Neuroendocrine Stress Response after Burn Trauma

ANDREAS LINDAHL
Some aspects of the stress response during acute intensive care for severe burns are described and quantified by measuring hormonal and neuroendocrine patterns and relating these to organ function in the short term. This includes an assessment of whether there are markers for the severity of stress that are better than conventional descriptors of the severity of a burn in predicting failing organ function.

P-CgA after a major burn injury is an independent and better predictor of organ dysfunction assessed as SOFA score than the traditionally used TBSA% burned. The results also suggest that the extent of neuroendocrine activation is related to organ dysfunction, and this motivates a more extensive effort to evaluate P-CgA as a prognostic marker with respect to mortality and long-term outcome.

P-NT-proBNP exhibited a complex pattern with considerable inter-individual and day-to-day variations. Values of P-NT-proBNP were related to size of burn, water accumulation and systemic inflammatory response. A considerable covariation with trauma response and SOFA scores was observed in day by day analyses, but with weight change only on day 2.

Maximum P-NT-proBNP showed a stronger correlation with SOFA score on day 14, with mortality, and with LOS, than did age and TBSA% burned. High values were also independent predictors of all subsequent SOFA scores up to two weeks after injury.

P-NT-proBNP and NT-proANP reflect and predict organ function after burn injury similarly, notwithstanding a significantly larger intra-individual variability for P-NT-proBNP. P-NT-proBNP, but not NT-proANP, reflects the systemic inflammatory trauma response.

Free cortisol concentration was related to the size of burns, as was the circadian cortisol rhythm. This effect of burn size was, at least in part, related to its effect on organ function.

This thesis points to the fact that the stress response is richly interwoven, and cannot be adequately assessed by one biomarker only. All biomarkers studied here can be viewed as representing efferent limbs of the stress reaction, and they would need to be supplemented by biomarkers representing individual physiologic responses that follow the stress signaling.

Keywords: Burn, Injuries, Neuroendocrine, Intensive Care, Cortisol, Chromogranin A, Natriuretic peptides

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To my parents
“Complete freedom from stress can be expected only after death.”
Hans Hugo Bruno Selye
List of Papers

The thesis is based on the following publications. They are referred to in the text by their Roman numerals.


III Lindahl A, Stridsberg M, Sjöberg F, Ekselius L, Gerdin B. Concentrations of ANP and BNP convey different types of information in Burn Intensive Care. Submitted for publication

IV Lindahl A, Stridsberg M, Sjöberg F, Ekselius L, Gerdin B. Circadian Cortisol Rhythm after Burn Injury. Submitted for publication

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABLS</td>
<td>Advanced Burn Life Support</td>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ANP</td>
<td>Natriuretic Peptide type A (previously known as Atrial Natriuretic Peptide)</td>
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<td>ATLS</td>
<td>Advanced Trauma Life Support</td>
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<tr>
<td>BICU</td>
<td>Burn Intensive Care Unit</td>
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<td>BNP</td>
<td>Natriuretic Peptide type B (previously known as Brain Derived Natriuretic Peptide)</td>
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<td>CCI</td>
<td>Chronic Critical Illness</td>
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<td>CgA</td>
<td>Chromogranin A</td>
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<tr>
<td>CRH</td>
<td>Corticotrophin-releasing hormone</td>
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<tr>
<td>CBG</td>
<td>Cortisol Binding Globulin</td>
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<tr>
<td>CV</td>
<td>Coefficient of Variability</td>
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<tr>
<td>HPA-axis</td>
<td>Hypothalamic-pituitary-adrenal axis</td>
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<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
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<tr>
<td>ICUAW</td>
<td>ICU-acquired weakness</td>
</tr>
<tr>
<td>LOS</td>
<td>Length of Stay (Days)</td>
</tr>
<tr>
<td>NP</td>
<td>Natriuretic peptide</td>
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<tr>
<td>NT-proANP</td>
<td>N-terminal proANP; an inactive pro-peptide co-released with ANP</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal proBNP; an inactive pro-peptide co-released with BNP</td>
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<tr>
<td>PTSD</td>
<td>Posttraumatic Stress Disorder</td>
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<tr>
<td>PVN</td>
<td>Paraventricular Nucleus</td>
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<tr>
<td>ROC</td>
<td>Receiver Operator Curve</td>
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<tr>
<td>SOFA</td>
<td>Sequential Organ Failure Assessment, formerly Sepsis Organ Failure Assessment</td>
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<tr>
<td>TBSA</td>
<td>Total Body Surface Area</td>
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Introduction

The term “stress” is used in this thesis to describe a non-specific response to extreme demands on the human organism. While it is true that individuals react differently to stress, it is equally true that there are common characteristics and universal features in the physiological stress response in the human organism.

Trauma, an important cause of morbidity and mortality worldwide, induces a strong stress response. Burn trauma is special in at least two respects: it is one of the most severe types of trauma imaginable, and it is quantifiable in a way other types of trauma are not.

The stress response is complex and is controlled by nervous and endocrine regulation. Its purpose is to secure survival when there is a severe and acute threat to the individual. After prolonged or excessive stress, however, destructive effects can be observed on the organism. The stress response modulates cardiovascular function, immune function and metabolism.

Scrutiny and elucidation of the stress response after burn injury will ultimately enhance our understanding of different types of posttraumatic pathology, as well as advance burn care.

Trauma

Trauma, originating from the Greek word meaning "wound", refers to "an injury (such as a wound) to living tissue caused by an extrinsic agent" [88]. While initially used in a purely somatic context the term has recently also been used more extensively to describe psychiatric consequences of somatic or psychiatric events, "psychiatric trauma". In the present thesis, the term is used in its strict somatic context, if not specifically stated otherwise.

A trauma of a magnitude with a significant risk of ending up in disability or death is defined as a "major trauma". Examples of such injuries are serious head injuries, severe gunshot wounds and different types of accidents caused by high-energy forces. A major trauma demands optimal acute trauma care to combat organ- or life-threatening consequences of the injury. Such resuscitation of a trauma patient often involves multiple management procedures. Trauma is the sixth leading cause of death worldwide, accounting for 10% of all mortality, and is a serious public health problem with significant social and economic costs [82].
An excellent model for major trauma is the catastrophic experience of being afflicted with an extensive burn injury.

**Burn trauma**
A burn is a tissue injury caused by exposure to thermal energy. Exposure to caustic chemicals or radiation causes similar injuries that, from a practical point of view, are therefore regarded as "burn injuries".

**Epidemiology**
Burn injuries are among the most devastating of injuries and constitute a major global public health crisis [40]. Burns are the fourth most common type of trauma worldwide, following traffic accidents, falls, and interpersonal violence [140]. Approximately 90 percent of burns occur in low to middle income countries, regions that generally lack the necessary infrastructure to reduce the incidence and severity of burns [93]. In the United States, 4500 people die from burn injuries each year, and up to 10 000 die every year from burn related infections.

It is estimated that just over 30 000 burn injuries require medical attention each year in Sweden, and that roughly 1000 patients require hospitalization due to burn injuries, of whom about 300 are treated as part of National Specialized Medical Care in centers for treatment of severe burns. These figures mean that the current incidence of burn injuries requiring hospital admission in Sweden is about 11 per 100 000 inhabitants [134], which can be compared to 29 per 100 000 inhabitants in Great Britain [109] and 31 per 100 000 inhabitants in Southern Italy [9]. The most reliable figure for the entire United States is 14-15 per 100 000 [5].

The most common causes of burn injuries in the Western world are heat exposure due to scalding, flame and contact with hot objects [5; 19]. Among these, flame injuries are most common except in children under the age of five, who are more prone to scalding accidents. Electrical and chemical burn injuries represent a minority but can be very serious [18; 6]

Risk groups for burn injuries are children and the elderly, the disabled, individuals with alcohol- or drug abuse, and the socioeconomically disadvantaged. About one fourth of those who are admitted to a hospital require specialized burn care [6].

**Basic pathophysiology**
The key pathophysiologic features of tissue exposure to heat are related to immediate or late cell damage or death. An important feature is the virtually immediate alteration in connective tissue properties that results in a rapid
transport of intravascular water to the damaged site. In larger injuries the loss of intravascular water leads to severe hemoconcentration and burn shock, unless adequately corrected. The main aim during the resuscitation phase is to titrate the replacement of intravascular fluid to maintain an optimal circulation without detrimental consequences due to excessive water overload.

In parallel, there is activation of most inflammatory cascade systems in the body as part of an intense posttraumatic response, which is largely proportional to the magnitude of the burn, and which under unfavorable conditions may lead to failure of isolated or multiple organs over the forthcoming weeks. A serious effect is also an endogenous immunodepression, which has negative consequences regarding resistance to infections.

The resuscitation phase, which generally is 24 to 48 hours for a major burn, is followed by a phase dominated by the negative consequences of water overload, which in some patients may be 20 liters or more and is threatening to organ function.

During this time catecholamines are often manifoldly increased, leading to a state of hypermetabolism, insulin resistance and glucose intolerance, an increase in peripheral lipolysis, and catabolism of peripheral protein [142].

Towards the end of the first week the risk of infection with sepsis and subsequent organ failure becomes the main threat, and most of the in-hospital mortality occurs after this time.

Rehabilitative measures are undertaken from day one and become a greater focus as wounds heal during the later stages of the acute phase. Main issues include minimizing handicaps from scarring and improving physical and psychosocial function.

Stress response

History and concepts of stress

The history of stress has been summarized elegantly by George Fink [37]. The French physiologist Claude Bernard (1813-1878) was the first to formally explain how cells and tissues in multicellular organisms might be protected from stress. In 1859 he pointed out that the fixity, or stability, of the "milieu intérieur" is the prerequisite for free and independent life.

In the early 20th century, Walter Bradford Cannon suggested the designation "homeostasis" for the coordinated physiological processes that maintain steady states in the organism. He also coined the expression “fight or flight” to describe an animal’s response to threat by means of a general discharge of the sympathetic nervous system to prime the animal for fighting or fleeing. This was later recognized as the first stage, the acute stress response, of a
general adaptation syndrome (GAS) postulated by the “father of stress”, Hans Selye of Vienna. He observed that patients with a variety of illnesses had many of the same “nonspecific” symptoms that were a common response to stressful stimuli experienced by the body.

In the 1980s, Peter Sterling and Joseph Eyer advanced the concept of "allostasis", providing “stability through change” brought about by central nervous regulation of the set points that adjust physiological parameters to meet the stress/challenge [127]. This concept is in contrast to homeostasis, meaning “stability through constancy” around an already fixed set point.

At the center of the adaptation to stress are precise alterations in expression of a multitude of genes, and at the molecular level the cellular response to high temperatures and other types of stress is represented by a changed transcription pattern resulting in alterations in the synthesis of a variety of proteins [43]. Among such “stress proteins”, much attention has been focused on the heat shock proteins (HSPs) [43]. HSPs are involved in the maintenance of the folding of proteins and their purpose is to protect the protein structure and thereby all cellular functions when under environmental threat. The heat shock response is ubiquitous and highly conserved in all organisms.

Current concepts
The first and most generic definition of stress, “Stress is the nonspecific response of the body to any demand”, still holds. Stress involves a stressor and a stress response. The stressors are "identified" by, and the "response" is keyed by, the brain [22]. The term “allostasis” is less ambiguous than the term “stress response”. Allostasis is the meaningful physiological neuroendocrine response that helps us meet our everyday challenges as well as more threatening situations. “Allostatic load” and “allostatic overload” are terms designated for the situation of a dysregulated or protracted stress response that does not help the organism back to homeostasis, but rather has deleterious effects [58]. Nevertheless, the term “stress” is so firmly established that it will be used in this text. While body injuries cause various degrees of acute stress, chronic stress is more often ascribed to psychological (or experiential) factors. Depending on the type of stimulus, the time and the individual factors that are involved, the stress response pattern varies widely. Acute stress is characterized by an overactive sympathetic nervous system and an increased output of various hormones. More protracted physical [138] and psychological [14] stress involves an exhausted response. In major depression there is a similar exhaustion [106]. It is of interest that traumatic events in childhood affect the stress response pattern in adult life [95].

There are considerable individual differences in how stress is handled and responded to. The term “resilience” can be viewed as a reciprocal characteristic or antonym to the concept of “vulnerability”. Resilience is a crucial
component in determining the way in which individuals react to and deal with stress [23]. The expression locus of control, which can be either internal or external, is important. In the awake patient the experience of external locus of control is associated with chronicity of the stress response, i.e. allostatic overload. An internal locus of control is thought to have a stress relieving effect [37]

**Neuroendocrinology of the stress response**

The brain is the key organ in the response to stress and defines internal and external events that may be threatening and potentially harmful, as well as the physiological and behavioral responses. Those responses can be either adaptive or damaging [85]. Stress involves a two-way communication between the brain and the cardiovascular, immune, and other systems via neural and endocrine mechanisms. However, different stressors are sensed by different “afferent” systems. Some types of stress such as pain, for example, are sensed and keyed by the nervous system, but stressors that primarily affect the viability of cells, such as a burn or an infection, are sensed directly by affected cells in the damaged tissue and are propagated by endocrine mechanisms. The response is subsequently propagated by tightly coupled and integrated neurogenic as well as endocrine efferent mechanisms. In addition, the stress response can be initiated or potentiated by the cortex of the brain when it interprets experiences as threatening. Stress in relation to physical trauma is regulated mostly on an unconscious level, and is therefore present in the sedated patient during intensive care, as well as in the awake patient.

A hallmark of the stress response is activation of the autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis – see below, which leads to the “fight-or-flight” response, the classical way of envisioning the behavioral and physiological response to a threat. This occurs regardless of the type of danger, whether it is an attacking lion, a hostile attack by another person, a car accident, or the perception of a danger that in reality does not exist.

Neurogenic acute responses are mediated by the autonomous nervous system and are dominated by sympathetic activation in the form of epinephrine and norepinephrine release from sympathetic nerves or the adrenal medulla.

The endocrine effector response involves signaling from the hypothalamus, the pituitary gland, which has been called “the conductor of the neuroendocrine orchestra” [36], as well as from peripheral organs.

There are many mediators participating in allostasis. They are linked together in a network of regulation that is nonlinear, meaning that each mediator has the ability to regulate the activity of the other mediators, sometimes in a biphasic manner. An intrinsic phenomenon in this nonlinearity is that when any one mediator is increased or decreased, there are compensatory
changes in the other mediators. These related changes depend on the time course and level of change of each of the mediators. Unfortunately, biomedical technology cannot yet measure all components of this system simultaneously and must rely on measurements of only a few of them in any one study [85]. Nevertheless, the nonlinearity must be kept in mind when interpreting the results that are obtained.

An example of nonlinearity is the release of the major “stress hormone” cortisol, produced by the adrenal cortex in response to the adrenocorticotropic hormone (ACTH) from the pituitary gland, which in turn is stimulated by corticotrophin-releasing hormone (CRH) from neurons originating in the hypothalamus. It has recently been demonstrated that positive feedback from neurosteroids onto CRH neurons is required to mount the physiological response to stress [116]. The effect is mediated by GABA receptors on these neurons. Further, it has been shown that by turning off a subunit of these receptors, no stress response can be initiated. The release of a number of pro- and anti-inflammatory cytokines from a variety of cells that regulate each other, and that in turn are regulated by glucocorticoids and catecholamines, are other examples. Whereas catecholamines can increase the production of proinflammatory cytokines [13], glucocorticoids are known to inhibit this production [115]. There are exceptions, however, such as proinflammatory effects of glucocorticoids that depend on dose and cell or tissue type [31; 79].

The parasympathetic nervous system also plays an important regulatory role in the nonlinear network of allostasis, since it generally opposes the sympathetic nervous system. It has a negative chronotropic effect on the heart as well as anti-inflammatory effects [15; 133].

There are enormous individual differences in the response to stress that are based on genetics [112] and on the experiences of the individual in early life [50]. Early life maternal care in rodents is a powerful determinant of lifelong emotional reactivity and stress hormone reactivity, and increases in both are associated with earlier cognitive decline and a shorter life span [21; 41]. It is not uncommon to hear that hardships have “aged” a person, and, indeed, the “weathering hypothesis” [45] proposes that stressful life experiences accelerate aging.

Studies on endocrine mediators of stress reactions have so far mostly been focused on the HPA-axis and on the role of glucocorticoids, above all cortisol. However, knowledge concerning the regulation of established stress hormones and of the HPA-axis is continuously growing. In addition, and supported by the view of the complexity of the stress system, new mediators, and new functions of established mediators, have recently become evident. In addition to an outline of the HPA-axis and glucocorticoid regulation, three such mediators with characteristics that make them candidates for a role in the acute stress response after injury, are described below.
HPA-axis and cortisol
The hypothalamic-pituitary-adrenal (HPA) axis is a key adaptive neuroendocrine system that is fundamental to all responses to stress in the human organism. This tightly coupled hormone axis is highly enlaced with the central nervous system, and it exerts control over glucocorticoid (cortisol in humans) secretion, which is essential to combating all forms of allostatic load in mammals and is critical to life [135; 84].

The HPA-axis can be described as a feedback loop that includes the hypothalamus, the pituitary and the adrenal glands. Structurally, it consists of the paraventricular nucleus of the hypothalamus (PVN), which secretes corticotrophin-releasing hormone (CRH), and also arginine vasopressin (AVP); the pituitary gland, which responds to incoming controlling stimuli, and produces ACTH; the adrenal gland cortex, which secretes the glucocorticoids, mainly cortisol in humans, and dihydroepiandrosterone (DHEA) in response to interaction with ACTH. Hormones from all different levels of the HPA-axis act within the axis by modulating release of hormones from other levels, and/or from central or peripheral sites outside the axis.

Control of cortisol secretion
Corticotrophin Releasing Hormone (CRH), a 41 amino acid peptide, is the main physiological regulator of the HPA axis. It is produced in a subset of neurons in the paraventricular nucleus (PVN) of the hypothalamus, which is a key center of the central nervous system that integrates neuroendocrine responses to allostatic load. Once secreted, CRH enters the pituitary portal system and reaches its anterior lobe where it stimulates another subset of neurons, corticotrophs, to release ACTH [48]. ACTH release is also stimulated by AVP, but its role is not as generally well accepted. The current perspective is that AVP serves as an additive controller of ACTH release from the pituitary [48]. There are two principally different vasopressinergic systems in the brain. First, AVP produced in the supraoptic nucleus and in magnocellular cells in the PVN is transported to the neurohypophysis (posterior pituitary), which delivers AVP and oxytocin to the peripheral circulation where they regulate water homeostasis and blood pressure. Second, AVP is produced in parvicellular neurons in the PVN, from where AVP is secreted via the local portal system to the anterior pituitary. AVP seems to interact with CRH in controlling release of ACTH [48].

Numerous afferent neural pathways carry information about the stressor and thereby influence regulation of these CRH neurons, which are also regulated through negative feedback mechanisms that are central to the autoregulation of the HPA-axis. Examples of afferent neural inputs are projections from ascending catecholaminergic pathways, from the limbic system, the hippocampus and the amygdala [53]. There are also indirect pathways from sensory systems in the forebrain that may stimulate CRH neurons.
by activating stimulatory (glutamatergic) or repressing inhibitory (GABA-ergic) interneurons [52].

The central sensors of feedback to the HPA-axis are two corticosteroid receptors, the high-affinity mineralcorticoid receptors (MR) and the low-affinity glucocorticoid receptors (GR), which are expressed in the brain and on the corticotrophs, i.e. the basophilic cells of the anterior pituitary responsible for ACTH secretion. A direct inhibitory effect of glucocorticoids on CRH transcription underlies the central mechanisms of feedback regulation and desensitization of the HPA-axis to stress responses [3].

The CRH that enters the anterior lobe induces secretion of adrenocorticotropic hormone (ACTH). ACTH triggers release of cortisol and dihydroepiandrostendione (DHEA) from the adrenal cortex. There is evidence that the mechanistic link between ACTH and cortisol is less tight than previously thought since there are studies indicating dissociation between the two factors [28]. Cortisol acts on specific receptors present in most peripheral tissues and the brain and triggers the metabolic, immune, neuromodulatory, and behavioral changes needed to cope with the impact of the stressors [78].

To underline the complexity of this stress response system alone, it is necessary to clarify that the adaptive influence of the HPA-axis under stress is realized not only through cortisol, but also through ACTH and the hypothalamic neuropeptides CRH and AVP that are released in the brain, where they are responsible for behavioral and autonomic responses to stress. Furthermore, to fully understand regulation of HPA-axis activity, network approaches must be taken as brain regions do not function as monolithic activators or inhibitors [53].

In the resting condition, cortisol secretion has a circadian rhythm that is a net result of ultradian changes with pulsatile secretion that are greater in amplitude during the phase of wakefulness. This leads to higher average levels of glucocorticoids during the day in humans. Rhythmicity in the HPA-axis is essential for the normal functioning of the brain and of all other glucocorticoid responsive organs, and this is an example of a key adaptation to cope with the different environmental challenges the organism faces at different times of day, such as changes in lighting conditions and temperature, food availability or the presence of predators [28]. These circadian changes are driven by an endogenous biological pacemaker made up of two components: one central pacemaker in the suprachiasmatic nucleus (SCN) and one peripheral pacemaker in the adrenal cortex affecting sensitivity to ACTH [28]. AVP is believed to regulate circadian rhythms [61] and is expressed in the SCN [66].

**Measurements of cortisol**

Assessment of the peripheral concentration of cortisol has long been a way of estimating the extent of activation of the HPA-axis, and thus the stress
response. Decades ago, accumulative measurements of the metabolic end products 17-hydroxycorticosteroids in the urine were introduced as an integrated measure of corticosteroid production. As modern technologies emerged, however, direct measurement of cortisol in plasma or serum was made available. This measurement only partly reflects the concentration of cortisol available for cells, as most of the cortisol is bound to Cortisol Binding Globulin (CBG), and to some degree also to albumin. The free fraction is not commonly measured with methods used in clinical practice, and requires ultrafiltration of samples or equilibrium dialysis. To handle this problem a number of mathematical models are used to estimate the free serum cortisol fraction [24; 32]. Attempts have recently been made to measure the interstitial concentration of cortisol, but there is currently no intellectual framework for interpretation of those data.

The cortisol concentration in saliva correlates well with the free concentration in plasma or serum and has frequently been used as a proxy for these measurements.

**Chromogranin A**

The concentration of chromogranin A (CgA) in plasma (P-CgA) is a biomarker of stress and sympathetic overactivity [26]. CgA is a glycoprotein/prohormone which is co-secreted with epinephrine and norepinephrine by chromaffin cells from the adrenal medulla and also together with other hormones from the neuroendocrine system [131].

CgA is not only a biomarker. Both the parent molecule and peptides derived during its proteolysis, such as vasostatin, pancreastatin, catestatin, and serpinins, have biological effects [51; 74]. For example, both CgA and vasostatin-1 are important modulators of the endothelial barrier function [74], while both vasostatin-1 and catestatin directly suppress myocardial inotropy and lusitropy, exerting remarkable anti-adrenergic modulation [83], while the serpinin peptides appear to act in an opposite, β-agonist-like manner [74]. A hypothesis is that, via its vasostatin-1 and catestatin domains, the normal levels of circulating CgA may provide a homeostatic buffer function against effects of an excessive stress response [51].

The concentration of CgA in plasma responds to large-scale perturbations of the sympathetic nervous system, appears relatively insensitive to short-term behavioral challenge, and seems to be an early marker of severity in critically ill patients [148]. P-CgA responds to significant alterations in the sympathetic nervous system, but is said to be relatively insensitive to acute stress [29]. However, it has been observed that the concentration of CgA has a close relation to the release of norepinephrine, which is why it can be seen as a reflector of sympathetic activity [30].

It was recently shown that the concentration of CgA [148; 113], and of its downstream product vasostatin-1[117], were strong and independent predic-
tors of mortality [148; 113] in patients with critical illness. However, the integrated role of CgA in clinical stress reactions has not been elucidated.

**Natriuretic peptides**

Natriuretic peptides constitute a peptide family which, in addition to peripheral effects on the regulation of water balance, have receptors throughout the CNS and constitute a neuromodulatory system with influence on emotional behavior, for example anxiety and arousal. They are also involved in the regulation of the HPA-axis and the autonomous nervous system [57; 141].

There are currently three structurally related hormones in this family that are coded by separate genes. The first to be discovered was ANP, originally named “atrial natriuretic peptide” [62], but later “A-type natriuretic peptide”, followed by BNP, “brain natriuretic peptide” [114], renamed “B-type natriuretic peptide”. Years later the third member of the family, C-type natriuretic peptide, CNP, was discovered [128]. They all utilize a common receptor family with three members mediating their effect and/or their internalization and degradation [107]. In addition to their widespread distribution in the vasculature, the adrenals and the kidney, there is abundant evidence that natriuretic peptides and their receptors are widely distributed in the central nervous system, suggesting possible roles in modulating physiological functions of the CNS [20].

The natriuretic peptides increase venous capacitance and promote a natriuresis that reduces extracellular fluid volume. This is partly due to direct effects on both glomerular and tubular functions of the kidney and partly on suppression of the angiotensin–aldosterone axis [73]. They also reduce sympathetic tone in the peripheral vasculature. This reduction is probably caused by dampening of baroreceptors, by suppression of the release of catecholamines from autonomic nerve endings, and especially by suppression of sympathetic outflow from the CNS [73]. CNP inhibits aldosterone secretion, but it has little effect on arterial pressure or salt and water excretion [54].

ANP is mainly stored and released from granules in the atrial myocardium [34], while BNP is stored and released from granules in both the atria and ventricles [80]. Less is known concerning CNP, but it is the most widely expressed NP, with synthesis in the brain, chondrocytes and endothelial cells. CNP is thought to act locally as a paracrine/autocrine regulator, since it is cleared rapidly from the circulation and present at very low concentrations in plasma [77]. In chronic heart failure ventricular synthesis is upregulated, above all for BNP, which is why BNP is known as a ventricular hormone [55; 76; 92]. ANP, BNP, and CNP are formed by enzymatic cleavage of a prohormone to form similar amounts of the carboxy-terminal biologically active peptides and amino-terminal biologically inactive fragments [47; 80; 77]
Higher concentration of the A-type natriuretic peptide (ANP) than in controls has been observed in burn patients, but the reason for the increased level is unclear [97]. The B-type Natriuretic peptide (BNP) has recently been extensively investigated as a biomarker for a failing ventricular myocardium, where it is primarily synthesized [80]. Here, gene expression of the BNP prohormone is rapidly increased during periods of ventricular wall stretch. The concentration in plasma is therefore considered to reflect volume load on the heart, and is used in diagnosing heart failure. The BNP molecules activate surface receptors, the activation of which results in a decreased cardiac pre- as well as after-load by several mechanisms including that of a reduced sympathetic tone.

In ICU settings and in chronic inflammation, however, data suggest that the concentration of BNP is also affected by non-heart-related mechanisms. BNP concentrations have been suggested as markers of hydration state in patients with severe burns and predictors of outcome in critically ill patients, but results are contradictory.

Effects of long-standing stress
Severe stress affects the structure and function of the nervous system. Chronic stress in animal models causes atrophy of neurons in the hippocampus and prefrontal cortex, brain regions involved in memory, selective attention, and executive function, and causes hypertrophy of neurons in the amygdala, a brain region involved in fear and anxiety as well as aggression [85]. Several studies, for example [16], also correlate stress with atrophy of the hippocampus in man, due at least partly to an increased release of glucocorticoids via glutamatergic toxic effects [70]. There are also receptors for glucocorticoids in the hippocampus [44]. It seems that such an effect of glucocorticoids on the hippocampus is reversible [126]. To complicate this issue, recent data have made previous studies difficult to interpret as it seems that the pre-exposure size of the hippocampus is actually related to the susceptibility to stress [46]. The hippocampus plays a role in shutting off the HPA stress response, and damage to or atrophy of the hippocampus impairs the shut off and leads to a more prolonged HPA response to psychological stressors [52].

Stress also has an impact on other cortical and subcortical brain structures [78]. Finally, factors other than glucocorticoids also play a role in the impact of stress on the CNS [84].

Sleep deprivation produces an allostatic overload that can have deleterious consequences. Sleep restriction to four hours of sleep per night increases blood pressure, decreases parasympathetic tone, increases evening cortisol and insulin levels, and promotes increased appetite, possibly through the
elevation of ghrelin, a pro-appetitive hormone, along with decreased levels of leptin [72; 124; 125].

To summarize, chronic stress has negative consequences for learning, memory, and decision-making, as well as increasing levels of anxiety and aggression. The adult brain is a malleable organ and adapts structurally and functionally to experiences that are stressful and potentially deleterious. Some of these changes do not necessarily constitute “damage” but may nevertheless be long lasting.

Trauma, burn trauma, ICU care and the stress response

A severe burn trauma is one of the most stressful conditions that can befall man. It affects all main integrating systems in the body and from an overall perspective it can be viewed as an excellent model for a severe trauma as such. The acute stress response after burn trauma involves catecholamines [142] as well as cortisol [137] and glucagon [59]. The stress response, the altered vascular dynamics, and pain are the three most important reasons why severely burned persons are treated in Burn Intensive Care Units (BICU).

Injured individuals exhibit widely differing premorbid characteristics that affect their ability to combat stress. They have different psychiatric histories, personalities and socioeconomic backgrounds, somatic health and fitness, types and perceptions of care, and they differ concerning their adaptation and outcome.

There are several factors to consider when analyzing why treatment in a BICU is associated with considerable, often long-standing and iterative stress. First, the condition that motivates ICU care is associated with a stress response. Second, burn patients are commonly subjected to secondary insults in the form of surgery and infections. Third, ICU patients often present with disturbances in sleep [99], loss of a normal melatonin secretion pattern [96] and a disruption in circadian rhythms of the HPA-axis [138]. Fourth, various components of the care, for example repeated painful procedures [101] and the expectation of pain from such procedures [105], have been suggested to be contributory. In fact, frightening experiences during ICU care and length of stay in the ICU have been related to late problems in the form of depression or PTSD [111; 110]. However, how the underlying illness as such and the length of the ICU period affect the characteristics of the stress response has not yet been determined.

Patients treated in intensive care units are often victims of the so-called ICU syndrome, which is comprised in the definition of a delirious state [86]. Delirium during ICU care is actually quite common, and has been related to a longer length of stay and an increased mortality. Well established causes are infections and medication, but there are also reports of delirium in cases
of psychic stress and sleep deprivation [86]. ICU patients often suffer from a poor quality of sleep [42], which is considered to be at least partly related to an inappropriate pattern of release of melatonin [122], a disturbed circadian rhythm of the HPA-axis, and the release of endogenous opiates [87]. During burn ICU care more or less severe psychic symptoms, including sleep problems, psychoses, behavioral problems, anxiety and depression are common [100]. A protracted course under a high stress load in critical care may lead to the development of chronic critical illness (CCI) [25]. CCI is a relatively common clinical entity characterized by prolonged ventilatory support, neuromuscular weakness, brain dysfunction, malnutrition, endocrinopathies and symptom distress [94]. A major complication of critical illness, also thought to be related to aspects of a sustained severe stress load, is ICU-acquired weakness (ICUAW), which is probably a generalized progression of critical illness polyneuropathy and myopathy with consequent progressive muscle atrophy [33].

There are important characteristics of a burn injury that contrast with a “true” fight or flight situation. Prolonged hormone and neurotransmitter elevation, absence of increased muscle work, and presence of massive tissue injury are exacerbated by surgical debridement of burn wounds and skin grafting procedures. Many questions remain regarding this prolonged activation, but it is clear that neuroendocrine changes regulate metabolic, cardiovascular and immune function, and ultimately wound healing, recovery and survival. As critical care advances and intensive care unit mortality declines, the number of survivors of critical illness is increasing. These patients frequently experience long-lasting complications, and it is therefore important to understand why they appear and to implement evidence-based practices to minimize them.

In spite of rapidly growing knowledge concerning components of the stress response, there is currently no integrated description of the stress activation patterns in patients receiving burn intensive care, or of which endocrine pathways are more activated than others, or of the relationships between different pathways. Furthermore, treatment strategies for severe burns have changed dramatically during the last few decades, which is why previously obtained fragmentary knowledge concerning hormonal changes after burns needs to be revised.

Assessment of severity of illness in the ICU

The need to describe the severity of illness in patients in the ICU in a manner that allows for comparisons based on large patient cohort data has motivated the development of ICU-scoring systems. The models can be categorized into general-purpose severity models and models of organ dysfunction. In the first group, the Acute Physiology and Chronic Health Evaluation
(APACHE) [65], the Mortality Prediction Model (MPM) [71], and the Simplified Acute Physiology Score (SAPS) [69] have become the most well accepted, further developed and widely used models. The most important systems in the second group are the Sequential Organ Failure Assessment (SOFA) [145], the Multiple Organ Dysfunction Score (MODS) [81] and the Logistic Organ Dysfunction System (LODS) [68].

The first group of scores predicts mortality based on physiological variables collected in the first 24 h of ICU stay, ignoring the fact that morbidity and mortality are very closely correlated, and that changes in the initial parameters may influence patient outcome [60]. The fundamental premise of these systems is that clinical variables at or near the time of ICU admission are associated with the risk of hospital mortality [132].

The second group, the organ dysfunction scores, assesses severity of illness over time as organ systems show signs of worsening function or failure [143], and these scores are also used for prediction of mortality. It was therefore possible to use these models as a measure of outcome in this thesis. However, during the development of these scoring models burns were often excluded [64; 63].

The Sepsis-related Organ Failure Assessment score was originally designed to quantitatively describe organ dysfunction over time and to evaluate morbidity in septic patients [145]. It was later understood that the score could also be applied in non-septic patients, which is why the acronym "SOFA" now refers instead to Sequential Organ Failure Assessment [144]. The SOFA score is the sum of subscores for the following six organ systems: respiratory, circulatory, renal, hematology, hepatic and central nervous systems, depending on the level of dysfunction (rated as 1 to 4 points) for each. The SOFA score has also been used to predict mortality, although it was not originally developed for this function. The prognostic performance of the SOFA score used in various models for predicting mortality in adult patients in medical and/or surgical ICUs was recently systematically reviewed [89].

There are few studies on the use of SOFA in burns. Pavoni et al reported that survivors had lower SOFA scores on admission than non-survivors [102]. Palmieri et al found that the maximum SOFA score was an independent risk factor for mortality [98]. In an assessment of 439 burn patients, Lorente et al [75] used SOFA at day 4 as a measure of late organ dysfunction, as previous studies suggested that dysfunction developing soon after trauma reflects, to a greater extent, reversible derangement in organ function induced by the inciting event or incomplete resuscitation, while later measurement of organ function would better define multiple organ failure and prognosis [90]. They observed that the SOFA scores at days 1 and 4 were independent predictors of mortality.
Current investigation

Context

This study is part of a broad approach to assess the effect of a burn injury on the organism. The research group has previously produced an array of papers focusing on the psychosocial outcome after a severe burn injury, including studies on individual factors that affect the outcome. These studies have pointed to the fact that many individuals suffer from cognitive or other late psychiatric sequels after severe burns.

The overall concept is that a multitude of factors interact in defining the final integrated outcome in the long term, including preinjury individual factors, the type and extent of burn trauma, and the sometimes immense stress on the organism during the long period of intensive care, and also events during the long rehabilitation phase (Figure 1).

![Figure 1](image_url)

**Figure 1.** The interactive mechanisms defining the final outcome after a severe burn.

Based on recent literature describing similar long-term consequences of other types of protracted life-threatening states, the general research questions concern possible causative relations between the extent of physical stress during acute care and late neurofunctional sequels. In order to approach this hypothesis in a serious manner, more detailed knowledge is needed concerning components of the physiological stress response during acute care.
Aims

The main aim of this thesis is to elucidate, describe and quantify some aspects of the stress response during the acute intensive care phase for severe burns by measuring hormonal and neuroendocrine patterns, and to elicit how those aspects are related to organ function in the short term. This includes an assessment of whether there are markers for the severity of stress that are better than conventional descriptors of the severity of a burn in predicting failing organ function.

The overall aim of this thesis can be summed up in four points:

- to investigate the release pattern of CgA in acute care of severe burns and to relate this to organ function
- to investigate the release pattern of BNP in acute care of severe burns by measuring its co-released propeptide NT-proBNP, and to relate this to water load, inflammatory response, and organ function
- to investigate the relationships between circulating concentrations of NT-proANP and NT-proBNP in an attempt to reveal different mechanisms in their release and turn-over
- to investigate the diurnal variability of cortisol in severe burns and to assess mechanisms affecting this variability
Material and Methods

Patients
The patients reported on in this thesis were recruited consecutively during 2003 through 2009 from the ICU at The Burn Center at Uppsala University Hospital, which is one of two national centers responsible for the in-hospital care of severe burn victims in Sweden, which has a population of 9.3 million. The Uppsala Burn Center is a referral center for patients primarily from the northern and middle parts of Sweden.

Inclusion criteria were i) age between 18 and 60 years; ii) burn injury exceeding 10 % TBSA; iii) admission to the Burn Center within 48 h after injury; iv) without ongoing steroid treatment; v) no ongoing steroid intake and vi) no known neuroendocrine tumor. Before enrollment, information about the study was given to the patient, or in sedated patients to a next of kin, by a research nurse who also secured formal approval for participation.

Fifty-two patients fulfilled the inclusion criteria. Only one of these declined participation, resulting in the inclusion of 51 patients. In paper II and paper III sufficient data were not obtained for one of the male subjects, resulting in the inclusion of 50 patients. In paper IV 2 male subjects were excluded for the same reason, resulting in the inclusion of 49 patients. Table 1 shows the study population.

Procedure
The Uppsala and Linköping Burn Centers together treat all severe burn injuries in Sweden, and are now also responsible for longitudinal multiprofessional follow-up. This is a new situation that will ensure population based recruitment for interdisciplinary studies. Since 2000, all severely burned patients admitted to the Uppsala University Hospital who have given their consent have been enrolled in the Uppsala Burn Center Research Program.

The investigations of these patients include many psychiatric evaluations and tests outside the scope of this thesis, and also include an assessment of the physical stress load during the ICU period and during recovery.
Table 1. Characteristics of the individuals included in the studies. Data are given as integers or median and range.

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
<th>Study 3</th>
<th>Study 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Included</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Did not want to participate</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Insufficient data</td>
<td>-12</td>
<td>-1</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>Study population</td>
<td>39</td>
<td>50</td>
<td>50</td>
<td>49</td>
</tr>
</tbody>
</table>

|                          |         |         |         |         |
| Gender (female/male)     | 12/27   | 15/35   | 15/35   | 15/34   |
| Mean age (years)         | 45 (20-82) | 41 (19-60) | 41 (19-60) | 41 (19-60) |
| TBSA²                    | 31 (10-96) | 31 (10-96) | 31 (10-96) | 31 (10-96) |
| TBSA full thickness²     | 13 (0-95)   | 14 (0-95)   | 14 (0-95)   | 14 (0-95)   |
| Length of stay (days)    | 21 (4-183) | 20 (4-230) | 20 (4-230) | 21 (4-230) |
| Died during stay         | 4        | 4        | 4        | 4        |

| Cause of injury          |         |         |         |         |
| Fire³                    | 32       | 41       | 41       | 40       |
| Scald                    | 3        | 4        | 4        | 4        |
| Electrical               | 2        | 3        | 3        | 3        |
| Chemical                 | 2        | 2        | 2        | 2        |

¹Data are given as integers or median and range, ²Percent, ³Including explosion and contact.

Blood samples were taken four times daily at 6 am, 12 pm, 6 pm, and 12 am on days 1, 2, 3, 4, 5, 7, 10, and 14 of ICU care, and then once a week until discharge. For some analyses samples were collected only at 6 am and 6 pm, or at 6 am only. The samples were sent to the certified Clinical Chemistry Laboratory (CCL) at the University Hospital, where they were analyzed immediately and also stored frozen in aliquots for later analysis. Arterial blood gas analysis was also performed at all sampling times.

Data were later imported from the CCL for scrutiny and validation and subsequently included in a relational database (Microsoft® Access). This database also includes demographic data, summaries of chart text, daily SOFA scores, and other variables such as vital parameters, arterial blood gas measurements, ongoing septic processes, concurrent surgical procedures, TBSA %, length of stay and mortality. Clinical information required for intensive care scoring and other important information was documented as part of a structured ICU-routine and later included into the database. Building, expanding, restructuring and validating this database has been a prioritized part of the work throughout the writing of this thesis. Although the number of included patients is large with respect to measuring molecular
biomarkers in burn patients, it still is a fairly small number with respect to statistical modeling. Therefore, great care has been taken to maximize the quality of our study database.

Measurements

Samples of serum (S-) and EDTA-plasma (P-) were obtained at predetermined sampling times, transported to the CCL, analyzed and then stored frozen at −70°C in aliquots for future reference.

P-Cga was determined using a commercial radioimmunoassay (Eurodiagnostica, Malmö, Sweden).

P-NT-proBNP was analyzed on a Cobas E601 instrument (Roche Diagnostics, Basel, Switzerland). The sample was allowed to react with a biotinylated monoclonal NTproBNP-specific antibody and a ruthenium-complex labeled monoclonal NTproBNP-specific antibody. The complexes were bound to streptavidin coated micro particles and the amount of bound ruthenium complex was analyzed by chemoluminescence emission. The total imprecision was <4%. The results were converted to the same unit as S-NT-proANP (pmol/L) using a molecular weight of 8.5 kDa.

S-NT-pro-ANP was measured in an in-house ELISA with light stimulated fluorescence detection (DELFIA) [39]. The assay was of non-competitive design and had a total imprecision of <12%.

S-Cortisol was measured on a Cobas E601 instrument. The free cortisol concentration was estimated using the equation of Coolens et al [24] and based on the concept that the concentration of cortisol binding globulin decreased in parallel with the decrease in albumin concentration, and a normal concentration of CBG of 625 nM [32].

C-reactive protein in plasma (P-CRP) was used as a measure of physiological response to trauma. B-platelets, P-creatinine, and S-bilirubin were analyzed for the purpose of SOFA-scoring. The quality of all analyses was continuously controlled according to certification procedures.

Weight gain was used as a measure of accumulated edema, and weight loss after maximum weight was reached was used as a measure of net edema mobilized back into the circulation. SOFA scores were calculated according to the Swedish Intensive Care Registry (SIR) guidelines [1].

Clinical routine

The Parkland formula served as a resuscitative strategy during the first 24 post burn hours. Hourly diuresis of 0.5 ml/kg/h was aimed for. Patients were artificially ventilated upon arrival in the unit until no longer necessary. Ventilator support was guided by the “open lung concept” [2]. Routine burn
treatment consisted of early excision and grafting in all cases. Early enteral nutrition was based on calculated caloric needs. Regular wound revisions were done under general anesthesia using standardized dressings.

Statistical analyses

A major theme in this thesis is exploration of various neuroendocrine peptide concentrations at certain time points, as well as changes over time. These have then been related to each other as well as to more thoroughly studied parameters in intensive care such as age, TBSA % burned, organ dysfunction score, length of stay, mortality, and well-known blood work parameters such as CRP. Hence, appropriate tools have mainly been various regression techniques.

Techniques

The predominating techniques were multiple linear and multiple logistic regressions. A linear mixed model procedure (LMM), which accounts for the hierarchical nature of the data, was also an important tool.

For correlation analysis Pearson and Spearman tests were utilized. Parametric methods were used whenever possible. Variables significantly skewed judged from the Kolmogorov-Smirnov test were logarithmically transformed to obtain normality. The Chi-square test was used for categorical variables, and the Mann Whitney U-test was used for ordinal and continuous variables in paper I. The paired-samples t-test with Bonferroni correction was performed for each day to test for diurnal variations in paper II.

All calculations were performed using the IBM SPSS Software Statistics, versions 19 or 21. All analyses were defined a priori.

Data presentation

When the parameter times are presented in relation to time of admission to the Burn Center, they refer to actual assessment times, which were the same for all patients. Day zero was then defined as beginning the first time after admission that the clock showed 6 am. When the parameter time is presented in relation to time of injury, the closest match with respect to time after injury was used. Area under the curve (AUC) calculations for concentration vs. time curves was based on linear interpolation.

Dichotomization of SOFA scores was achieved by defining high SOFA as 4 or more, which is stricter than in some studies but was chosen to split the cohort as equally as possible; 44 % were in the high SOFA group. Receiver Operator Curve (ROC) analysis based on dichotomized models was used for evaluating overall adequacy of the risk predictions.
Missing data were replaced utilizing a last observation carried forward strategy (LOCF) when appropriate, but never when analyzing changes over time, i.e. regarding cortisol rhythmicity or CV calculations. CV was calculated as SD/mean.

Directed Acyclic Graphs
In a non-experimental setting, the goal of covariate selection is to remove confounding by only including appropriate covariates in equation modeling. This was done in a traditional way in the first three papers, i.e. through clinical reasoning combined with statistical selection through simple regressions. In paper IV a method for covariate selection predominantly used in epidemiological research was introduced, a directed acyclic graph (DAG) model. This approach is based on counterfactual theory for defining causal effects, and has been validated as efficient in selecting confounders [139]. Two good reasons for using DAGs are that the procedure is more transparent to the reader than other alternatives and is based entirely on biology and clinical reasoning, and also that through reductive logic a minimal sufficient adjustment set for estimating the direct effect between a proposed exposure and outcome is found [123]. Covariates are selected in view of their internecine biological relationships, and are not treated as monolithic entities that have to pass a mechanistic statistical test.

Ethics
The studies were approved by the Uppsala University Ethics Committee, and the investigations were performed according to the Declaration of Helsinki.
Results

For detailed results the reader may turn to the underlying publications.

Paper I

The aim was to assess characteristics of the release of CgA after burn injury and explore relations to organ dysfunction one week after injury.

There were considerable differences between different patients with respect to the secretion pattern over time. Two different secretion patterns could be discerned based on variability between measurements. About two thirds of the patients had a low variability in P-CgA concentration during the first 7 days, and the remaining third of the patients clearly had more variability over time. A high variability group, defined as the greatest difference between samples being more 6 nmol/L, had a greater overall increase in P-CgA during the first 7 days than a low variability group. These patients were also older, had larger deep burns and more frequent surgery during the first 7 days. They also exhibited a worse multiple organ function at day 7 (SOFA score 5.6±3.4 vs. 3.0±2.5; p = 0.007).

All four different measures of P-CgA used for predictions correlated with SOFA scores at day 7. In univariate regression models (Table 2), age, TBSA % burned and three of the different measures of CgA were found to be significant predictors of SOFA at day 7, while the fourth, P-CgA-AUC 1 to 7 days, was not.

In subsequent multiple regressions, age and TBSA% burned significantly predicted SOFA at day 7 with an adjusted R² of 0.27 (Table 3).

Adding P-CgA in the models improved prediction. The addition of the 24 hours after injury value, the clinically most practical measure of P-CgA, thus resulted in a prediction equation with an adjusted R² of 0.34. The addition of the mean or maximum P-CgA value resulted in similar predictions.

To better assess the relation between sympathetic stress measured by P-CgA and clinically relevant multiple organ dysfunction, the day 7 SOFA value was dichotomized into one group with high scores and one group with low scores in a way that as nearly as possible split the study population in half, which was done by defining low SOFA as less than 4 and high SOFA as 4 or more. Receiver operator curves showed that TBSA% burned and age weakly predicted having a high SOFA score respectively.
Table 2. Univariate regressions with age, TBSA burned, and the different measures of P-CgA as independent variables and SOFA scores at day 7 as the dependent variable. (1logarithmically transformed data)

<table>
<thead>
<tr>
<th>Covariates</th>
<th>beta</th>
<th>p-value</th>
<th>adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.27</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>TBSA partial thickness burn¹</td>
<td>0.03</td>
<td>0.86</td>
<td>-0.03</td>
</tr>
<tr>
<td>TBSA full thickness burn¹</td>
<td>0.31</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>TBSA burned¹</td>
<td>0.42</td>
<td>0.009</td>
<td>0.17</td>
</tr>
<tr>
<td>P-CgA at 24 hours¹</td>
<td>0.45</td>
<td>0.005</td>
<td>0.18</td>
</tr>
<tr>
<td>P-CgA maximum value up to day 7¹</td>
<td>0.47</td>
<td>0.003</td>
<td>0.20</td>
</tr>
<tr>
<td>P-CgA mean value up to day 7¹</td>
<td>0.42</td>
<td>0.008</td>
<td>0.16</td>
</tr>
<tr>
<td>P-CgA AUC 1 to 7 days¹</td>
<td>0.33</td>
<td>0.11</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table 3. Multiple regressions with age, TBSA burned, and different measures of P-CgA as independent variables and SOFA scores at day 7 as the dependent variable.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>beta</th>
<th>p-value</th>
<th>adjusted R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.38</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBSA burned¹</td>
<td>0.50</td>
<td>0.001</td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>Age</td>
<td>0.25</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBSA burned¹</td>
<td>0.44</td>
<td>0.004</td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>P-CgA at 24 hours¹</td>
<td>0.43</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.23</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBSA burned¹</td>
<td>0.47</td>
<td>0.002</td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>P-CgA mean value to day 7¹</td>
<td>0.35</td>
<td>0.026</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.22</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBSA burned¹</td>
<td>0.41</td>
<td>0.006</td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>P-CgA maximum value to day 7¹</td>
<td>0.34</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBSA burned¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-CgA AUC 1 to 7 days¹</td>
<td>0.26</td>
<td>0.18</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

¹logarithmically transformed data; ns = not significant
Each of the four CgA related variables were better predictors, with AUCs under the ROC curve varying between 0.75 and 0.80. While TBSA % and age together gave a value of 0.81, the best prediction was achieved with those two in addition to CgA at 24 hours, with a value of 0.90.

**Paper II**

The P-NT-proBNP showed large day to day and between patient variations during the first 14 days after injury. The general pattern was that of a gradual increase for a few days and then a certain decline after reaching a maximum value on day 5 (5; 4-8) (Figure 2).

![Figure 2](image)

**Figure 2.** Individual concentration vs. time curves for NT-proBNP. Concentration is expressed as % of maximum value for each patient and the curves are smoothed to visualize individual patterns.

The peak concentration varied greatly between individuals and was clearly elevated in all cases compared to established cut-offs for congestive heart failure [56].

The maximum weight occurred on day 2 (median 2; 1-2) and timing correlated negatively with TBSA% burned, i.e. those with larger burns reached their maximum weight earlier. On the contrary, the time for maximum P-NT-proBNP correlated positively with TBSA%, i.e. those with larger burns reached their maximum value later. Furthermore, maximum P-NT-proBNP occurred later in patients with greater total weight gain, in patients who had lost more weight, i.e. mobilized more edema, at their maximum concentration, and in patients with higher maximum concentrations. TBSA% was correlated with maximum weight gain.
Maximum P-NT-proBNP correlated with the total amount of resuscitation fluid administered during the first 24 hours and with the amount of edema at maximum weight gain, but not with the weight gain at the time for maximum P-NT-proBNP. Nor did P-NT-proBNP at the time for maximum weight gain correlate with the value for maximum weight gain.

To discern whether the extent of edema mobilization had an immediate impact on P-NT-proBNP, weight loss for every 24-hour period was tested against morning P-NT-proBNP at the end of the same period. The only significant correlation was on day 2.

Similar to P-NT-proBNP, the P-CRP value showed a peak formation. Maximum P-CRP occurred on day 5 (median 5; 3-7). Time for peak P-CRP correlated with time for maximum P-NT-proBNP, i.e. these parameters showed covariation within individuals. Time for peak P-CRP correlated with TBSA%, i.e. larger burns had a later P-CRP peak. Furthermore, daily morning P-NT-proBNP and P-CRP correlated significantly on day 0, and on day 2 until and including day 6.

Organ dysfunction measured as SOFA deteriorated the first few days with the highest SOFA score on day 3 (median 3; 2-4), after which it reached a plateau with a very slow decline (Figure 6). Time for maximum SOFA correlated with TBSA, i.e. maximum SOFA was reached later in larger burns.

P-NT-proBNP correlated significantly with concomitant SOFA scores from day 3 until and including day 11, and on day 14.

Maximum P-NT-proBNP measured within the first 14 days after burn injury correlated with the SOFA score on day 14 after injury and with length of stay, and was also significantly related to mortality (t-test; p<0.001).

In linear regressions together with age at injury and TBSA, P-NT-proBNP assessed on days 3 to 8 was an independent predictor for every SOFA score measured one or more days later up to day 14 (Table 4). TBSA and age at injury assessed the first two days were independent predictors of later SOFA scores, after which P-NT-proBNP and TBSA both contributed to prediction of subsequently assessed SOFA scores. Age appeared to be an independent predictor early after injury and for short-term prediction as compared to the other two predictors.

**Paper III**

Both S-NT-proANP and P-NT-proBNP showed a time dependent pattern with an increase followed by a plateau phase (Figure 3 top). Variability over time was, however, much less for S-NT-proANP than for P-NT-proBNP. The mean inter-individual CV, calculated over all 14 days, was 81 %; SD 14 % for S-NT-proANP and 1572 %; SD 329 % for P-NT-proBNP (p< 0.001), and the corresponding mean intra-individual CV was 44 %; SD 19 % vs. 651%; SD 227 % (p< 0.001).
Table 4. Regressions with SOFA on different days as dependent variables and P-NT-proBNP assessed at different days, TBSA% and age at injury as independent variables. Only significant p-values are displayed.

<table>
<thead>
<tr>
<th>Day</th>
<th>BNP</th>
<th>SOFA Day</th>
<th>TBSA</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BNP</td>
<td>0.006</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>TBSA</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.002</td>
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<tr>
<td>8</td>
<td></td>
<td>0.000</td>
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</tr>
</tbody>
</table>

1Enter model; p-values for independent variables are shown. Age = Age at injury; BNP = P-NT-proBNP

P-NT-proBNP, but not S-NT-proANP, was also related to the size of the burn; r=0.40, p<0.01 for P-NT-proBNP and r=0.23, p=0.11 for S-NT-proANP, and reached its highest concentrations earlier than S-NT-proANP. (Figure 3 bottom).
Figure 3. S-NT-proANP and P-NT-proBNP expressed as pmol/L during the 14 days of Burn Intensive Care. Top: All patients; Bottom left: Patients with a TBSA <20% (n=13); Bottom right: TBSA ≥20%. (n=37). Mean ± SEM. No values are calculated for day 12 due to the low number of samples.

The absolute values for the two analytes correlated only moderately well (r = 0.38 – 0.67 for different days; p = 0.012 to <0.001). The corresponding day-to-day changes also correlated moderately well. This suggested that the two analytes were controlled and expressed differently.

To elucidate these differences more clearly the quotient between S-NT-proANP and P-NT-proBNP was plotted. This revealed two essential features. First, on a group level there was a temporary decrease in the S-NT-proANP/P-NT-proBNP ratio after 3 to 7 days, most pronounced at days 4 and 5, and thereafter a return to the initial level (Figure 4). Second, there was considerable inter- and intra-individual variability in the ratio, and also substantial day-to-day fluctuations (Figure 5). The fluctuations of P-NT-proBNP, assessed as the day-to-day change, only correlated with those of CRP at days 4 and 5 (p=0.01; r = 0.38 both days), and not at all with those of S-NT-proANP.
Figure 4. Natriuretic peptide (NP) ratio, calculated as S-NT-proANP (pmol/L) / P-NT-proBNP (pmol/L), and P-CRP (mg/L) during the 14 days of Burn Intensive Care. Mean ± SEM.

Figure 5. Natriuretic peptide (NP) ratio, calculated as S-NT-proANP (pmol/L) / P-NT-proBNP (pmol/L) for each patient the first 14 days after injury.

As differences in the relation to clinical variables might shed light on possible differences in regulation of the two NPs, simple regressions were made for the different days in the BICU. This revealed differences between S-NT-proANP and P-NT-proBNP. The most consistent observations were that the
age of the individual was a predictor of both NPs measured during the entire course of the illness. Furthermore, P-CRP only predicted S-NT-proANP at day 2, but it predicted P-NT-proBNP from day 2 up to and including day 6. Cardiovascular SOFA scores predicted S-NT-proANP at days 3 to 9 and days 13 and 14, and P-NT-proBNP at days 2 to 9. Respiratory SOFA scores also predicted both NPs, although with less statistical strength and only at 4 of the 14 days of observation for each of them. Finally, Renal SOFA scores predicted both NPs; P-NT-proBNP from day 5 and thereafter, and S-NT-proANP from day 7 and thereafter. A somewhat unexpected observation was that the extent of burn injury described as TBSA burned only predicted P-NT-proBNP at two time periods, days 7 and 8. Weight change the last 24 hours was only related to P-NT-proBNP at day 2 and to S-NT-proANP at day 14, and current excess weight was not related to P-NT-proBNP at any time in this analysis.

There was no difference in either NP between genders (not shown).

Paper IV

Although immense variations in S-cortisol between individual patients were immediately obvious, a number of initial statistical approaches suggested that the free cortisol concentration was related to the size of the burn. First, in a linear regression approach, the daily mean free cortisol concentration was significantly related to burn size for all days except for days 2 and 4 (data not shown). Furthermore, when utilizing values from all study days, burn size correlated with mean free cortisol ($r^2=0.31$, $p<0.001$), as well as maximum free cortisol ($r^2=0.25$, $p<0.001$).

As seen in Figure 6, there were very large differences in cortisol concentration patterns on day 1, which corresponded to a very large variability in time since injury until patients arrived at the BICU, from two to 36 hours with a median of eight hours, and until time for the first morning value, from five to 56 hours, with a median of 24 hours (Table 1). Therefore, some patients had their first morning concentration assessed very early after injury, and during the resuscitation phase, while others had it long thereafter. Cortisol dynamics during the first 24 hours in the BICU were therefore considered too unreliable in an assessment of disturbances in circadian rhythm, and the resuscitation phase was deliberately excluded in all patients, and detailed assessments were made from day 2 and onwards.

The free cortisol concentrations behaved differently in patients with burns larger than 20 % compared to those with burns smaller than 20 %. It was initially very high in those with large burns, after which a slight decline occurred, and both burn size groups had similar concentrations over the forthcoming days. Subsequently the concentration declined in those with small burns, while it remained high in those with larger burns (Figure 6).
When constructing slope variables it was clear that the circadian zenith for free cortisol frequently occurred later than would be expected in a healthy population. Median time for daily peak cortisol was 12pm, rather than the expected 6am. Circadian zenith in different patients occurred at all four assessment times, but distribution was heavily skewed towards early and late morning. The time of circadian zenith was not explained by burn size group, ongoing ventilator care, or surgery the preceding day (data not shown). It was, however, related to the current SOFA score (p=0.035).

A DAG was performed to identify a set of confounding variables with a potential impact on cortisol slope (Figure 7). A minimal sufficient adjustment set for estimating the direct effect of burn size group on cortisol slope was found to include age, P-NT-proBNP, P-CgA, and SOFA score. Thus these variables were included as covariates in subsequent analyses. Multi-level modeling revealed that burn size explained cortisol slope. Patients with large burns had significantly flatter slopes than patients with small burns (p=0.033; Table 6). Adjustments for age and gender and for covariates from the DAG excluding SOFA score did not diminish the effect (p=0.028 and 0.030, respectively; Table 6). After adjustments for all covariates from the DAG, including the SOFA score, there was no independent effect of burn size group. Rather, the only significant covariate in that model was the SOFA score itself (Table 6).

Furthermore, burn size also explained the cortisol slope after adjustment for ventilator treatment. Here, ventilator treatment had an independent effect, both together with burn size (Table 6), and also alone (p< 0.013; data not
Figure 7. The DAG obtained in the DAGitty software as basis for the multilevel statistical analysis.

...shown). Surgery the preceding day did not explain the cortisol slope (data not shown).

Thereafter an analysis of whether the difference in slope was related to specific differences at any defined time point in the day was made. This was done using free cortisol concentration measurements at the four assessment time points as dependent variables. Differences between large and small burns were only noticed at 6pm (Table 6), while there was no significant difference in free S-cortisol between burn size groups at 6am, 12pm or 12am (data not shown). This suggests that the burn-size-dependent flattening of the slope was due primarily to a slower decline in free cortisol from morning to evening. When adjusting for all covariates from the DAG, including SOFA score, slope at 6pm was explained by burn size group as well as by P-CgA and SOFA score (Table 6).

Finally, two secondary outcome measures were assessed, the coefficient of variability (CV) in free cortisol concentration as a measure of variability over the day, and AUC as a measure of the magnitude of cortisol release each day. Burn size group explained daily CV for free cortisol after adjusting for all covariates from the DAG (Table 6). In that model, SOFA score was also significantly related to the CV. Similarly, burn size group explained AUC after adjustment according to the DAG (Table 6). In addition, in the adjusted model P-CgA and SOFA score independently explained AUC.
Table 6. Multilevel analysis of the effect on cortisol related measures for days 2 to 7 after burn in the 49 patients.

<table>
<thead>
<tr>
<th>Burn size group</th>
<th>TBSA &gt; 20%</th>
<th>TBSA ≤ 20%</th>
<th>Fixed effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome and significant covariates</strong></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Free cortisol slope</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No adjustments</td>
<td>-0.009</td>
<td>0.002</td>
<td>-0.017</td>
</tr>
<tr>
<td>Adjusted by age and gender</td>
<td>-0.009</td>
<td>0.002</td>
<td>-0.017</td>
</tr>
<tr>
<td>Adjusted as DAG(^1) without SOFA</td>
<td>-0.009</td>
<td>0.002</td>
<td>-0.017</td>
</tr>
<tr>
<td>Adjusted as DAG(^1)</td>
<td>-0.009</td>
<td>0.002</td>
<td>-0.014</td>
</tr>
<tr>
<td>- in that model: SOFA score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for ventilator treatment</td>
<td>-0.010</td>
<td>0.003</td>
<td>-0.019</td>
</tr>
<tr>
<td>- in that model: ventilator treatment</td>
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<td></td>
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<tr>
<td><strong>Log free cortisol concentration at 6pm(^2)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No adjustments</td>
<td>2.479</td>
<td>0.051</td>
<td>2.620</td>
</tr>
<tr>
<td>Adjusted as DAG(^1)</td>
<td>2.513</td>
<td>0.044</td>
<td>2.618</td>
</tr>
<tr>
<td>- in that model: Burn size group</td>
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<tr>
<td>- in that model: P-CgA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- in that model: SOFA score</td>
<td>2.479</td>
<td>0.051</td>
<td>2.620</td>
</tr>
<tr>
<td><strong>Coefficient of variation for free cortisol concentration</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No adjustments</td>
<td>0.34</td>
<td>0.02</td>
<td>0.46</td>
</tr>
<tr>
<td>Adjusted as DAG(^1)</td>
<td>0.34</td>
<td>0.02</td>
<td>0.46</td>
</tr>
<tr>
<td>- in that model: SOFA score</td>
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<td></td>
<td></td>
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<tr>
<td><strong>AUC for free cortisol concentration(^3)</strong></td>
<td></td>
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<td></td>
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<tr>
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<tr>
<td>- in that model: P-CgA</td>
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</tr>
</tbody>
</table>

\(^1\) age, P-NT-proBNP, P-CgA, and SOFA score, \(^2\) values are in Log (nmol/L),

\(^3\) values are in Log (nmol/L) * h.
Discussion

The primary aim of the present thesis was to acquire more knowledge concerning the neuroendocrine stress response after burn injury. It must be kept in mind that this response is regulated by a system with many interlaced parts that work together in a non-linear fashion. Ideally, therefore, network based approaches with interventions with respect to important variables should be the best way to elucidate relationships between different interlaced parts. When a patient with a serious burn injury is admitted to the BICU, however, opportunities for controlled randomized interventions are small, as every possible measure is focused on optimizing the physiologic variables known to influence survival. Therefore this thesis is strictly based on observational studies – with a plethora of interventions that have to be adjusted for. Furthermore, burn injuries are relatively rare in Sweden, which limits the number of study subjects. Consequently, results must be interpreted humbly and carefully.

Specific discussion

Chromogranin A

Burn care is demanding in terms of resources and time, and the inflow of patients cannot be controlled or planned. The course of care is often long, with different phases that are influenced by varying interventions and complications, and it is therefore difficult to predict. Tools for predicting different outcome variables such as LOS in the ICU, LOS in the hospital, and mortality are important when optimizing care for patients. Currently, the gold standard for prediction in burn care is based on TBSA and age. Prediction models can be marginally improved with the addition of other currently available variables, but at the price of complexity, and a model is good only if it is used.

Paper I was based on the concept that many aspects of care, and the effects of complications that arise during care, add to the burden of the inciting event, and the net result of all these stressors in combination with the patient’s ability to handle them determines the outcome. The hypothesis in paper I was that CgA may be a biomarker for such aggregated stress after
burn injury. Since CgA is synthesized and co-released with catecholamines in neuroendocrine tissue [30], it is believed that it can serve as an integrated measure of sympathetic activation. Simply measuring S-catecholamines would introduce new problems, as large exogenous amounts of amines are irregularly administered as part of burn intensive care. Also, adrenergic beta-receptor blockers are commonly used as part of the ICU routine, which could affect the peripheral concentration of amines. However, it has been observed that exogenous administration of adrenergic beta-receptor blockers does not affect the P-CgA concentration [130].

Mortality, a crude and dichotomous measure of outcome, has decreased considerably in hospitalized burn patients during the past few decades [19; 108]. Mortality in the present cohort was four of the 51 patients, which limits statistical power. In this work, a decision was made to measure overall organ dysfunction in terms of the SOFA score, and use this as a proxy measure. The SOFA score was originally designed to quantitatively describe organ dysfunction over time in patients with sepsis [143]. It was later understood that the score could also be applied in non-septic patients [144]. The SOFA score, which is the sum of sub-scores for six organ systems, has also been used to predict mortality, although it was not originally developed for this function. The prognostic performance of the SOFA score when used in various models for predicting mortality in adult patients in medical and/or surgical ICUs was recently systematically reviewed [89]. SOFA score on admission, or various models of combinations of sequential SOFA scores, were confirmed to predict mortality in patients in general medical and/or surgical ICUs.

A subgroup of patients in this study was classified as having a high variability in CgA concentrations. It was observed that this population was older, had larger deep burns, more often underwent surgery and had higher SOFA scores than the other patients. Although few studies have been conducted on the intra-individual variability of P-CgA, the variability in the present study is larger than previously reported in healthy individuals or in individuals not treated in ICUs. For the sake of perspective it should be noted that even under intense to extreme acute sympathoadrenal stimulation, CgA levels do not increase more than twofold [26; 35]. The large variability observed in the present cohort must therefore be interpreted as intense and protracted sympathoadrenal stimulation. The fact that CgA variability was positively related to organ dysfunction, together with the other observations, indicates that CgA is a valid reflector of stress activation in BICU populations. However, measuring variability is not practical in clinical settings.

The present work also supports the hypothesis that high P-CgA values during the first week after a serious burn injury correlate positively with SOFA scores one week after injury, and therefore can be used to predict organ dysfunction. Further, it is known that organ dysfunction after one week in the ICU constitutes a great risk that the patient will not leave the
These data are convincing, since not only a point measure 24 h after injury, but also the mean, maximum and integrated values of P-CgA during the first 7 days correlated with the SOFA score at day 7. The data support P-CgA as a stronger predictor of organ dysfunction than TBSA burned, and that both are independent predictors of organ dysfunction. The results clearly show that not only the extent of the burn injury, but also characteristics of the individual’s response to injury, define the risk for organ dysfunction during acute intensive care. A similar approach has recently obtained considerable support, as the serum protein pattern assessed by proteomic methodology was found to be an independent prognostic factor with respect to survival in burned children [38].

Natriuretic peptides

A large burn injury is followed by changes in microvascular characteristics with increased leakage of water, solutes and proteins from the blood to the interstitium, which lead to hemoconcentration and hypovolemic shock. Treatment is based on the conception of upholding acceptable circulatory characteristics during the first 24 hours, during which most leakage to the interstitium occurs. After that time period there is mobilization of the excess edema that was formed during the first 24 hours.

The discovery of the natriuretic peptide system gave a rational explanation for the mode by which the central circulation can mediate control of water homeostasis. These discoveries conveyed a picture of the heart as an endocrine organ that releases natriuretic peptides from the myocardium based on atrial and ventricular distension. In this thesis three key questions concerning NPs have been addressed. First, what are their concentration dynamics in response to burn trauma? Second, do they convey the same or different types of information? Third, how are they related to outcome in terms of organ dysfunction?

Concerning the first question, there was a palpably large variability in NP secretion in terms of CV, both between patients and within patients over time, and especially in the case of NT-proBNP. Mechanisms underlying this variability have been only partially elucidated. In fact, even in non-critically ill patients with stable chronic heart failure there is significant between-day variation in P-NT-proBNP [17; 11]. Surprisingly, this phenomenon has been insufficiently analyzed, but it indicates a very phasic effect of BNP in maintaining stable atrial and ventricular wall tension by virtue of volume control.

The most likely mechanism for differences in concentration dynamics is related to differences in storage, synthesis and mechanisms of release. ANP has a larger storage pool that can rapidly be released, but induction of synthesis is faster for BNP. Theoretically, this may lead to a temporary dominance of BNP once storage pools for ANP become depleted. This is supported by the inverse relation seen when the NP ratio for the first sample ob-
tained is plotted versus time after injury.

Concerning the second question, both NPs were weakly related to cardiac, lung and kidney function, with minor differences. The most striking difference between the two NPs concerning their relation to other variables was that NT-proBNP, but not NT-proANP, was related to the systemic inflammatory trauma response measured as CRP.

Notably, TBSA showed no significant relation to S-NT-proANP in the present study, in contrast to a weak relation to P-NT-proBNP. It may be argued that the relation between TBSA and P-NT-proBNP is related to an effect of the systemic inflammatory response on P-NT-proBNP.

The somewhat surprising answer to the third question concerning the relation to outcome is that despite their volatility, both NPs were related to forthcoming overall organ dysfunction, in fact very consistently, and starting early after injury in the case of NT-proBNP. This is in line with previous observations that BNP is elevated in critically ill patients who lack any other observations suggesting incipient myocardial failure, and increased levels are related to mortality [121].

Although no immediate clinical utility for either NP has been revealed, future possibilities are implied. Clearly, NP concentrations hold information that determines forthcoming overall organ dysfunction, and thus can aid the clinician in determining prognosis. It is premature to say in what way this should be done. However, given the high variability, stable predictions will likely demand serial sampling. The explained variance in regressions between various specific organ dysfunctions and either NP as outcome were in general low, with adjusted $R^2$ values of less than 0.10. Mechanisms not included in the variables investigated here must therefore affect NP concentration. This, and the immense day-to-day variability in both NPs as well as in the NP ratio, indicates that hoping for organ-specific clinically valuable information from NPs in trauma patients may be futile.

The significance of these findings is that they clarify the immense variability in NP concentration after trauma. This challenges the interpretation of numerous previous studies based on only one or a few measurements from each patient. Additionally, this work provides texture to the volatile secretion of these NPs, which seems chaotic on outset. The conclusion that both NPs are similarly related to overall organ dysfunction, but not well suited to differentiate cardiac from lung dysfunction, or monitor fluid balance, is also an important contribution to the understanding of burn stress.

Generalization to other types of trauma may not be appropriate, since NPs are strongly involved in the regulation of water homeostasis, which is clearly very challenged in burn trauma.
Cortisol

Cortisol, perhaps the most investigated of the stress hormones, is secreted in a circadian fashion and is involved in the regulation of a diverse number of processes related to development, homeostasis, metabolism, cognition and inflammation [12]. It can therefore be thought of as a reference hormone in biochemical characterization of a stress response. In addition to furthering the understanding of cortisol secretion and rhythmicity after burn injury, the present work firmly positions burn research in surrounding stress and glucocorticoid research and provides a comparative measure of stress intensity.

The main finding was that rhythmicity was more disturbed in large than in small burns. More specifically, large burns had smaller daily CV values, flatter cortisol slopes and an inability to downregulate cortisol secretion late in the day, as seen in small burns and healthy subjects. Furthermore, and in accordance with observations made by other groups, S-cortisol levels were well elevated after burns, and more so for larger burns.

The disruption of circadian rhythm observed during the initial seven days of ICU care reflect considerable allostatic load, as validated by our CgA study. While no conclusions about long-term negative effects on the organism can be drawn based on the present data, they establish that clear alterations of cortisol rhythmicity, which indicate loss of regulatory competence of the HPA-axis [119], occur early and are related to the size of the burn injury. This may have serious implications, since there is accumulating evidence that the rhythmicity of cortisol secretion is of fundamental importance for an optimal functioning of the cortisol signaling system [28; 147]. Furthermore, cortisol slope has previously been shown to predict mortality as well as other clinical outcomes in various patient populations, which makes the present findings particularly interesting.

The effect of burn size on cortisol rhythmicity was overshadowed by the effect of the SOFA score in statistical modeling. The SOFA score can be viewed as a consequence of all stressors a patient is subjected to, which makes it plausible that cortisol rhythm, just like CgA and NPs, can be viewed as an integrated measure of multiple stressors. Which of the four that is the most optimal reflector of overall stress remains to be determined.

It has previously been stated that thermal injury of more than 20% of TBSA leads to disturbances in cortisol metabolism and the equilibrium of the hypothalamic-pituitary-adrenal axis [103], but to our knowledge this is the first study on burn stress that utilizes cortisol slope, the most prevalent measure in circadian cortisol research in various patient groups [120]. The present study is also based on a larger patient material and utilizes a more sophisticated statistical strategy to reveal patterns in cortisol rhythmicity than in previous assessments. The present results further support the generally accepted view that injury of more than 20% of TBSA leads to serious allostatic load, and should be treated in specialized burn units.
A separate rhythm disturbance was observed that was not associated with burn size, surgery or intubation: the cortisol zenith occurred later in the morning than would be expected in a healthy population. The data do not supply an answer regarding which mechanisms underlie the delay, but this phenomenon has been observed by others in burn patients [104]. One reason could be related to the critical illness as such, as there was a weak relation to SOFA score. Other tentative reasons could be features of care, such as sleep disturbances, delayed wake up times or exposure to stressful care situations which usually occur before lunch time. External factors as well as internal changes in chronobiological rhythmicity must be considered, as this phenomenon occurred very early after the inciting traumatic event.

The significance of this work is that it elucidates the cortisol stress response after burn injury by means of a more general approach to measuring cortisol rhythmicity and more advanced statistical modeling than in previous burn research. Furthermore, the most relevant fraction of cortisol, the free fraction, has been analyzed. Interestingly, this did not change results compared to calculations using total cortisol, but it slightly improved levels of significance.

Further improvement in our understanding of the biological consequences of altered cortisol dynamics probably require approaches to measure the cellular response in a more detailed manner than today, i.e. in relation to receptor activation and cortisol specific transcription events, as has also been concluded by others [12].

General discussion

The neuroendocrine afferent and efferent communication between the brain and the rest of the body, which is central to the stress response after trauma, is complex, highly interlaced, and impacts metabolism, immune function and the circulatory as well as the nervous system. This web of communication is not specifically designed for or used in combating trauma, but is a universal system for maintaining allostasis in any situation where there is a threat to the organism, perceived or real. A stress response can be initiated by widely differing stressors and by activation steps in several locations in the neuroendocrine system. Consequently, research in many different fields has contributed to the understanding of the physiological processes aimed at combating allostatic overload.

The integrated neuroendocrine response

The stress response system is a truly integrated system characterized by a large number of interactions and interrelations between agonists and their receptors. Individual characteristics affect details of the stress response sys-
tem, and consequences of stress system activation for the individual. There is, thus, genetic variability in various signaling systems in the stress response. Such is the case for the tumor-necrosis factor (TNF)-α and -β gene [7; 129], but also for the BDNF-gene, where a distinct polymorphism, Val66Met, is related to an attenuated HPA-axis and cardiovascular reactivity to stress [4]. Burn survival has been related to polymorphism in the Toll like receptor 4 gene and the TNF-α gene [8]. In addition, the cortisol levels are subjected to a large number of genetic influences [10]. These studies all point to the fact that there are a multitude of putative gene polymorphisms affecting physiological responses to somatic and psychological stressors in a way that may have an impact on late perceived outcome. Obviously, therefore, the stress response that is initiated affects individuals differently. This is established with respect to the risk of developing somatic organ damage after septic episodes, for example, where certain genetic features are related to an increased risk for multi-organ failure. As a corollary to this observation, the question arises as to whether there are individual differences in the ways the stress response harms different organ systems, i.e. whether there are characteristics that leave some individuals with renal damage and other individuals with pulmonary sequels, for example, after a similar somatic insult. This question has not been scientifically investigated.

There are various questions concerning survivors of critical illness that are related to the integrative and individual nature of the stress response and to which there are not yet any good answers. Why do some patients remain catabolic after wounds have healed? What determines whether patients will acquire CCI? Are there sequels to an inadequate stress response? Are the late cognitive problems that sometimes arise a consequence of the stress response in the brain? Why do some patients never return to work or become lethargic, even if they have healed well? Why do some, but not others, get PTSD? How can structural and permanent adverse modification of the stress system be prevented? Our data cannot answer these questions, but this thesis is a stepping-stone on the long journey towards understanding and handling adverse consequences of the post-traumatic stress response.

This integrative nature of the stress system must be considered with respect to the present work, which should not be viewed as representing four completely different aspects of the stress response. For example, there is evidence that there is co-localization of CgA and BNP in secretory granules of cardiomyocytes, and interactions between the down-stream products of CgA, vasostatin-1 and catestatin, NPs and catecholamine receptors are proposed as a paradigm of the heart’s capacity to organize complex integration processes for maintaining homeostasis under stress [83]. There are also observations suggesting that release of CgA is more closely connected to the myocardial release of natriuretic peptides and the inflammatory response than to activation of the sympathoadrenergic system in patients with heart failure [67]. Free cortisol and BNP were recently found to correlate strongly
in septic ICU patients [5]. The neuroendocrine response also depends on whether a stressor occurs in the ascending or descending phase of a cortisol secretory pulse [54], in that the response is stronger when the stressor acts on the ascending phase. This suggests that stochastic processes are involved in the interplay between glucocorticoid receptor activation and stress response. All these findings severely complicate the interpretations of our findings.

Finally, all present measurements were made outside the blood brain barrier, which precludes any attempt to understand details in the central regulation of the response.

The neuroendocrine response and late sequels

Trauma continues to be a serious public health problem that invokes a strong stress response that is effective most of the time. Modern intensive care of trauma patients is more effective than at any previous time in history, and many patients now survive horrendous traumas that previously would have led to certain death. One price these patients pay is having to undergo extensive periods of life supporting interventions and hence of allostatic overload. From an evolutionary perspective such prolonged and intense stress responses have not been compatible with survival, and it is generally accepted that long periods of life-saving ICU care have adverse consequences. Prior studies on the stress response during critical illness have, however, largely focused on the immediate physiological effects and consequences of the critical care itself in order to ascertain survival and minimize life threatening secondary organ injuries. As survival from severe critical illness has become the expected outcome for most patients in the past few decades, a number of recent studies have focused on the late sequels that represent non-life-threatening organ damage. Compromised lung and renal function, and ICUAW constitute such damage.

It has been confirmed to some extent that the protracted and intense stress response in itself can generate different types of pathophysiology. Problems these patients face, which may or may not be caused or exacerbated by stress, include catabolism and weight loss, asthenia, dyspnea, joint stiffness, anxiety and depression [49]. Some of these problems may be unrecognized during care, but become apparent once the patient returns home.

Attention has recently focused on whether the long-standing stress that accompanies ICU care for critical illness also results in organic consequences for the brain. A number of arguments support such a notion, while no arguments indicate why the brain should be free from organ damage when other organs “fight for their lives”. White matter lesions have been observed by MRI in the aftermath of critical illness [91]. It was recently noted that severe somatic stress in the form of sepsis [118] or critical illness in general [91] was sufficient for such lesions to appear. It has also been observed that higher functions are truly affected, both in the form of cognitive impairment,
which occurs in up to one-third of ICU-survivors [127], and in the form of properly diagnosed psychiatric conditions, both during and after general intensive care [27; 39]. With respect to burns, those afflicted have smaller hippocampus volumes than controls [146], which is an indication of a severe stress lesion in the brain.

Summing up

How should the stress response after trauma best be understood?

Major trauma results in multiple organ dysfunction and elicits a strong stress response and, in accordance with what has been discussed, activation of all branches of the neuroendocrine stress response system. The interlace-ment of branches of the stress system obstructs research focused on specific aspects of the stress response.

In this thesis the stress response has been assessed from four different perspectives. A common feature seems to be that if anyone of the biomarkers studied signals a strong stress response, this reflects and predicts that the patient is or will become more affected by the disease or trauma in question than would otherwise be the case. It is, however, futile to believe that the richly interwoven stress response can be adequately assessed by one biomarker only. All biomarkers studied can be viewed as representing efferent limbs of the stress reaction, and they would need to be supplemented by biomarkers representing afferent response signaling, and by measures of the ensuing individual physiologic changes that follow the stress signaling.

One question that arises from analyzing our data is reminiscent of the chicken and the egg - which came first? A trauma results in wounds and yields a stress response – that weakens immune function and induces a catabolic state – that yields infection and sepsis – that worsens wounds and yields more stress – that results in organ dysfunction…and so on. Obviously, a stress response is both a consequence and the etiology of tissue and organ damage.

This thesis has clarified in certain ways some co-variations between stress and different clinical parameters, but based on the nature of the underlying data it cannot provide any definitive answers about causality. From an assessment and prediction perspective the question of causality does not have to be answered; the stress response and organ dysfunction seem to progress in tandem. Other issues, however, such as manipulating the stress response through hormone therapy, need to be viewed from a causality perspective and will have to be dealt with in future studies.
Conclusions

- P-CgA after a major burn injury is an independent and better predictor of organ dysfunction assessed as SOFA score than the traditionally used TBSA% burned.

- The results suggest that the extent of neuroendocrine activation is related to organ dysfunction and they motivate a more extensive effort to evaluate P-CgA as a prognostic marker with respect to mortality and long-term outcome.

- P-NT-proBNP exhibited a complex pattern with considerable inter-individual and day-to-day variations.

- Values of P-NT-proBNP were related to size of burn, water accumulation and systemic inflammatory response. A considerable covariation with trauma response and SOFA scores was observed in day by day analyses, but with weight change only on day 2.

- Maximum P-NT-proBNP showed a stronger correlation with SOFA score on day 14, with mortality, and with LOS, than did age and TBSA% burned. High values were also independent predictors of all subsequent SOFA scores up to two weeks after injury.

- Both NPs reflect and predict organ function after burn injury similarly, notwithstanding a significantly larger intra-individual variability for P-NT-proBNP.

- P-NT-proBNP, but not NT-proANP, reflects the systemic inflammatory trauma response.

- Free cortisol concentration was related to the size of burns, as was also the circadian cortisol rhythm. This effect of burn size was, at least in part, related to its effect on organ function.
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