic corticotropin-releasing hormone (CRH) that stimulates the corticotrophs of the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH) which drives cortisol synthesis and secretion from the adrenal cortex. Cortisol exerts feedback inhibition at the pituitary and the hypothalamic level, hence controlling its own release.

In patients suffering from critical illness, an extreme example of physical stress, the increased availability of cortisol does not seem to originate from this typical pattern of an entirely activated HPA axis. We recently demonstrated that plasma ACTH concentrations are in fact lower than in healthy subjects, while plasma cortisol concentrations remain elevated from the first day in ICU throughout at least the first week of critical illness.[1] This is known as the 'ACTH-cortisol dissociation' during critical illness. Furthermore, we showed that these elevated plasma cortisol concentrations are only to a limited extent explained by increased cortisol production. Instead, increased cortisol availability during critical illness is largely brought about via suppressed cortisol breakdown.[1] Consequently, maintaining these high circulating cortisol levels via reduced cortisol metabolism could potentially exert negative feedback inhibition on the central components of the HPA axis, as such explaining the low plasma ACTH concentrations. This would be similar to the inhibition of both ACTH synthesis and secretion from the pituitary corticotrophs and suppressed hypothalamic CRH synthesis and secretion in response to a prolonged exposure (24h or more) to high doses of exogenous corticosteroids.[2]

However, the hypothalamic-pituitary feedback regulation appears much more complex than the initially proposed simple closed loop feedback system.[3] For example, whereas TSH becomes completely unresponsive to TRH when thyroid hormone levels are high[4], CRH can break through the feedback inhibition exerted by high cortisol levels.[5,6] Furthermore, many afferent inputs from different suprahypothalamic brain regions, also targeted by cortisol, can influence CRH neuronal function in the hypothalamus, thereby regulating the set-point of pituitary responsiveness to cortisol.[3] An important regulator is the hippocampus, which is capable of inhibiting stress-induced activation of the HPA axis response, and which in turn is activated by cortisol.[7] Given the fact that prolonged elevation of cortisol evokes a reduction in hippocampal neurons and glucocorticoid receptors, this may cause a loss of feedback sensitivity of the HPA axis during chronic stress.

Our recent insights reshaped the understanding of HPA axis regulation during critical illness. In a mixed ICU population we documented that hypercortisolemia, during the first week of critical illness, coincided with suppressed nocturnal pulsatile ACTH and cortisol secretion rates, whereas the cortisol response to endogenous ACTH was overall unaltered.[8] Therefore, these findings argue against the dogma of an activated HPA axis, and could in fact be explained by a suppression of the central components of the HPA axis in response to critical illness. However, it remains unknown

whether and to what extent the elevated plasma cortisol concentrations in ICU patients evoke feedback-inhibition at the level of the hypothalamus and/or the pituitary, and whether and how this suppressive effect evolves with the duration of critical illness. One could speculate that the longer the feedback-inhibition persists, the more CRH and/or ACTH synthesis and secretion would be suppressed. However, the progressive loss of responsiveness of the HPA axis to negative feedback regulation, due to the degenerative changes in the hippocampus, could also play a role.[7] On the other hand, long-term administration of exogenous glucocorticoids or endogenously elevated plasma cortisol concentrations in patients with Cushing syndrome have been shown to cause tertiary, and not secondary, adrenal insufficiency by prolonged suppression of the hypothalamic CRH neurons and/or its higher regulatory inputs.[9]

Given the important function of ACTH in ensuring adrenal functional and structural integrity, a sustained central CRH and/or ACTH deprivation could explain the increased incidence of absolute adrenal insufficiency in the prolonged phase of critical illness.[10] The CRH stimulation test has been used to diagnose adrenal insufficiency and to differentiate between primary or secondary causes, by measuring the ACTH and cortisol response to intravenous CRH injection.[11] Currently, the test is commonly used but only few studies were done in critically ill patients.[12-15] Unfortunately, large enough and well-designed prospective clinical studies of CRH stimulation tests performed in critical ill patients at different time points during the course of their illness are currently lacking. As such, documenting a change in ACTH and/or cortisol response to CRH over time could be highly informative to understand illness evolution and might open perspectives for new therapeutic strategies.

3. Study objectives and Design

3.1 Study objectives

Our *primary objective* is to compare the ACTH and cortisol responses to exogenous CRH in patients with increasing duration of critical illness. Therefore we will investigate 3 cohorts of critically ill patients, matched for baseline characteristics (equal proportions of primary diagnostic category on ICU admission), during the acute, the intermediate and the prolonged phase of critical illness and compare these with a matched healthy control group. Cortisol binding proteins will also be assessed to calculate free cortisol. This will allow us to gain further insights in the potential (loss of) suppression of the central HPA axis in response to critical illness as well as any eventual evolution to (absolute) adrenal failure. To further reduce the impact of confounding covariates, each patient will serve as his or her own control in a crossover design. Our *secondary objective* is to describe the possible causes/consequences of such changes within the HPA-axis.

3.2 Study Design

A randomized, double-blind, placebo-controlled crossover study

4. Selection of subjects

4.1 Patients

We will study unique patients from different time cohorts, with an increasing duration of critical illness, in order to have 3 sets of patients who represent 3 different time points in the course of critical illness:

Cohort 1: acute phase of critical illness: ICU day 3 to 6.

Cohort 2: intermediate phase of critical illness: ICU day 7 to 16.

Cohort 3: prolonged phase of critical illness: ICU day 17 to 28.

If a patient, who was included in an earlier cohort, is still in ICU at the time point of the next cohort recruitment, and still meets the inclusion criteria, the patient will be studied again according to the same study protocol. The results from these analyses within patients over time will not be included in the primary study to avoid bias due to multiple comparisons, but will be analyzed separately. The availability of such sequential responses over time within one patient will allow an additional longitudinal analysis of HPA-axis alterations during the time course of the disease.

Two times each week, all adult (age ≥18y) patients from the population present in 5 ICUs from 2 departments (medical and surgical) in the University Hospital of Leuven will be screened for eligibility in the study. Patients will be included when written informed consent by the patient or patients' next of kin is given.

Inclusion criteria

All adult (age ≥18y) patients at the surgical or medical intensive care units, with ongoing intensive care dependency, a stable condition for at least 48h, and an expected stay in ICU for at least 48h, are eligible.

Exclusion criteria

- Predisposing factors of adrenal insufficiency
  - Cerebral disease with intracranial hypertension threatening the neuroendocrine system
  - Pituitary disorders including (pan)hypopituitarism
  - Known adrenal disease (Cushing's syndrome or Addison's disease)
  - Chronic treatment with glucocorticoids, other steroids or anti-steroid chemotherapy within the last 3 months
  - IV administration of glucocorticoids within the last 72 hours
  - Use of etomidate within the last 72 hours
  - Use of azoles within the last 7 days

- Other drugs predisposing to adrenal insufficiency: phenytoin, rifampicin, glitazones, imipramin, phenothiazine, phenobarbital

- Patients known to be pregnant or nursing
- No arterial line or central venous catheter in place
- Ethical restrictions
  - Moribund
  - Declined participation

4.2. Controls

The healthy control subjects will be searched in a broad social network of colleagues, family, friends and acquaintances of the study participants and/or researchers and will be matched with the general ICU population for age, sex and BMI. Control subjects with a recent history of treatment with HPA-axis interfering drugs will be excluded. Any relationship with the researchers is never of that kind that there is a situation of "dependency". In no way is there any kind of pressure on patients or volunteers. At all times every measure is taken to guarantee a free decision. Every individual can withdraw his/her consent at any time prior or during the study.

4.3. Expected duration of the study

a. Time window of monitoring per patient:

Patients from each cohort will be studied during a total period of 4.5 hours, with repetitive blood sampling during 2 consecutive mornings from 10.45-13.00 h, after receiving an alternate injection of CRH or placebo each morning. To use the crossover design, the effects of the intervention during the first period (i.e. effect of CRH) must not carry over into the second period.[16] Firstly, taking the half-life of hCRH (30.5 +/- 3.3 min) into account, the requirement for a wash-out of 5 times the drug half-life (152.5 min) is sufficient to meet this assumption.[17] Secondly, based on the fact that the cortisol clearance is reduced with at least 50% in critical ill patients than in healthy individuals, it is justifiable to perform the second alternate injection on the morning after the first injection.[1]

b. Calculated sample size:

Based on the available literature [9], we calculated to require 20 patients per cohort in order to detect a potential Cushing-like suppression of the ACTH response to CRH in critically ill patients, in comparison with the response of 20 healthy volunteers, with an alpha error of 1% (to correct for the multiple comparisons of each of the 3 patient cohorts with the healthy controls) and >80% power. In more detail: we expect an effect size of 1.20 based on previous findings, reporting a difference of mean delta AUC ACTH of 2159 to 795 pmol L^{-1} min, with a common standard deviation of 1136 pmol L^{-1} min. [9] Power calculation was performed with an independent T-test for comparison of 2 means, assuming equal variances.

To take into account a potential increase in variation due to the heterogenous critical illnesses, we will double the number of patients to 40 per cohort. With 40 patients per cohort, we can detect a reduction of 30% in the ACTH responses to CRH between 2 patient cohorts, with an alpha error of 5% and >80% power (power calculation was performed as explained above, based on a reduction of 30% of 2159 pmol L^{-1} min with the same standard deviation[9]). Recruitment of patients will continue until the preset number of 40 patients is reached in the 3 patient cohorts, with equal proportions of ICU admission categories among the groups.

c. Study duration:

Based on a retrospective analysis of the mean stay of patients in the ICU, we estimate that the study will take approximately one year to finalize recruitment.

5. Study Procedures and sample collection

5.1 Patients group

After informed consent, each patient from each cohort will be randomized for the order of CRH or placebo injection into two crossover study groups. Consecutive patients will be randomly assigned to 'first placebo' or 'first CRH' using blinded envelopes, stratified according to the 3 'time in ICU' cohorts. At 11.00 am, after 2 baseline blood samples (EDTA, 4ml) (-15 and 0 min), 100µg CRH or placebo (0.9% NaCl) will be injected as an intravenous bolus. Blood samples (EDTA, 4ml) will then be obtained at 5, 10, 15, 30, 45, 60, 90, and 120 minutes after injection to determine plasma ACTH and cortisol concentrations. The next day, each patient will receive the alternate injection, and the same blood sampling protocol as the day before (Figure 1). Since for each participant the measured effect is the difference between the response to the injection of CRH and placebo, variability is reduced.[16]

Injections of CRH or placebo will be given through a peripheral or central venous catheter, followed by a flush with ±5 ml (depending on the distance between the injection point and the insertion point of the catheter) of normal saline (NaCl 0.9%) to guarantee a full injection of the entire dose, and blood samples are drawn from an arterial or central venous catheter, all in situ and inserted for clinical purposes. The total amount of blood sampled per session is 40ml (Good Clinical Practice (GCP) consists of 40 ml per day). Some patients may experience mild, brief facial flushing immediately after injection, but at this dose level no other side effects have been reported.[18] Allergic reactions have also not been reported.

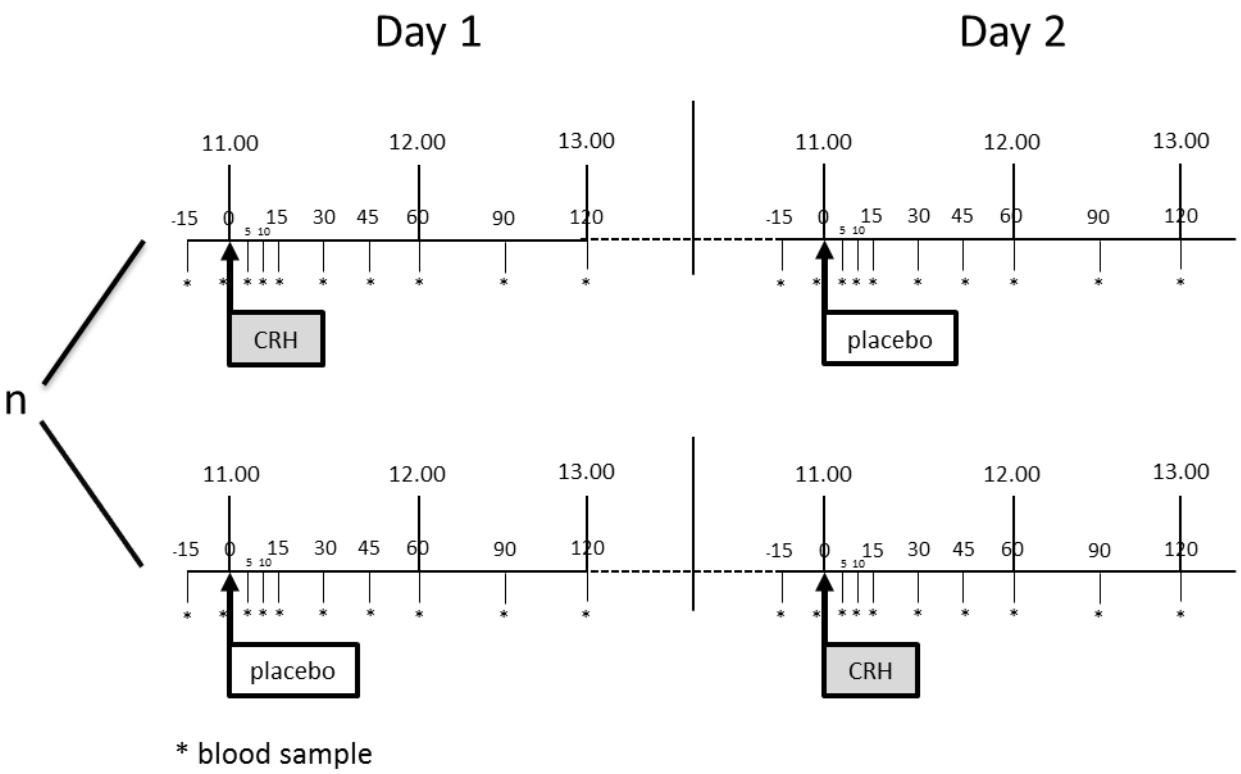

Figure 1: Study protocol. Patients from the 3 cohorts will be randomized for the order of CRH or placebo injection into two crossover subgroups. Each patients from each subgroup will be studied during a total period of 4.5 hours, with repetitive blood sampling during 2 consecutive mornings from 10.45-13.00 a.m.

The ACTH and cortisol responses to CRH/placebo will be correlated with available demographic and clinical data. Such stored patient data include:

Patient characteristics and outcome parameters: demographics (age, gender, BMI), medical history (diabetes, malignancy, renal replacement therapy), admission characteristics (reason for ICU admission, APACHE II score, SIRS and sepsis on admission), patient characteristics during ICU stay (clinical evidence of adrenal failure, SIRS and sepsis), ICU and hospital stay, ICU and hospital mortality.

Clinical parameters: Among others vital parameters (mean, systolic and diastolic blood pressure, pulse, temperature), mechanical ventilation, mean daily glycemia and renal function (daily urinary volume, creatinine clearance), infection.
Treatment information: all relevant treatments such as vasopressors and inotropes, treatment with opioids, anti-coagulants, insulin, and types and amounts of nutrients.

5.2 Control group

Time profiles of ACTH and cortisol response to exogenous CRH are not clearly defined in the literature. Especially data from healthy volunteers with a comparable age, gender and BMI as our patient population are lacking. To compare the evolution of the ACTH and cortisol response during critical illness with healthy reference values, we will use the same study protocol as for the patients in 20 demographically matched healthy volunteers, with repetitive blood sampling during 2 consecutive mornings from 10.45-13.00 h, after receiving an alternate injection of CRH or placebo each morning. Volunteers will be asked to limit their morning nutritional intake to a European breakfast.

6. Compensation for control group

Healthy control subjects will be compensated for all expenses made to participate in the study, such as transportation cost and costs for parking space, and will be offered a meal on each study day and an overnight stay in Leuven. Furthermore, a gift card (value card worth 50 EUR) will be offered as an additional reward.

7. Statistics

First, the response to CRH injection (changes over time in plasma ACTH and cortisol concentrations) will be corrected for its corresponding placebo test result (changes over time in plasma ACTH and cortisol concentrations), resulting in a delta AUC value. The delta AUC values will be compared between each of the 3 cohorts and the healthy reference group, with correction for multiple comparisons with use of ANOVA (Bonferroni correction of the alpha error to correct for the multiple comparisons of each of the 3 patient cohorts with the healthy controls), after transforming the data to normally distributed values if needed. If the latter is not possible, non-parametrical tests will be used.

Second, for those patients from whom multiple CRH test results were collected, changes in delta AUC values within these patients will be analyzed with use of mixed models / repeated measures ANOVA after transforming the data to normally distributed values if needed.
Third, correlations between the ACTH and cortisol responses to exogenous CRH and clinical risk and outcome parameters will be done with the use of Pearson tests. Fourth, these measurements will be compared in patients with a favorable and a non-favorable clinical outcome.

8. Ethics and regulatory approvals

The study will be conducted in compliance with the principles of the Declaration of Helsinki (2008), the principles of GCP and in accordance with all applicable regulatory requirements. This protocol and related documents is will be submitted for review to Ethics Committee.

The study can and will be conducted only on the basis of prior informed consent by the subjects, or their legal representatives, to participate in the study. We will obtain a signed informed consent form (ICF) for all patients prior to their enrollment and participation in the study in compliance with all applicable laws, regulations and the approval of the Ethics Committee. We will retain such ICFs in accordance with the requirements of all applicable regulatory agencies and laws.

The investigators shall treat all information and data relating to the study disclosed to the Investigators in this study as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the performance of the study. The collection, processing and disclosure of personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data (Directive 95/46/EC and Belgian law of December 8, 1992 on the Protection of the Privacy in relation to the Processing of Personal Data).

Total blood loss per patient will be limited to 80 ml (GCP is 40 ml per day). Samples will be taken via the arterial line or central venous catheter, already in place as part of the standard of care for ICU monitoring, and thus no additional discomfort will be caused.

All laboratory measurements specifically required for this study will be performed in the Laboratory of Intensive Care Medicine and will thus not generate any cost for the patients or healthy control subjects.

9. Data Management

For reasons of data integrity and internal control during data input, the patient name will be stored in a separate table linked to the eCRF (electronic Case Report Form). However, these data will only be accessible for the authorized local research staff and the principal database manager on an individual login/password base. When the database is finalized, the identity data will be detached from the eCRF and stored at the local site. Data transferred within the research group will always be anonymous and limited to the data needed to address the question of the researcher.

10. References

1. Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden YM, Spriet I, Wouters PJ, Vander PS, Langouche L, Vanhorebeek I, Walker BR, Van den Berghe G: Reduced cortisol metabolism during critical illness. N Engl J Med 2013; 368:1477-1488

2. Keller-Wood ME, Dallman MF: Corticosteroid inhibition of ACTH secretion. Endocr Rev 1984; 5:1-24

3. Watts AG: Glucocorticoid regulation of peptide genes in neuroendocrine CRH neurons: a complexity beyond negative feedback. Front Neuroendocrinol 2005; 26:109-30

4. Gemsenjager E, Staub JJ, Girard J: Pituitary thyroid recovery following surgery in TRH-unresponsive patients with uni- and multinodular goiter. Horm Res 1977; 8:139-47

5. Axelrod J, Reisine TD: Stress hormones: their interaction and regulation. Science 1984; 224:452-9

6. Uchoa ET, Aguilera G, Herman JP, Fiedler JL, Deak T, de Sousa MB: Novel aspects of glucocorticoid actions. J Neuroendocrinol 2014; 26:557-72

7. Sapolsky RM, Krey LC, McEwen BS: The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev 1986; 7:284-301

8. Boonen E, Meersseman P, Vervenne H, Meyfroidt G, Guiza F, Wouters PJ, Veldhuis JD, Van den Berghe G: Reduced nocturnal ACTH-driven cortisol secretion during critical illness. Am J Physiol Endocrinol Metab 2014; 306:E883-92

9. Gomez MT, Magiakou MA, Mastorakos G, Chrousos GP: The pituitary corticotroph is not the rate limiting step in the postoperative recovery of the hypothalamic-pituitary-adrenal axis in patients with Cushing syndrome. J Clin Endocrinol Metab 1993; 77:173-7

10. Boonen E, Langouche L, Janssens T, Meersseman P, Vervenne H, De Samblanx E, Pironet Z, Van Dijck L, Vander Perre S, Derese I, Van den Berghe G: Impact of Duration of Critical Illness on the Adrenal Glands of Human Intensive Care Patients. J Clin Endocrinol Metab 2014:jc20142429

11. Kronenberg H, Melmed S, Polonsky KS, PR. L: Williams Textbook of Endocrinology, 11th edition. Philadelphia, PA, Saunders Elsevier, 2008, pp 482

12. Reincke M, Allolio B, Wurth G, Winkelmann W: The hypothalamic-pituitary-adrenal axis in critical illness: response to dexamethasone and corticotropin-releasing hormone. J Clin Endocrinol Metab 1993; 77:151-156

13. Schroeder S, Wichers M, Klingmuller D, Hofer M, Lehmann LE, von Spiegel T, Hering R, Putensen C, Hoeft A, Stuber F: The hypothalamic-pituitary-adrenal axis of patients with severe sepsis: altered response to corticotropin-releasing hormone. Crit Care Med 2001; 29:310-6

14. Dimopoulou I, Alevizopoulou P, Dafni U, Orfanos S, Livaditi O, Tzanela M, Kotanidou A, Souvatzoglou E, Kopterides P, Mavrou I, Thalassinos N, Roussos C, Armaganidis A, Tsagarakis S: Pituitary-adrenal responses to human corticotropin-releasing hormone in critically ill patients. Intensive Care Med 2007; 33:454-9

15. Schuster KM, Macleod JB, Fernandez JB, Kumar M, Barquist ES: Adrenocorticotropic hormone and cortisol response to corticotropin releasing hormone in the critically ill-a novel assessment of the hypothalamic-pituitary-adrenal axis. Am J Surg 2012; 203:205-10

16. Friedman LM, Furberg CF, DL. D: Fundamentals of Clinical Trials, 4th edition. New York, Springer, 2010, pp 79-80

17. Saphier PW, Faria M, Grossman A, Coy DH, Besser GM, Hodson B, Parkes M, Linton EA, Lowry PJ: A comparison of the clearance of ovine and human corticotrophin-releasing hormone (CRH) in man and sheep: a possible role for CRH-binding protein. J Endocrinol 1992; 133:487-95

18. Nieman LK, Cutler GB, Jr., Oldfield EH, Loriaux DL, Chrousos GP: The ovine corticotropin-releasing hormone (CRH) stimulation test is superior to the human CRH stimulation test for the diagnosis of Cushing's disease. J Clin Endocrinol Metab 1989; 69:165-9