Summary & general discussion
Summary

In this thesis we explored the concept of relative adrenal insufficiency (RAI) in critically ill patients. We studied predictors for RAI, and cross-sectionally investigated the predictive value of RAI for mortality and effects of corticosteroid treatment in septic and non-septic critically ill patients. We used repeated adrenocorticotropic hormone (ACTH) testing to explore the predictive value of RAI longitudinally. Finally, we studied the effect of therapeutic hypothermia in the prognostic value of components of the pituitary-adrenal axis such as ACTH, cortisol and the cortisol response to ACTH in comatose patients after cardiopulmonary resuscitation (CPR) for cardiac arrest.

Chapter 1
One of the body's most important regulatory systems needed to achieve adaptive responses to stress is the hypothalamic-pituitary-adrenal (HPA) axis, with cortisol as one of the main end products. Cortisol is important for cardiovascular reactivity, metabolism, and anti-inflammatory effects. Although highly activated, the HPA axis activity can be insufficient for the degree of stress, a state which may be denoted as RAI. During critical illness, many factors can impair the cortisol response to ACTH. This HPA response to stress and thus RAI is principally diagnosed in the critical care setting by dynamic adrenal testing with help of the standard short ACTH stimulation test. Diagnostic tests evaluating the entire HPA axis, such as the insulin tolerance test and the metyrapone test, are not routinely used in the intensive care unit (ICU) as these tests are complex, cumbersome and carry some risk. RAI may have prognostic significance, since treatment with corticosteroids in patients with RAI may have beneficial effects by improvement in haemodynamics and a reduction in the need for vasopressor therapy, and RAI may predict reduction of mortality by hydrocortisone therapy. There are some controversies in the concept of RAI, such as the dose of ACTH administered, appropriate cutoff levels for the diagnosis of RAI, and thereby about the prognostic value with regard to mortality and corticosteroid treatment; a random/baseline cortisol level may not adequately reflect the 24-hour secretory profile in the critically ill; total serum cortisol levels may underestimate the free cortisol levels which are responsible for the physiological activity; finally, repeated ACTH testing may have poor reproducibility. These controversies can be linked to the aims of this thesis: exploring the concept of RAI in terms of predictors for RAI, the predictive value of RAI for mortality and beneficial effects of corticosteroid treatment, the predictive value of RAI in repeated ACTH testing, and the effect of therapeutic hypothermia in the prognostic value of the pituitary-adrenal axis in comatose patients after CPR for cardiac arrest.

Chapter 2
We review criteria of abnormal 250 µg ACTH-induced cortisol patterns which are
commonly used to define RAI/non-responsiveness to ACTH in the critically ill, and evaluate the value of these patterns, in predicting haemodynamic responses to treatment with corticosteroids and mortality rates. RAI has not been defined clearly, and a wide variety of cutoff levels has been used to indicate hyporesponsiveness to ACTH, by many regarded as the main diagnosticum for RAI. Random or baseline cortisol levels, peak cortisol levels upon ACTH testing, increments from baseline to peak cortisol levels after ACTH, or combinations are used to indicate RAI. This heterogeneity of cutoff levels subsequently leads to the reported varying prevalences (0-84%). Furthermore, there is no consensus about the predictive value of the different cortisol levels for steroid-responsiveness, nor about prognostic values regarding to disease severity or mortality.

Chapter 3
We describe whether there are predictors for RAI, which may help to gain insight in the pathophysiology of RAI and to select patients for ACTH testing. A mixed population of 405 critically ill patients who underwent a 250 µg ACTH stimulation test because of prolonged hypotension and/or need for vasopressor/inotropic therapy was studied in a retrospective setting and comprised the largest series on ACTH testing hitherto reported. Clinical variables were collected at admission and at the test day. In multivariate analyses, we found that low pH/bicarbonate, low platelets, the severity of disease and organ failure, and absence of prior cardiac surgery are predictors of a subnormal adrenocortical response to ACTH defined as a cortisol increase of less than 250 nmol/L or a cortisol peak value of less than 500 nmol/L, independently of baseline cortisol values and cortisol binding capacity in blood. Furthermore, baseline cortisol/albumin ratios, as an index of free cortisol, related directly and the increases of cortisol/albumin inversely to disease severity indicators. These data suggest involvement of metabolic acidemia and coagulation disturbances in the pathophysiology of adrenocortical dysfunction, particularly in non-cardiac (surgery) patients.

Chapter 4
In this chapter, we report on our investigation to evaluate the concept of RAI in septic shock. In 218 patients with septic shock who underwent a short 250 µg ACTH test because of longer than 6 hours hypotension requiring repeated fluid challenges and/or vasopressor/inotropic treatment, we explored different cortisol cutoff levels for mortality prediction and investigated whether there are beneficial effects of treatment with corticosteroids. Firstly, we found that in these patients a cortisol response to ACTH of less than 100 nmol/L, independent of blood cortisol binding by albumin, appeared to be related to severity of disease and thereby mortality. However, ACTH/cortisol responses did not predict outcome independently of disease severity, and thus a low cortisol response to ACTH may be a marker rather than a mediator of severe disease and
associated mortality. Secondly, the cortisol response of less than 100 nmol/L was able to predict a haemodynamic and outcome benefit from corticosteroid treatment. These findings favour the concept of RAI occurring in patients with septic shock.

Chapter 5
Whether the findings found in Chapter 4 could be confirmed in non-septic critically ill patients as well, is described in this chapter. In a retrospective study on 172 non-septic patients of whom 51% after trauma or surgery, we found that baseline cortisol levels, in particular when higher than 475 nmol/L, predict mortality, partly dependent of disease severity, suggesting sufficient adaptation to stress, independent of cortisol binding in blood. We explored the cortisol cutoff level with the best discriminative value for mortality, but the resultant cortisol response to ACTH of less than 200 nmol/L had little value to predict a beneficial effect of corticosteroid treatment on haemodynamics and mortality, which argues against RAI identifiable by ACTH/cortisol testing and necessitating corticosteroid treatment in non-septic hypotensive ICU patients.

Chapter 6
This study investigated the significance of temporal changes in cortisol responses to repeated ACTH testing with regard to disease severity, mortality, and corticosteroid treatment during the course of critical illness. We retrospectively identified 54 critically ill patients who underwent two or more ACTH tests with an interval of more than 24 hours because of prolonged hypotension requiring repeated fluid challenges or vasopressor/inotropic treatment for both tests. We found that a higher cortisol response of the second ACTH test compared to the first ACTH test using repeated ACTH testing in the course of critical illness, relates to less sepsis and lower disease severity, beneficial effects of corticosteroid treatment and survival, independent of serum baseline cortisol levels and cortisol binding in blood. Thus, the changes in cortisol responses to ACTH in time during critical illness are not likely to be caused by poor reproducibility or altered baseline levels, but more likely by varying degrees and reversibility of sepsis-induced RAI. These findings argue in favour of the concept of potentially harmful RAI necessitating early and prolonged corticosteroid therapy and showing reversibility in survivors, during sepsis.

Chapter 7
This chapter reports on our prospective study to investigate the effect of therapeutic hypothermia in the prognostic value of the pituitary-adrenal axis and cortisol responses to ACTH in 29 comatose patients after cardiac arrest who were successfully resuscitated and treated with induced hypothermia. We measured cortisol and ACTH levels prior to, during and after therapeutic hypothermia, and did short ACTH stimulation tests during and after therapeutic hypothermia. ACTH and (free) cortisol levels were elevated, but higher in non-survivors than in survivors, whereas cortisol
responses to ACTH did not differ. Levels of ACTH and (free) cortisol decreased in time, but the relative difference between outcome groups was maintained. In multivariate analysis, only baseline cortisol levels prior to and during therapeutic hypothermia independently contributed to prediction of survival time, whereas ACTH, ACTH-induced increases in cortisol levels and hydrocortisone treatment did not. Negative somatosensory evoked potentials as prognosticators of irreversible cerebral damage were predicted by baseline cortisol levels prior to therapeutic hypothermia. Thus, in comatose patients resuscitated from cardiac arrest, the pituitary-adrenal axis is activated particularly in non-survivors, irrespective of therapeutic hypothermia. Hence, activation of the pituitary-adrenal axis may be a marker of fatal cerebral damage. Hypothermia does not seem to have a suppressing effect on pituitary-adrenal function. There is no evidence for RAI contributing to death in patients after cardiac arrest who were successfully resuscitated, and a transiently blunted cortisol response to ACTH in non-survivors may be attributed to higher baseline cortisol values.

General discussion

Adequate adrenocortical function is essential for the human organism to maintain homeostasis during stress. However, adrenal failure was once considered a rare diagnosis in the ICU, but is now increasingly reported in a broad group of critically ill patients, in particular those with septic shock, but also in patients with pneumonia, multitrauma, head injury, burns, human immunodeficiency virus infection, pancreatitis, liver failure, brain-dead, and following the use of etomidate [1-9]. Adrenal failure may be absolute due to structural damage to the adrenal gland, pituitary or hypothalamus, or relative, as many critically ill patients suffer from a transient and reversible failure at any point in the HPA axis. There is discussion whether or not the terms 'absolute' versus 'relative' are most appropriate with regard to adrenal dysfunction and alternative terms as critical illness-related corticosteroid insufficiency (CIRCI) have been proposed very recently, indicating inadequate corticosteroid activity for the severity of the patient’s illness [10]. We still use the term relative adrenal insufficiency (RAI) as we focus on its diagnosis and the value of the short ACTH test in this regard. RAI has therefore been defined as a clinical picture of prolonged hypotension with vasopressor-dependency in combination with a subnormal response to 250 µg of ACTH.

RAI, which may be diagnosed with help of the standard short ACTH test and in which serum cortisol levels, although high in absolute terms, are insufficient to maintain homeostasis, has been described as a harmful entity in the critically ill patient due to the associated increased risk of death, although this can be debated, as reviewed in Chapter 2. The reported incidence of this so called RAI varies widely (0-84%) depending on definitions, the population being studied and the diagnostic
tests involved. There is general agreement that RAI develops in at least 10-20% of patients in a general ICU, approaching 60% in septic shock patients [11]. Diagnosis of RAI with help of the ACTH test is hampered by some difficulties. For example, the dose of ACTH is controversial and the ‘normal’ response to ACTH is unknown. Assays are not uniform and show wide variations in test characteristics. Furthermore, the ACTH test does not assess the integrity of the entire HPA axis, the response of the HPA to other stresses, or the adequacy of stress cortisol levels. Indeed, even 50% of healthy volunteers have a cortisol increase upon ACTH of less than 250 nmol/L [12]. In spite of these limitations the ACTH test has found its way from classical endocrinology toward critical care medicine, with a proven value in several studies [13-15].

**RAI: its predictors and a predictor itself**

The main focus in the chapters 3 to 7 was to evaluate predictors of a subnormal ACTH-induced cortisol response and to investigate the capacity of RAI to predict disease severity, mortality and beneficial effects of corticosteroid treatment in these patients.

Clinical features, manifestations and pathophysiology of RAI are poorly understood. However, we showed in Chapter 3 that low pH/bicarbonate and platelets, and the severity of disease and organ failure are predictors of a subnormal adrenocortical response to ACTH, and that none of the classic signs and symptoms associated with adrenal insufficiency such as fever, hyponatraemia and hyperkalaemia were predictive for RAI. Metabolic acidosis can directly suppress the adrenal cortisol synthesis [16], which may explain our surprising finding that a low pH and bicarbonate were predictive for RAI. Indeed, acid-base disturbances showed a dissociation between ACTH, plasma renin activity, aldosterone and cortisol responses, as described by Yamauchi et al. [16]. Other factors than ACTH and plasma renin activity could be involved in the decline of serum cortisol levels, such as acidosis and hyperkalaemia, but in our study hyperkalaemia did not appear to be a predictor for RAI. As shown, the function of the adrenal cortex may further be impaired by coagulation disturbances. The contribution of low platelets to a low cortisol response upon exogenous ACTH, independently of sepsis or infection, may be caused by circulating factors promoting platelet aggregation and impairing adrenal function, or may be associated with adrenal microcirculatory thrombosis or bleeding, which can impair cortisol synthesis [17]. With the knowledge of predictors for RAI, we may better select patients for ACTH testing, and gain insight into the pathophysiological mechanisms of a low cortisol response to ACTH.

In two retrospective studies we explored the main controversies of the concept of RAI: whether RAI can predict disease severity, mortality and beneficial effects of corticosteroid treatment in the critically ill, and thus, should we use the ACTH test to select and diagnose those patients who could benefit from this treatment? In Chapter
4 it was demonstrated that in septic shock patients, a low cortisol response (<100 nmol/L) related to severity of disease and to mortality. Moreover, it predicted a haemodynamic and outcome benefit of corticosteroid treatment for these patients, confirming previous findings by Annane et al. [15], but contrasting the recent CORTICUS study [18]. In this large randomized, double-blind, placebo-controlled trial, 233 of 499 patients who did not respond to ACTH (cortisol increase of less than 250 nmol/L), were assigned to treatment with moderate-dose hydrocortisone or placebo. Hydrocortisone did not improve survival or reversal of septic shock overall nor in non-responders. However, although this study on corticosteroids in septic shock is the largest trial on this subject, it was not adequately powered to detect a substantial reduction of relative risk of death [19]. The 95% confidence interval for the relative risk of death overlapped the risk from the Annane et al. [15] study, and thus the controversy remains unsolved, and ACTH testing may still show a subset of patients who could benefit from moderate-dose corticosteroid therapy.

Whereas in septic shock patients low cortisol responses upon ACTH predicted disease severity and mortality, in non-septic critically ill patients (Chapter 5), high baseline cortisol levels predicted mortality and this was partly dependent of disease severity. This is compatible with a sufficient adaptation of the adrenal function to stress. Because in these as well as in the septic shock patients ACTH/cortisol responses did not predict outcome independently of disease severity, a low cortisol response to ACTH may be a marker rather than a mediator of severe disease and associated mortality, which implies that abnormal cortisol levels are not causally related to mortality, but are signs of severe disease. In this regard, ACTH testing lacks preference over scoring systems to mark the severity of disease in non-septic critically ill patients. Furthermore, in the non-septic patients, there was little independent predictive value of a low cortisol response and moreover, no discernable mortality-reducing effect of corticosteroids. However, an improvement in haemodynamic status was demonstrated by moderate-dose corticosteroid treatment, particularly in less severely ill patients with low baseline cortisol values and normal increases upon ACTH. This beneficial effect of corticosteroid treatment may relate to increased vascular sensitivity for endogenous and exogenous catecholamines and other vasopressor drugs [20]. All together, in non-septic critically ill patients, RAI was not identifiable by ACTH testing which implies ACTH testing is not warranted for prediction of mortality and beneficial effects of corticosteroid treatment, as opposed to septic shock patients, even if associated with severe disease and associated stress.

Chapter 6 confirmed the former results, favouring the concept of potentially harmful RAI during sepsis, necessitating early moderate-dose corticosteroid therapy and showing reversibility in survivors. This study, of retrospective design, is one of the few on repeated ACTH testing and it was shown that reversible RAI, as denoted by an increase in the cortisol response using repeated ACTH testing at at least two time-points, is associated with a decrease of sepsis and severe disease, and increase of
survival, particularly after early corticosteroid treatment. Changes in the cortisol response to ACTH during critical illness are thus not likely to be caused by poor test reproducibility or altered baseline levels, as being debated in the controversies of RAI [21-23].

Cardiac arrest, perhaps the ultimate stress for the human body, is often followed by low baseline cortisol levels and/or low cortisol increases upon exogenous ACTH [24-29]. Since therapeutic hypothermia for coma following cardiac arrest was implemented in clinical practice in 2002, it is hard to conclude on the effect of hypothermia on the pituitary-adrenal axis, and the effect of hypothermia on the adrenal responsiveness will remain controversial. However, we demonstrated (Chapter 7) that baseline cortisol levels were elevated on admission and decreased during the following days, and were higher in non-survivors than in survivors, even when therapeutic hypothermia was discontinued. Furthermore, RAI was not demonstrable in our patients who were successfully resuscitated after cardiac arrest, since neither low baseline cortisol values nor low responses to exogenous ACTH predicted mortality. Thus, as shown in Chapter 3 and 5 as well, cardiac arrest and resuscitation do not appear to be independent risk factors for RAI, and RAI is not identifiable in non-septic critically ill patients.

Methodological considerations
The studies on the capacity of RAI to predict disease severity, mortality and beneficial effects of corticosteroid treatment and on the reproducibility of ACTH testing were of retrospective design and thus the decision to perform the ACTH tests in these studies were based on clinical and not investigational grounds. Furthermore, we did not use the low-dose (1 µg) ACTH test, but measured cortisol responses upon 250 µg of exogenous ACTH which is considered as supranormal. The low-dose test gives a maximal response already and was proposed as a more sensitive test [30]. However, no superiority of the low-dose test was found in secondary adrenal insufficiency [31] and the low-dose test is hampered by problems related to dilution and is therefore liable to errors. Interestingly, in septic shock patients, 50% of high-dose responders failed to respond to the low-dose test possibly reflecting a subset of patients with a worse outcome [32]. All together, we preferred not to use this 1 µg ACTH test for comparison with extended literature on the 250 µg ACTH test.

Corticosteroid-binding globulin (CBG) and free cortisol levels were not measured in most of the studies presented in this thesis. We may thus have underestimated baseline free cortisol levels and rises upon ACTH, since low levels of cortisol binding proteins and hypoalbuminaemia in the course of severe disease may lower total cortisol levels and responses to ACTH, independently of free cortisol levels and in the absence of RAI [33-37]. However, we used albumin levels to estimate free cortisol, since albumin binds cortisol, albeit less than CBG, and both albumin and CBG levels may decrease to the same extent in critical illness [33-37].
Moreover, in a recent study on patients with community acquired pneumonia, serum free cortisol levels did not seem superior to serum total cortisol levels as predictors of disease severity and outcome [8].

**Future perspectives**

We identified metabolic acidaemia and coagulation disturbances as predictors for RAI in the critically ill patient, but we do not know the cellular and/or tissue mechanisms directly leading to RAI. Does underlying circulatory insufficiency and perhaps adrenal hypoperfusion cause RAI, or does metabolic acidosis directly suppress adrenal cortisol synthesis? Which are the circulating factors involved promoting platelet aggregation and impairing adrenal function, or is RAI perhaps caused by adrenal microcirculatory thrombosis or bleeding? We need to stress that our studies were of retrospective design and the results on the effects of corticosteroids on mortality in septic shock and non-septic hypotensive patients should be evaluated prospectively. The literature on this is still inconclusive in spite of several (positive) meta-analyses, which heavily rest on the Annane-study, but, as described, recently being contradicted by the CORTICUS results [15,18,38-40].

We retrospectively demonstrated that an increase in the cortisol response to repeated ACTH testing is associated with a decrease of sepsis and severe disease, and increase of survival, but it needs to be investigated whether the reproducibility of ACTH testing could be demonstrated in a prospective setting as well, which is one of the questions to be answered by an ongoing project in our institution. Furthermore, future studies could include investigating the effects of corticosteroid treatment on adrenal responsiveness by repeated ACTH testing in a predefined time interval. Are differences discernable between septic (shock) and non-septic patients, and are differences maintained after decrease of disease severity and sepsis?

As lined out, comparing results of ACTH testing with 250 versus 1 µg of exogenous ACTH may show a subset of patients with a worse outcome. Thus, comparisons may be made of data between cortisol responses upon 250 µg of exogenous ACTH which is considered as supranormal, and 1 µg of exogenous ACTH. Furthermore, studies comparing free cortisol levels with total cortisol levels and their increases upon exogenous ACTH in their capacity to predict disease severity and outcome in septic shock and non-septic patients will give more insight into pathophysiological mechanisms of serum cortisol and its bio-active forms. Data on free cortisol levels are underway in our institution.
Final conclusions

Relative adrenal insufficiency may be related to disease severity and mortality and beneficial effects of corticosteroid treatment in patients in the intensive care unit, but the concept is not well defined. In this thesis we have gained insight into risk factors for RAI and possible pathophysiological mechanisms such as metabolic acidaemia and coagulation disturbances. RAI particularly seems to occur in patients with septic shock, whereas in non-septic critically ill patients and patients after cardiac arrest who were successfully resuscitated and treated with induced hypothermia, ACTH/cortisol patterns do not predict beneficial effects of corticosteroid treatment on haemodynamics and mortality. Furthermore, the change in cortisol response to ACTH in time during critical illness is not likely caused by poor reproducibility or altered baseline levels, but by varying degrees and reversibility of sepsis-induced RAI. These findings may help to select patients who benefit most for moderate-dose corticosteroid treatment, and may give insight for future prospective studies and investigation regarding cellular and tissue mechanisms leading to RAI.
References
