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# Metabolic and Endocrine Response in the Acute Stage of Subarachnoid Hemorrhage

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### **Abstract**

Nyberg, C. 2017. Metabolic and Endocrine Response in the Acute Stage of Subarachnoid Hemorrhage. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1357. 61 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0038-2.

The rupture of an aneurysm in subarachnoid hemorrhage (SAH) is a dramatic event causing a severe impact on the brain and a transient or permanent ischemic condition. Several types of responses to meet the challenges of SAH have been found in the acute phase, including activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, elevated levels of brain natriuretic peptide (BNP), and disturbances in cerebral and systemic metabolism.

Cerebral metabolism and the endocrine stress response in the ultra-early phase was investigated in a novel porcine model of SAH in which autologous blood was injected to the anterior skull base. Early activation of the HPA axis was found with rapid elevation of adrenocorticotrophic hormone, cortisol and aldosterone. The peak values of these hormones were early and may be impossible to catch in patients. There were indications of a sympathetic nervous response with excretion of catecholamines in urine as well as plasma chromogranin-A elevation. Cerebral microdialysis suggested immediate substrate failure followed by hypermetabolism of glucose. The animal model seems suited for further studies of aneurysmal SAH.

NT-proBNP was investigated in 156 patients with SAH, there was a dynamic course with increasing levels during the first 4 days of the disease. Factors predicting high NT-proBNP load included female sex, high age, high Troponin-I at admission, angiographic finding of an aneurysm and worse clinical condition at admission. High levels of NT-proBNP were correlated to factors indicating a more severe disease, suggesting the initial injury in aneurysmal SAH is an important factor in predicting high NT-proBNP during the acute stage of the disease.

Measurements with indirect calorimetry were performed daily during the first week after SAH on 32 patients with SAH. There was a dynamic course with increasing energy expenditure (EE) the first week after SAH. Comparisons with three predictive equations indicated that measured EE generally is higher than predicted, but considerable variation exists within and between patients, indicating that prediction of EE in SAH is difficult.

Altogether, the studies demonstrate a complicated response in acute SAH that needs to be further studied to increase possibility of good outcome in SAH patients.

*Keywords:* Subarachnoid hemorrhage, Energy Expenditure, BNP, Animal Model, Cortisol, Microdialysis

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*Till Ludvig, Nils och Erika*



# List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Nyberg C., Karlsson T., Hillered L., Ronne Engström E. (2014)  
Metabolic Pattern of the Acute Phase of Subarachnoid Hemorrhage in a Novel Porcine Model: Studies with Cerebral Microdialysis with High Temporal Resolution.  
*PLoS ONE* 9(6):e99904
- II Nyberg C., Karlsson T., Hillered L., Stridsberg M., Ronne Engström E. (2016)  
The Early Endocrine Stress Response in Experimental Subarachnoid Hemorrhage.  
*PLoS ONE* 11(3):e0151457
- III Nyberg C., Karlsson T., Ronne-Engström E. (2014)  
Predictors of increased cumulative serum levels of the N-terminal prohormone of brain natriuretic peptide 4 days after acute spontaneous subarachnoid hemorrhage.  
*Journal of Neurosurgery*. 120(3):599-604.
- IV Nyberg C., Ronne Engström E., Hillered L., Karlsson T.  
Daily Energy Expenditure in the Acute Phase of Aneurysmal Subarachnoid Hemorrhage.  
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# Abbreviations

ACTH	Adrenocorticotrophic hormone
AP	Arterial pressure
AUC	Area under curve
BNP	Brain natriuretic peptide
CPP	Cerebral perfusion pressure
CRH	Corticotropin releasing hormone
DCI	Delayed cerebral infarction
DIND	Delayed ischemic neurological deficit
EBI	Early brain injury
EE	Energy expenditure
GOS	Glasgow Outcome Scale
H-B	Harris-Benedict equation
HPA	Hypothalamic-pituitary-adrenal
IC	Indirect calorimetry
ICP	Intracranial pressure
MAP	Mean arterial pressure
NT-proBNP	N-terminal prohormone of BNP
PSU-98	Penn State University 1998 equation
SAH	Subarachnoid hemorrhage
VMA	Vanillylmandelic acid
WFNS	World Federation of Neurosurgical Societies



# Introduction

Spontaneous subarachnoid hemorrhage (SAH) is a condition in which blood is pathologically located in the cerebral subarachnoid space. The incidence is around 9/100000 person-years<sup>1</sup>. There are, however, variations depending on age and ethnicity.

A majority of patients are younger than 60 years<sup>2</sup>, SAH thus has a significant impact on the population since death and dependence is a common resulting outcome<sup>3</sup>.

The most common cause of spontaneous SAH is rupture of an arterial cerebral aneurysm. This event leads to leakage and pooling of blood in the subarachnoid space. At the same time, the arterial pressure (AP) is transferred over the arterial wall into the intracranial space, leading to increase of the intracranial pressure (ICP). An increase in ICP subsequently leads to decrease of the cerebral perfusion pressure (CPP). Decrease in CPP may result in reduction of the cerebral blood flow, and render permanent or transient cerebral ischemia. Compensatory mechanisms to maintain cerebral perfusion includes cerebral autoregulation and elevation of the arterial pressure.

When the aneurysm ruptures and ICP increases, the pressure gradient between the artery and the subarachnoid space will gradually decrease, and in most cases a blood clot will form and temporarily seal the leakage of arterial blood. When this happens in a patient, the brain may have suffered an ischemic time period. This ischemic period is most likely global and of varying length and magnitude. In some cases, the rupture of an intracranial aneurysm leads to immediate death. However, in most patients the aneurysm is sealed by clotting of the blood and the cerebral circulation is normalized. These patients have suffered from a varying degree of brain injury. Even those who wake up in good condition are at risk for severe brain injury and poor outcome.

After rupture of a cerebral aneurysm and formation of a clot, there is a substantial risk of re-bleeding<sup>4</sup>. Therefore, the aim of most centers is to treat and permanently occlude the aneurysm in the early stage of the disease.

A ruptured aneurysm can be treated by two different general principles; surgical clipping or endovascular coil occlusion. Surgical clipping is the traditional method requiring a craniotomy, dissection is performed to reach the aneurysm which is then treated by applying a clip over the neck of the aneurysm, thus excluding it from the cerebral circulation. Endovascular treatment is performed by reaching the aneurysm from the arterial circulation with a microcatheter. Through the microcatheter, several platinum coils are placed

and detached inside the aneurysm. At a certain point, the density of the coils in the aneurysm will be high enough to prevent circulation of blood in the aneurysm and a clot will be formed. Endovascular coil treatment<sup>5</sup> was introduced in the early 1990s, and since then there has been a tremendous development in techniques and materials used in the procedure. The International Subarachnoid Aneurysm Trial<sup>6</sup> demonstrated better outcome in patients treated with endovascular coiling compared to surgical clipping. At present time, endovascular coiling is the first treatment option in many centers. However, surgical clipping is still a safe and effective treatment. There are many ruptured aneurysms unsuitable for endovascular treatment and the role of surgical clipping of ruptured aneurysms remains.

## Early brain injury and delayed cerebral infarction

The aneurysm rupture and the cascade of events immediately following thereafter causes a severe impact on the brain. The injury sustained at this time is referred to as early brain injury (EBI). Patients with immediate loss of consciousness persisting for an extended period of time after aneurysm rupture have likely suffered from severe EBI. On the other hand, patients that presents with headache as the only symptom after aneurysm rupture and likely moderate EBI, are still at risk of developing ischemic lesions. Ischemic lesions that develop later than within the first 3 days are referred to as delayed cerebral infarction (DCI) or delayed ischemic neurological deficits (DIND).

## Cerebral vasospasm

Cerebral vasospasm is a description of arterial narrowing of the cerebral circulation that can be observed with angiography. Cerebral vasospasm typically presents a few days after SAH and is a feared complication after SAH, leading to decreased cerebral blood flow in affected parts of the cerebral vasculature<sup>7</sup>. Ischemic infarcts may develop and the outcome may be fatal. It is commonly accepted that vasospasm is a major factor in development of DIND, but angiographic vasospasm and DIND not always co-exist, and the causes of DIND are not fully understood. Alternative mechanisms suspected to play a role in DIND are spreading cortical depression, micro-thrombosis and other factors related to EBI<sup>8</sup>.

## Neurointensive care

The rupture of an aneurysm in SAH patients leads to cerebral injuries because of events taking place immediately after onset of the disease. These early injuries may be permanent and impossible to affect even if best possible care is

provided. The focus of neurointensive care is instead to optimize conditions in the acute stage of the disease. This is achieved by multimodal monitoring and careful evaluation of factors that may affect the brain and its metabolism. Factors complicating the course of the disease may lead to secondary damage of the brain, sometimes referred to as secondary insults.

Examples of secondary insults in SAH<sup>9</sup> are events causing decrease of cerebral blood flow, thus increasing the risk of developing ischemic lesions. Such an event is elevation of ICP because of hematoma, re-bleeding, cerebral edema, hydrocephalus, seizures or pyrexia. Factors directly influencing cerebral blood flow, such as vasospasm or arterial hypotension, may also produce a secondary insult. Hypoxemia or increased cerebral energy demand because of seizures or pyrexia also increases the risk of secondary brain damage.

Another important factor is plasma glucose concentration. In several studies, elevated plasma glucose concentrations have been reported in SAH patients and correlated to vasospasm and poor outcome<sup>10-13</sup>. However, it is not clear whether elevation of plasma glucose itself affects outcome or not. Plasma glucose elevation could be secondary to the stress response after SAH and related to the severity of the disease. There are indications that insulin administration to normalize plasma glucose leads to low cerebral glucose concentrations<sup>14</sup> and that tight management of plasma glucose may be related to worse outcome<sup>15</sup>.

## Endocrine response to SAH

The anterior lobe of the pituitary gland is the source of adrenocorticotropic hormone (ACTH). The release of ACTH from the pituitary is regulated by corticotropin releasing hormone (CRH) that is produced in the hypothalamus. ACTH affects the adrenal cortex, stimulating secretion of cortisol and also to some extent aldosterone. The cortisol produced in the adrenal cortex has inhibitory effect on secretion of CRH and ACTH, thus forming a negative feedback loop.

Activation of the hypothalamic-pituitary-adrenal (HPA) axis is seen with critical illness in general<sup>16</sup>. It is considered to be a physiologic response to stress, leading to elevated blood cortisol levels and is most likely of great importance to meet the challenges of critical illness, such as SAH.

Another physiological response to stress is activation of the sympathetic nervous system, which is a part of the autonomous nervous system. The sympathetic activation leads to increased concentrations of circulating catecholamines which in turn prepares the body to meet a stressful event.

The rupture of an intracranial aneurysm is followed by a complex chain of events. It is generally thought that the main trigger of the systemic changes is the increase in ICP leading to transient or permanent global cerebral ischemia. A major stress reaction follows, including the release of adrenocorticotropic

hormone (ACTH), cortisol, and catecholamines<sup>17-21</sup>. The stress reaction is a response in order to restore cerebral blood flow, but it can also cause adverse effects.

The blood in the subarachnoid space is commonly distributed in the basal cisterns with close relation to the pituitary gland. Disturbances in the pituitary functions have been demonstrated, both in the acute phase<sup>17,18,22-24</sup> and in long-term follow-up<sup>25-28</sup>. This could be a consequence of a general cerebral injury caused by the sudden increase in ICP, decrease in CPP and transient global brain ischemia at the time of aneurysm rupture. It is also possible that the proximity of the blood to the pituitary gland and the hypothalamus directly affects the pituitary.

Elevated cortisol concentrations have been found early in the acute phase in SAH patients, but it remains unclear whether the increase of cortisol is impaired because of pituitary dysfunction or is an adequate response to the trauma. Low levels of cortisol have been demonstrated, both in the acute phase and in long-term follow-up. It has also been found that the normal diurnal cortisol excretion may be altered in patients with SAH<sup>18,29</sup>.

In several studies, indications of early pituitary deficiency have been found<sup>22,23</sup> and cortisol concentrations have been shown not to be directly linked to the severity of the disease in SAH<sup>25</sup>, also indicating a possible insufficiency in the pituitary-adrenal response. It has also been demonstrated that episodes of low cortisol are associated with increased risk of poor outcome<sup>22</sup>.

Various cardiac abnormalities in patients with SAH, such as troponin-I release, ECG changes and arrhythmias, are well described<sup>30</sup>. It is generally thought that cardiac symptoms are secondary to the catecholamine burst at the aneurysm rupture. Pathological cardiac findings are often evident already at time of admission. Cardiovascular abnormalities after SAH have been shown to be predictors for in-patient death<sup>31</sup>. Increased concentrations of brain natriuretic peptide (BNP), as well as other natriuretic peptides, have been observed in patients with SAH. The biological effects of BNP include diuresis, vasodilatation and inhibition of renin and aldosterone production<sup>32</sup>. BNP is produced from a prohormone that is split to BNP, and NT-proBNP. NT-proBNP has no known biological effect. The half-life of NT-proBNP is longer than for BNP, thus making NT-proBNP a good marker of total BNP production. BNP is mainly produced in cardiac myocytes. BNP has been found in brain tissue, but it is not clear whether it is also produced in the brain.

In SAH patients, the effect of BNP on fluid and salt balance has been studied<sup>33-35</sup>. In studies of BNP and vasospasm, the results indicated it was clinical apparent brain ischemia rather than angiographic or transcranial Doppler defined vasospasm that was related to the BNP increase<sup>36-38</sup>. Elevated BNP was associated with cerebral infarction on CT<sup>39</sup>. BNP has also been explored as a marker of cardiac injury in SAH patients and it was found that high BNP at

admission as well as high Troponin-I at the third day was related to increased in-patient mortality<sup>31</sup>.

## Energy expenditure in SAH

Energy expenditure (EE) is the amount of energy the body needs to maintain its functions. In medical literature, EE generally means the amount of energy the body uses over a given time period (normally 24 hours). The intake of energy should ideally match EE. If EE is higher than the intake of energy, a negative energy balance occurs, leading to catabolism of energy-rich molecules such as carbohydrates, proteins or fatty acids.

Providing adequate nutrition to critically ill patients is of great importance. These patients are often unconscious and cannot regulate energy intake themselves. Nutritional balance may affect outcome after critical illness in general, including stroke<sup>40,41</sup>. Both underfeeding and overfeeding may be harmful.

IN SAH patients, EE has been shown to be elevated and signs of protein catabolism with negative nitrogen balance have also been present<sup>42</sup>.

The fact that surgical interventions and other types of severe stress affects energy expenditure raises a suspicion that energy demand may differ significantly between patients with SAH and also in the same patient depending on the time after ictus and other factors such as presence of clinical vasospasm or infections.

Ideally, calorimetric measurements should be done daily on every patient, and energy content in the nutrition matched to this measurement. Measurements of EE is, however, not a standard method in most intensive care units and predictive equations to estimate EE are used instead.

## Predictive equations of energy expenditure

Predictive equations to estimate nutritional need have been developed and refined since the early 1900s. The original equation to calculate the basal metabolic rate by Harris and Benedict (H-B) is still being used<sup>43</sup>. Several equations have been proposed to make more accurate predictive calculations including different factors in disease that may influence energy balance. A patient with severe illness, sedation and need of mechanical ventilation presents an even more complicated situation. Equations to predict EE in this heterogeneous group have been suggested, but, generally, correlation to measured EE is poor<sup>44,45</sup>. The predictive equation from Penn State 1998<sup>46</sup> (PSU-98) has previously been shown to correlate with measurements by indirect calorimetry (IC) for patients with severe traumatic brain injury<sup>47</sup>. Using 25 kcal per kg body weight<sup>48</sup> is a simple approach that likely is widely used in ICU patients.

## Measurements of energy expenditure

Indirect calorimetry is currently considered to be the best easily available method for measuring EE in critically ill patients<sup>49</sup>. In IC, oxygen consumption and carbon dioxide production is measured and used to calculate energy expenditure. The equipment is, however, expensive and in most centers IC is not routinely used.

In the literature, measurements with IC on patients with hemorrhagic stroke have been reported. In these studies, single short measurements have been performed once or twice during the acute stage of the disease<sup>42,50-53</sup>. For patients with SAH, EE has generally been elevated compared to values predicted by equations.

## Animal models of SAH

The events occurring at the time of and directly following aneurysm rupture cannot be studied in the clinical setting since the patients are unavailable for measurements and examinations at onset of disease. A patient with SAH is generally admitted to a local hospital, diagnosed with SAH and transferred to a secondary hospital. Considerable time will pass before monitoring and measurements can be started.

The very early events occurring after aneurysmal SAH have to be studied in animal models of acute spontaneous SAH. Most animal models described in the literature<sup>54-59</sup> are designed for questions targeting later occurring intracranial events, such as vasospasm. The rat has been used frequently, but it can be questioned how well rat physiology applies to human conditions. It has been shown that rats are resistant to glucose depletion, even during a prolonged period of brain hypoxia<sup>60</sup>, suggesting that the cerebral energy metabolism of the rat could differ from the human metabolism. Rabbits have also been used in SAH models<sup>56,57</sup>. However, both the rat and the rabbit are lissencephalic animals, and have smaller relative volume of the cortex compared to humans. We wanted a model in which we could perform studies of both intracranial and systemic changes in the very acute stage of SAH. This required a robust animal with a physiology resembling human conditions. The larger relative neuronal volume of a gyrencephalic brain like that of a pig may render it more sensitive to ischemia and generally closer resembling a human brain in terms of energy metabolism. The pig is an experimental animal that offers several advantages compared to smaller animals. Since the pig is a relatively large animal, it is easy to study and monitor vital parameters. The larger size also makes collection of blood, urine and other body fluid samples possible with lower risk of hypovolemia and anemia as a consequence.

# Aims of the investigations

## General aim

The general aim of the thesis was to study the metabolic and endocrine response in the acute stage of subarachnoid hemorrhage.

## Specific aims

- To study cerebral metabolism at the actual time of aneurysm rupture and the first hours in experimental SAH. (Paper I)
- To study the endocrine stress response in experimental SAH in the ultra-early phase. (Paper II)
- To evaluate how NT-proBNP is related to the severity of the disease in SAH patients. (Paper III)
- To study energy expenditure in the acute phase of SAH, the relation to predictive equations and how energy expenditure changes over time in the first week after SAH. (Paper IV)

# Methods

## Paper I and II

### Animals and anesthesia

The study was performed on a series of 11 pigs. The animals were of mixed breed and of both sexes. The age of the pigs was 8-10 weeks with a mean weight of 24.9 (23.1-28.8) kg. At induction of anesthesia, the animals received a bolus fluid infusion with 30 mL/kg of Ringer-acetat (Fresenius Kabi, Uppsala, Sweden). Thereafter, fluid infusion was administered with Ringer-acetat at 8 mL/kg/h and Rehydrex 25 mg/mL (Fresenius Kabi) at 10 mL/kg/h. Before transportation to the laboratory, the animals were given intramuscular injections of 50 mg xylazine (Rompun vet, Bayer Health Care, Leverkusen, Germany).

Induction of anesthesia was performed with intramuscular injections of tiletamine 3 mg/kg and zolazepam 3 mg/kg (Zoletil 100, Virbac, Carros, France), xylazine 2.2 mg/kg (Rompun vet, Bayer Health Care) and atropine 0.04 mg/kg (Atropin Mylan, Mylan, Stockholm, Sweden) in combination with intravenous injections of ketamine 100 mg (Ketaminol vet, Intervet International, Boxmeer, Netherlands) and morphine 1 mg/kg (Morfin Meda, Meda, Solna, Sweden). Maintenance of anesthesia was achieved by continuous intravenous infusion of ketamine 20 mg/kg/h, morphine 0.5 mg/kg/h and rocuronium bromide 2 mg/kg/h (Esmeron, NV Organon, Oss, Netherlands).

Immediately after the experiment was finished (140 minutes after induction of SAH), the animals were euthanized with potassium chloride while still under anesthesia. Thereafter, either a computed tomography scan or morphological examination of the brain was performed.

### Preparation

The animals were tracheotomized and mechanically ventilated. The arterial partial pressure of carbon dioxide was kept within 5.0-6.0 kPa. The inspired gas was set to contain 30% oxygen mixed with air. A central venous catheter and a pulmonary artery catheter were inserted. A cervical branch from the subclavian artery was used to introduce an arterial catheter. A suprapubic catheter was placed directly in the bladder to deviate urine. The body temperature was monitored and kept within normal limits (37-39 °C) throughout the experiment.

A midline incision was made on the skull; the scalp tissue and the periosteum were removed from the bone to visualize the coronal suture. One burr hole was made 1 cm left of the midline and 1 cm anterior to the coronal suture. A corresponding burr hole was made on the right side. In the left burr hole, an external ventricular drain (EVD) stylet was inserted and placed on the anterior skull base, aiming to get the tip in the midline. A catheter without side holes was inserted over the stylet to the skull base. The stylet was removed and the catheter was connected to a stopcock. The right burr hole was used to insert an intra-parenchymatous ICP monitor (Integra Camino, Integra Neurosciences, Plainsboro, NJ) that was secured with a screw-bolt into the skull bone.

About 2 cm anterior to the right burr hole, a third hole was made in which a microdialysis catheter (70 Brain Microdialysis Catheter, membrane length 10 mm, M Dialysis AB, Solna, Sweden) was inserted into the brain parenchyma. The catheter was connected to a 107 Microdialysis pump (M Dialysis AB) with a flow rate set at 1  $\mu\text{L}/\text{min}$  with Perfusion fluid CNS (M Dialysis AB). The burr holes were sealed with bone wax to prevent leakage of cerebrospinal fluid. Microdialysate was analyzed for glucose, lactate and pyruvate using a CMA 600 bedside analyzer or ISCUSflex Microdialysis Analyzer (M Dialysis AB). The correlation between CMA600 and ISCUSflex data from separate runs with the same control sample was found to be excellent (for all analytes used here the  $r$  was  $>0.987$ ) allowing for direct comparison of data from both instruments. Both analyzers were automatically calibrated when started as well as every sixth hour using standard calibration solutions from the manufacturer (M Dialysis AB). Imprecision values for between assay coefficient of variation was  $<10\%$  for all analytes.

The position of the catheter on the skull base was verified either with fluoroscopy, CT after the experiment or post-mortem brain tissue examination.

## SAH induction

After preparation and starting of microdialysate collection, at least 15 minutes of baseline data was collected before induction of SAH. Autologous blood was injected through the catheter placed on the anterior skull base. The blood was aspirated from a central venous catheter or from an arterial catheter immediately before induction of SAH. The blood was injected slowly during 1-2 minutes with a 20 mL syringe, while monitoring ICP closely. The injection continued until CPP was kept around 0 for one minute. ICP and CPP was then allowed to recover spontaneously.

## Data collection (Paper I)

The first 30 minutes after microdialysis catheter insertion, the collected dialysate was discarded, and thereafter microdialysate from the interstitial fluid in the brain was collected in fractions of 5 minutes. The collected dialysate was

analyzed for concentrations of glucose, pyruvate, lactate and urea; the lactate-pyruvate ratio was calculated. Urea was monitored to control probe performance<sup>61</sup>. Dead space in the microdialysis collection system and in vivo extraction efficiency (relative recovery) were not compensated for. Relative recovery in vivo for glucose, pyruvate and lactate with the microdialysis catheter and perfusion rate used here is typically 20-30% in human brain<sup>62</sup>. The corresponding normal values in human brain (mean±SD) are for glucose, 1.2±0.6 mmol/L; pyruvate, 70±24 μmol/L; and lactate, 1.2±0.6 mmol/L<sup>63</sup>.

Monitoring data containing arterial pressure (AP) and intracranial pressure (ICP) was collected and recorded with BIOPAC AcqKnowledge 3.9.1.6 (BIOPAC Systems, Goleta, CA). CPP was calculated using the formula  $CPP=MAP-ICP$ .

Monitoring data and microdialysate were collected for 135 minutes after SAH induction.

## Blood and urine sampling (Paper II)

Samples of blood and urine were collected from each animal at -10, +15, +75 and +135 minutes from time of SAH induction. The blood samples were drawn from an arterial line. Ten minutes before each sampling, urine collection started and samples were taken for analysis from this volume.

The blood samples were analyzed for concentrations of serum cortisol, plasma ACTH, plasma aldosterone and plasma chromogranin-A. Urine samples were analyzed for concentrations of cortisol, creatinine, adrenalin, noradrenalin and metabolites from catecholamines.

## Biochemical methods (Paper II)

Measurements of cortisol were performed on automatic immune analyzer (Cobas e601, Roche Diagnostics, Basel, Switzerland). The total assay variation was less than 9%. Measurements of ACTH were performed on automatic immune analyzer Immulite 2000 XPi (Siemens, Los Angeles, CA, USA). The total assay variation was less than 6%. Measurements of creatinine were performed on automatic immune analyzer Architect Ci8200® analyzer (Abbott, Abbot Park, IL, USA). The total assay variation was less than 4%. Measurements of aldosterone were performed with a manual radioimmunoassay (Coat-a-Count-Bio International, Codolet, France). The total assay variation was less than 9%. Measurements of catecholamines were performed on a high-performance liquid chromatography system<sup>64</sup>. The total assay variation was less than 10%. Measurements of chromogranin-A were performed with an in-house radioimmunoassay<sup>65</sup>. The assay, based on human amino acid sequences, measure the N-terminal part of the molecule, which has 100% cross reactivity to porcine chromogranin-A. The total assay variation was less than 10%. All

analyses were performed at the routine laboratory of the Department of Clinical Chemistry at the University Hospital in Uppsala. The laboratory is certified by a Swedish government authority (Swedac).

## Statistics

The change of AP, ICP and microdialysate concentrations were evaluated with the Wilcoxon matched pairs test. The change of concentrations of hormones from baseline values were evaluated with the Wilcoxon matched pairs test. The Friedman test was used for analysis of variance of hormone concentrations over time. Values of  $p < 0.05$  were considered significant.

## Paper III

### Patients

Patients admitted to the Department of Neurosurgery at Uppsala University Hospital with acute spontaneous SAH from 2006 through 2011 were eligible. A total of 156 patients were included (Table 1). An aneurysm was found in 138 patients. Endovascular treatment was used for 82 patients, surgical clipping for 50, and no treatment for 6. The aneurysm was located on the anterior communicating artery in 45 patients.

**Table 1.** Characteristics of the patients

Characteristic	No. (%)
Female	105 (67)
WFNS grade	
1–2	66 (42)
3–5	90 (58)
Fisher grade	
1-2	90 (58)
3–4	66 (42)
High troponin-I*	79 (51)
Angiographic result	
aneurysm	138 (88)
normal	18 (12)
Vasospasm treatment	31 (20)
Any infection	97 (62)
Neurological deficits	109 (70)
Outcome	
poor (GOS Score 1–3)	72 (46)
favorable (GOS Score 4–5)	84 (54)

*Table 1.* Mean ( $\pm$  SD) patient age was  $59.8 \pm 11.2$  years. GOS = Glasgow Outcome Scale score; WFNS = World Federation of Neurosurgical Societies.

\* Pathological plasma levels of troponin-I at admission.

## Study protocol

Patients were included if samples had been collected for serum NT-proBNP and troponin-I on Day 0 (day of ictus) and at least 4 daily samples of NT-proBNP had been collected altogether for Days 0–5. Data were recorded for sex, age, clinical condition at admission (using World Federation of Neurosurgical Societies [WFNS] grading of SAH)<sup>66</sup>, blood distribution on first CT scan according to the Fisher classification<sup>67</sup>, angiographic finding, treatment of aneurysm, documented clinical neurological deterioration, any verified infection, and treatment of vasospasm. Outcome after 6 months was measured with the Glasgow Outcome Scale<sup>68</sup>.

## SAH management (paper III and IV)

The patients were managed according to the Department of Neurosurgery's protocols for SAH<sup>69</sup>. Briefly, this includes early diagnosis and treatment of the aneurysm. Patients with impaired consciousness are intubated and normo-ventilated, and receive a ventricular catheter for drainage of cerebrospinal fluid and monitoring of intracranial pressure. Sedation in mechanically ventilated patients is obtained mainly with propofol and bolus doses of morphine. En-

teral nutrition is administered through a gastro-enteral tube. Intravenous nutrition is only used in selected cases. Nimodipine is administered for three weeks. Symptoms of delayed cerebral ischemia are considered to be due to vasospasm when other causes of deterioration have been ruled out. Vasospasm treatment is then given with increase in blood volume and blood pressure and in selected cases intra-arterial administration of nimodipine. Secondary insults putting further strain on the brain's supply and use of energy and oxygen are systematically detected and treated if present<sup>9</sup>.

## Statistical analyses

NT-proBNP was quantified by using the trapezoidal method<sup>70</sup> to calculate the AUC for each measurement on Days 0–4. If a measurement was missing, a value was interpolated by using previous and subsequent measurements. Statistica 10.0 (StatSoft, Inc.) was used for descriptive and analytical statistics. The clinical information was dichotomized as follows: male/female sex, presence/absence of high plasma troponin-I at admission, presence/absence of aneurysm, aneurysm on anterior communicating artery/on other location, endovascular/surgical treatment, presence/absence of verified infection, presence/absence of neurological deterioration, and treatment/no treatment of vasospasm. We also dichotomized WFNS, Fisher, and Glasgow Outcome Scale scores as follows: WFNS Grade 1–2 versus 3–5, Fisher Grade 1–2 versus 3–4, and Glasgow Outcome Scale Score 1–3 (poor) versus 4–5 (favorable). AUC for NT-proBNP was compared between the aforementioned groups by using the Mann-Whitney U-test. Spearman's rank-order correlation coefficient was used for analysis of correlation to age. Change of NT-proBNP over time was evaluated with one-way ANOVA. A generalized linear model with a multinomial ordinal response and a logistic link function was used to calculate the best subset of variables predicting a large AUC for NT-proBNP. This was done by using Akaike information criterion<sup>71</sup>. In this analysis, the natural logarithm of AUC for NT-proBNP was used, and treatment method and outcome were not included as input variables. A difference was considered statistically significant when  $p < 0.05$ .

# Paper IV

## Patients

Patients were included from October 2010 until July 2014. Intubated patients with spontaneous SAH were considered for inclusion. Thirty-two patients were included with at least one measurement using indirect calorimetry during the first week after SAH (Table 2). The patients were managed according to the section of SAH management described in paper III.

**Table 2.** Characteristics of the patients

Characteristic	%
Female	66
WFNS grade	
1	3
2	16
3	9
4	53
5	19
Aneurysm location	
Anterior circulation	84
Posterior circulation	16
Treatment	
Endovascular	75
Surgical	25
Consciousness at discharge*	
Conscious	53
Unconscious	34
Dead	13

*Table 2.* Mean patient age was 60 years (range 41-81).

\* Discharge from the neurosurgical unit.

## Predictive equations

Calculations of the metabolic rate was done for each patient with the Harris-Benedict equation, the Penn State University 1998 equation, and by using 25 kcal per kilogram body weight.

$$\text{H-B men: } 66.473 + (13.7516 \times W) + (5.0033 \times L) - (6.755 \times A)$$

$$\text{H-B women: } 655.0955 + (9.5634 \times W) + (1.8496 \times L) - (4.6756 \times A)$$

$$\text{PSU-98: } (1.1 \times \text{Harris-Benedict}) + (140 \times T_{\max}) + (32 \times \dot{V}E) - 5340$$

W is weight in kg, L is length in cm, A is age in years,  $T_{\max}$  is maximum temperature in °C in the past 24 hours,  $\dot{V}E$  is minute volume in L/min.

## Metabolic measurements

The energy expenditure was determined by measurements with the indirect calorimeter Quark RMR (COSMED, Rome, Italy). Before each measurement, the equipment was calibrated according to instructions from the manufacturer. The measurements lasted for approximately 4.5 hours. Data from the measurements was processed in the following manner to exclude invalid measurements; records without measurements of oxygen consumption or carbon dioxide production values were removed, extreme values were removed by excluding records that deviated more than two standard deviations from the mean. From the resulting data, daily energy expenditure was calculated by taking the median from all collected data points.

Measurements were started as soon as possible after admission, typically on the day after admission. Measurements continued daily the first week after SAH or until the patient no longer was mechanically ventilated or had respiratory problems requiring >50% oxygen fraction in inspired air. On some occasions, the patient was unavailable for measurement because of time-consuming examinations or procedures.

In order to compare metabolic measurements between patients, the ratio of the measured value to H-B was calculated. The ratio of the measured values were also calculated to PSU-98 and to body weight. To be able to compare early and late measurements despite missing measurements, the mean of day 2-3 and the mean of day 5-6 was calculated for all patients.

Urine was collected for 24 hours repeatedly for the patients and analyzed for urea. From this value, daily urinary nitrogen excretion was calculated.

## Statistics

The change in EE and nitrogen excretion over time was evaluated with the Skillings-Mack test. Wilcoxon matched pairs test was used to compare early and late measurements. Values of  $p < 0.05$  were considered significant.

## Ethics

The studies in this thesis were granted permission by the regional ethics review boards for animal or clinical research.

# Results

## Paper I and II

### Arterial and intracranial pressures

The amount of blood injected in order to achieve a CPP of 0 for one minute varied from 13-25 ml. ICP typically increased to around 200 mm Hg (Figure 1). The AP increased shortly after the elevation of ICP. The rise of AP and drop in CPP was very rapid, peaking at the time when the injection of blood stopped. In some of the animals, the elevation of ICP was accompanied by extension posturing as well as cardiac arrhythmia or agonal breathing pattern.

After the peak in ICP at time of SAH induction, ICP levels slowly decreased to close to normal levels, but were still elevated compared to baseline values. In nine animals, ICP levels thereafter slowly increased during the remainder of the study time. In these animals, AP before induction of SAH had a mean value of 91 (70-106) mm Hg, compared to the peak value during SAH induction of 176 (140-210) mm Hg ( $p=0.0077$ ), and the AP at the termination of the experiment of 87 (74-92) mm Hg ( $p=0.074$ ). ICP before SAH had a mean value of 9 (2-13) mm Hg, a peak value of 202 (122-267) mm Hg ( $p=0.0077$ ), and the end value was 30 (20-34) mm Hg ( $p=0.012$ ).

In the remaining two animals, ICP remained elevated throughout the study time. CPP also remained negative after SAH induction. In these animals, microdialysis data also indicated severe persisting brain ischemia after SAH induction, as described below.

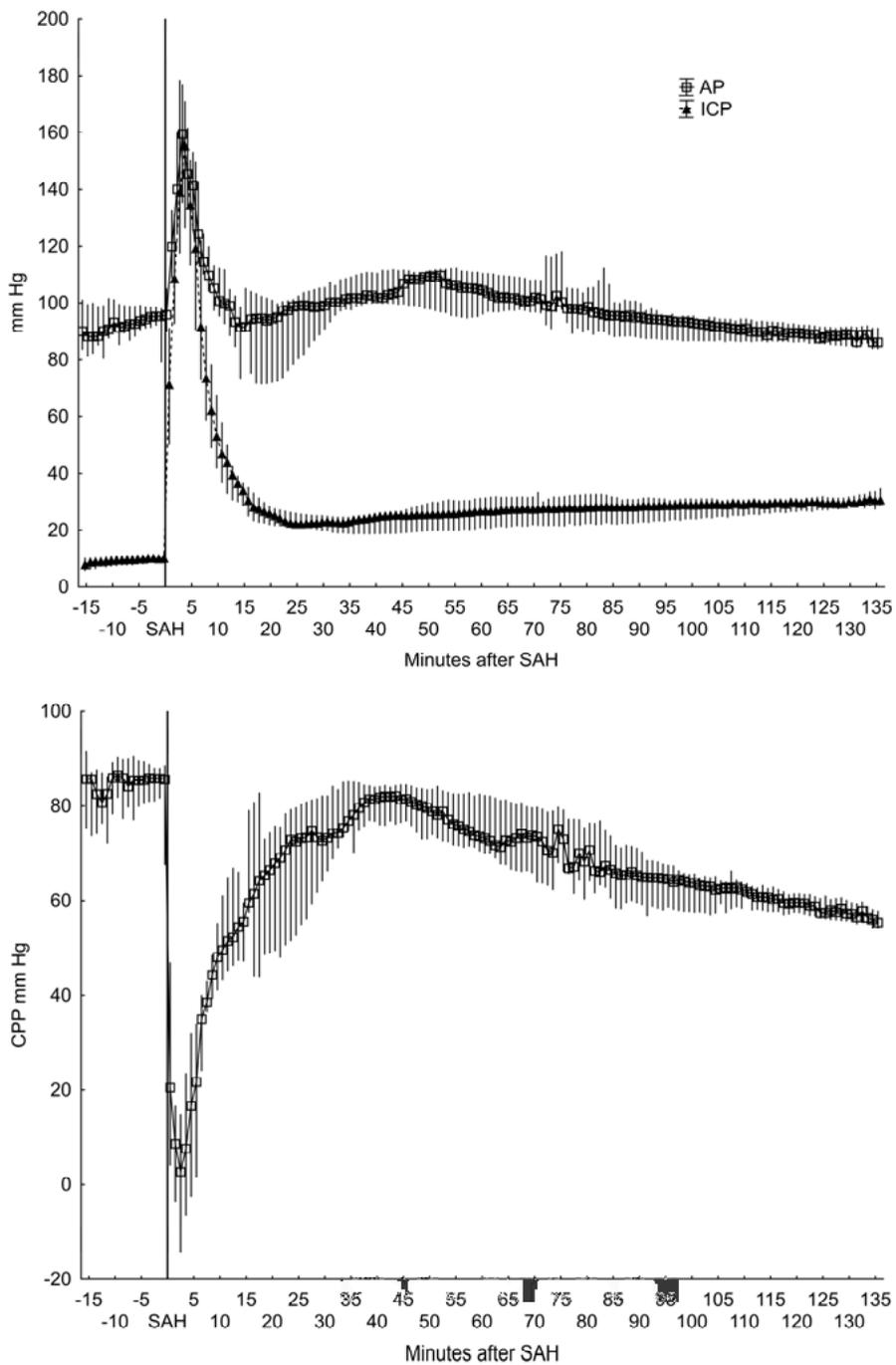


Figure 1. Arterial, intracranial and cerebral perfusion pressures in animals without persisting ICP elevation (n=9). Median values with 25th-75th percentiles. AP and ICP (upper), CPP (lower).

## Microdialysis

The analysis of the collected microdialysate showed two different patterns. Nine animals had a similar pattern in which glucose concentrations in the cerebral parenchyma dropped quickly after SAH induction (Figure 2). Glucose before SAH had a mean concentrations of 0.84 (0.51-1.13) mmol/L and decreased to 0.17 (0.02-0.45) mmol/L ( $p = 0.012$ ). Pyruvate levels initially also decreased, but then rapidly increased; mean pyruvate concentration before SAH was 32.9 (22.2-50.9)  $\mu\text{mol/L}$  and peaked at 25 minutes at 72.7 (37.5-105.8)  $\mu\text{mol/L}$  ( $p=0.0077$ ). Lactate concentrations increased rapidly after onset of SAH from 0.59 (0.51-1.13) to 2.6 (1.1-4.2) mmol/L ( $p=0.0077$ ) at 20 minutes. Consequently, the lactate-pyruvate ratio peaked in microdialysate collected 5-10 minutes after induction of SAH; initial lactate-pyruvate ratio had a mean value of 17.5 (13.3-23.5) and peaked at 89.9 (46.6-168.2) at 10 minutes ( $p=0.0077$ ). After these initials peaks, all analyzed metabolites slowly returned to concentrations similar to those collected from baseline data. The dead space in the microdialysis collection system is 5.1  $\mu\text{L}$ , meaning that the microdialysate results reflects conditions 5.1 minutes earlier than the time of collection since the flow rate is 1  $\mu\text{L}/\text{min}$ .

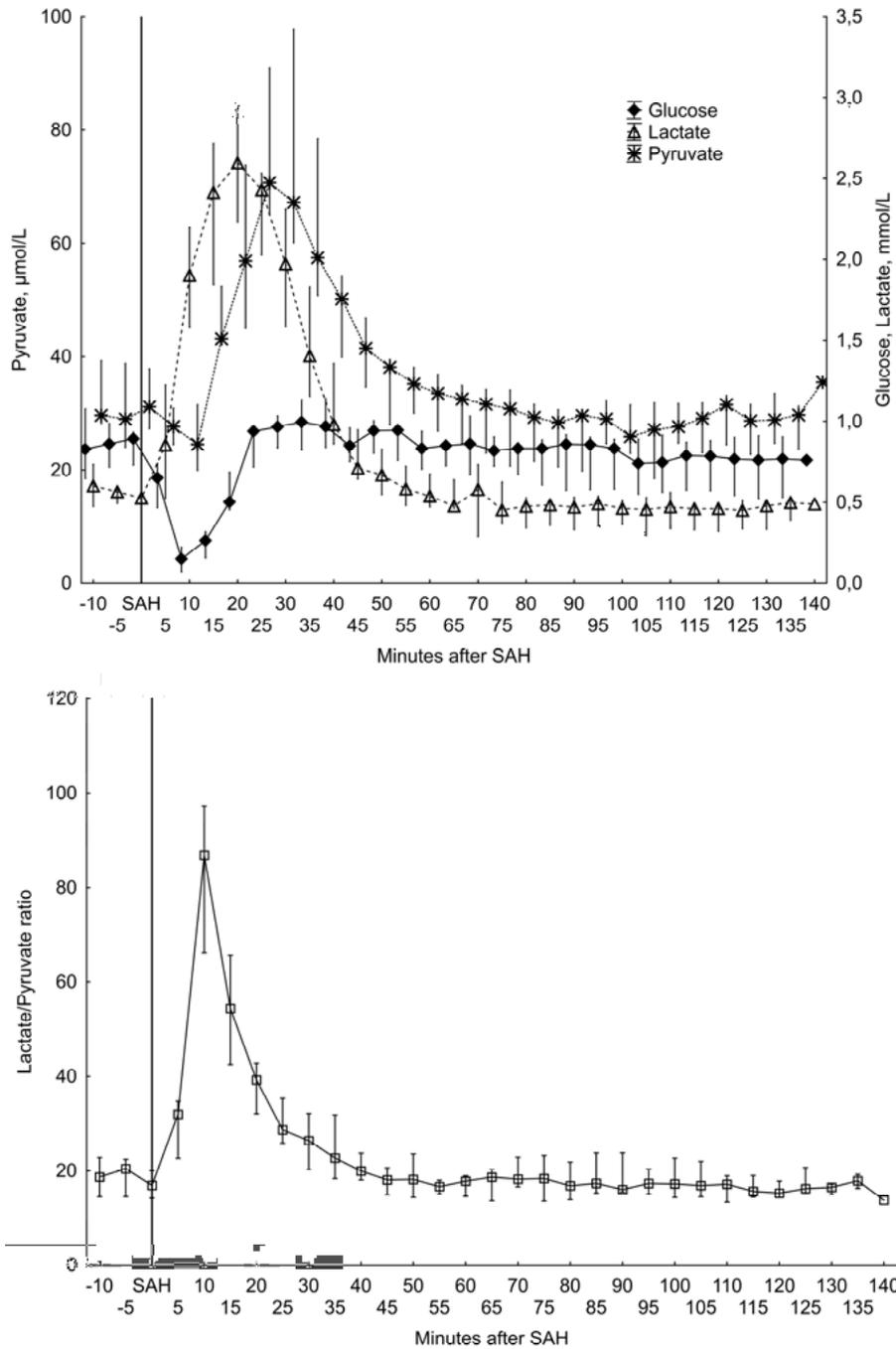


Figure 2. Microdialysis results from the animals without persisting ICP elevation (n=9). Median values with 25th-75th percentiles. Energy related metabolites (upper) and Lactate/Pyruvate ratio (lower).

In the two animals described above with persistent elevation of ICP, a typical microdialysis pattern of severe ischemia was seen<sup>72,73</sup>. In these cases, glucose and pyruvate dropped quickly to undetectable levels and remained at those levels throughout the study time. At the same time, lactate concentrations increased and remained elevated (Figure 3).

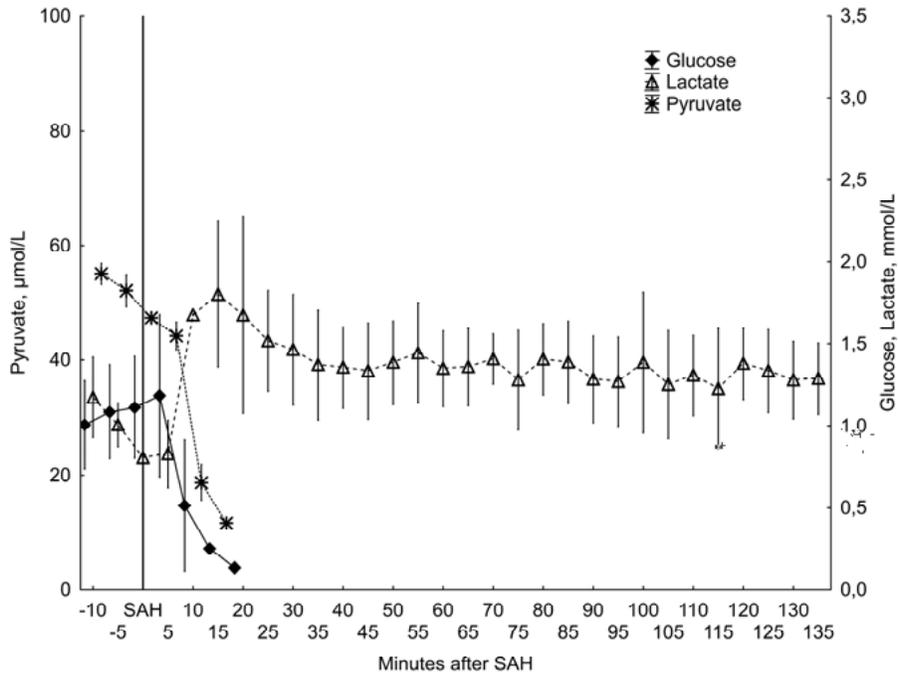
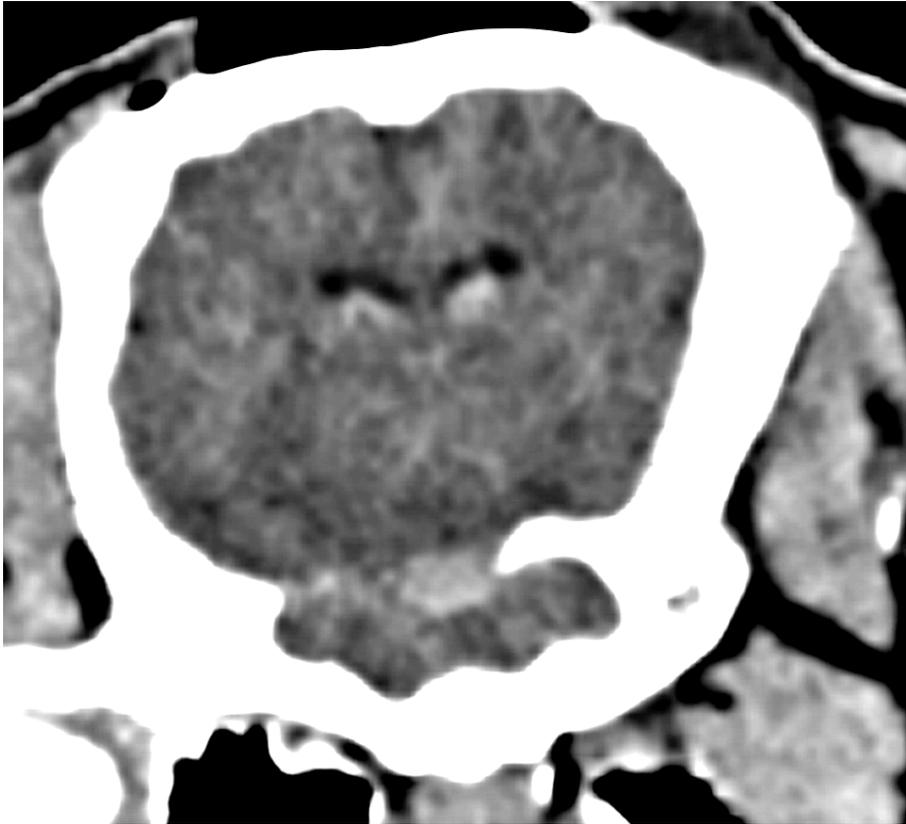


Figure 3. Microdialysis results from animals with persisting ICP elevation (n=2). Mean values with minimum and maximum values.

### CT and post-mortem morphological examination

The post-mortem examinations and the CT scans showed blood distribution in the basal cisterns in all animals. An example of the typical CT image is shown in Figure 4. In four animals, small amounts of coagulated blood were also found in the lateral ventricles. In one animal, there was a larger intraparenchymatous hematoma. This animal was also one of the two animals with persisting high ICP and ischemic pattern in microdialysis.



*Figure 4.* CT image after experiment verifying blood distribution in the basal cisterns. Blood is also visible in the lateral ventricles.

## Endocrine stress response (Paper II)

Because of the two different patterns in ICP reaction described above, the two pigs with persisting ischemia were analyzed separately from the other nine pigs.

The concentrations of analyzed hormones are presented in Table 3.

**Table 3.** Hormone concentrations

	Minutes after SAH	Mean	Median	Range	SD	<i>p</i>
P-ACTH <i>ng/L</i>	-10	18.8	7	5.7 - 101	30.98	
	+15	37.5	24	9.1 - 101	30.26	0.012
	+75	72.2	71	7.5 - 126	38.25	0.051
	+135	64.8	58	10.0 - 117	34.73	0.028
S-Cortisol <i>nmol/L</i>	-10	36.1	28	14.3 - 89.0	22.56	
	+15	156.8	181	13.9 - 252.0	79.55	0.011
	+75	240.6	275	5.82 - 329.0	97.93	0.011
	+135	202.2	224	6.57 - 327.0	100.88	0.017
P-Aldosterone <i>pmol/L</i>	-10	112.7	101	56 - 248	59.66	
	+15	196.9	200	84 - 311	82.66	0.012
	+75	268.9	294	101 - 409	94.12	0.012
	+135	221.3	221	101 - 426	108.91	0.028
P-Chromogranin-A <i>nmol/L</i>	-10	0.140	0.117	0.103 - 0.219	0.0450	
	+15	0.164	0.151	0.103 - 0.301	0.0593	0.441
	+75	0.111	0.107	0.037 - 0.208	0.0545	0.139
	+135	0.102	0.102	0.029 - 0.196	0.0546	0.069
U-Cortisol <i>nmol/L</i>	-10	131.8	142	35 - 232	79.23	
	+15	95.1	74	26 - 204	64.30	0.051
	+75	255.3	234	43 - 436	130.41	0.066
	+135	379.4	415	16 - 710	211.71	0.036
U-Cortisol/creatinine <i>μmol/mol</i>	-10	40.0	36	16.9 - 69	16.13	
	+15	38.6	32	15.3 - 68	17.74	0.678
	+75	97.1	88	9.9 - 203	65.75	0.021
	+135	167.6	163	15.9 - 300	95.94	0.017

*Table 3.* Hormone concentrations in the animals with transient ischemia (n=9) and significance testing of change from baseline (-10 minutes) using Wilcoxon matched pairs test.

### ACTH

After induction of SAH, plasma ACTH levels increased in the two following measurements. The highest concentrations of ACTH were found in the sample taken 75 minutes after SAH. In the last sample (135 minutes after SAH) the

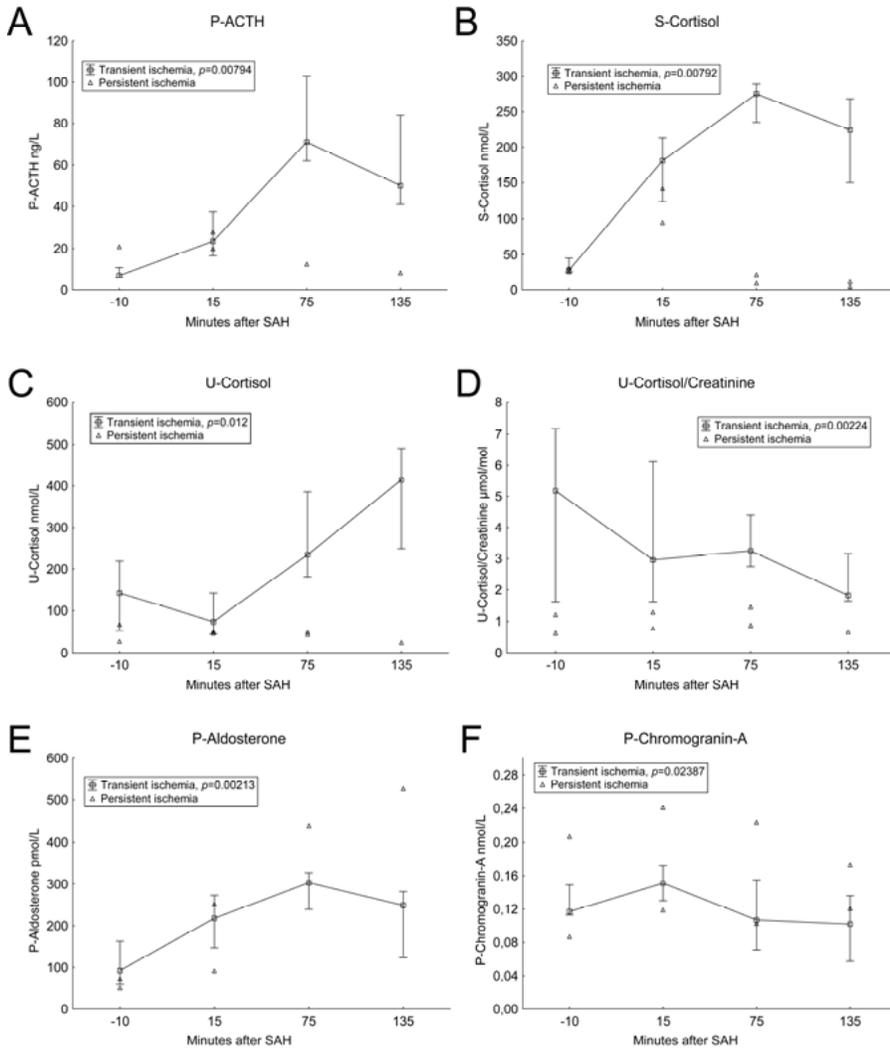
levels of ACTH had started to decrease, but the levels were still elevated compared to baseline values. The two animals with persisting ischemia did not show an elevation of ACTH levels after SAH. The dynamics of ACTH is shown in Figure 5A.

### **Cortisol**

Cortisol concentrations in serum increased in the same pattern as ACTH with increasing levels after SAH, peaking at 75 minutes after SAH induction (Figure 5B). The two animals with persisting signs of ischemia had an increase of cortisol levels in the measurements 15 minutes after SAH, but this was followed by decreasing levels. In urine, cortisol levels increased throughout the experiment (Figure 5C). However, when the quotient with urine creatinine was analyzed, the urine cortisol levels after SAH did not increase compared with baseline values (Figure 5D).

### **Aldosterone**

Concentrations of plasma aldosterone increased after SAH in a pattern similar to ACTH and cortisol, with the highest concentrations after 75 minutes. The two animals with persisting ischemia did not differ distinctively from the rest of the animals. Aldosterone concentrations are shown in Figure 5E.



*Figure 5.* Blood hormones. Median values with 25th and 75th percentiles. Animals with persistent ischemia are displayed separately. Significance testing of change over time with the Friedman test. Time scale is non-linear.

### Catecholamines and chromogranin-A

Adrenalin, noradrenalin and their metabolites were found in some urine samples. However, many samples were below detection level. Adrenalin, metadrenalin, methoxy-tyramine and vanillylmandelic acid were the only substances found in more than sporadic samples. Noradrenalin and its specific metabolites were only detected in a very small number of samples. The concentrations in urine of adrenalin and its metabolites are shown in Figure 6. No statistical analysis was performed because of the small number of samples.

Chromogranin-A concentrations in plasma (Figure 5F) increased after SAH induction, and thereafter decreased in the two following measurements.

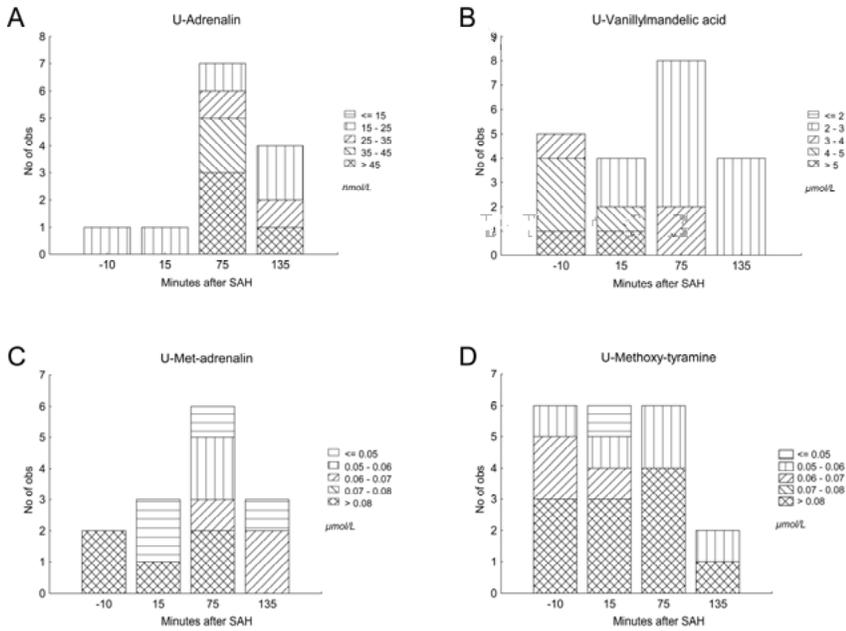


Figure 6. Catecholamines and metabolites in urine in animals with transient ischemia. Samples with detectable concentrations are shown.

### Paper III

At the time of patient admission, plasma troponin-I levels were increased ( $>0.022$  mg/L) in 51% of the patients. The highest value at admission was 8.2 mg/L. For 35 patients, troponin-I values were greater than 0.3 mg/L, indicating myocardial injury according to our hospital's standard.

Results from the univariate analysis are shown in Table 4.

**Table 4.** Univariate analysis results showing AUC NT-proBNP values

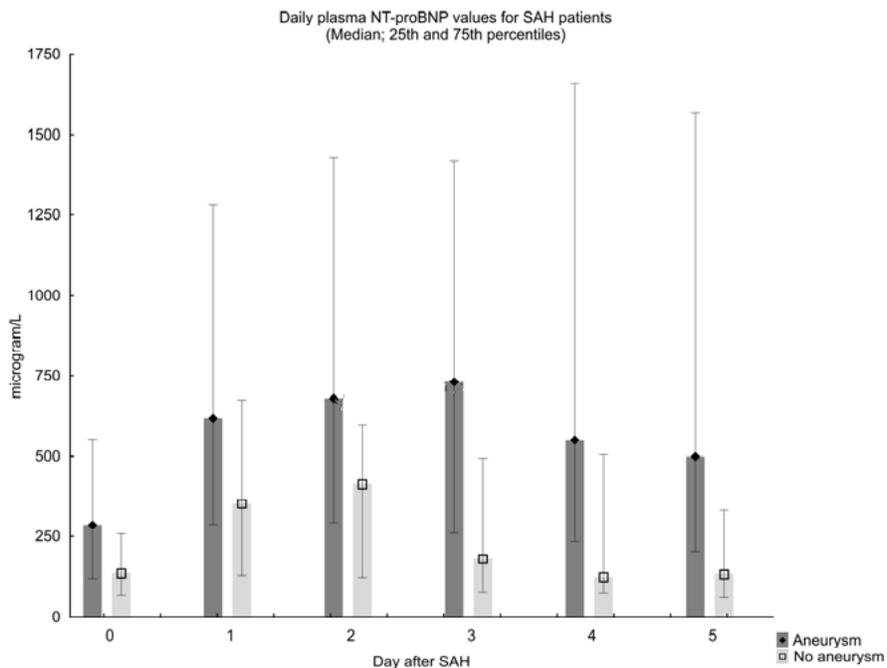
Patient Variable	Median Value (25th & 75th percentiles)		p Value
sex	male	female	<0.006
	3,610 (1,834 & 6,799)	5,102 (2,716 & 15,339)	
Fisher grade	1-2	3-4	<0.007
	2,041 (928 & 5,469)	4,888 (2,438 & 12,447)	
WFNS grade	1-2	3-5	<0.00003
	3,570 (1,486 & 5,424)	6,129 (2,995 & 16,817)	
angiographic finding	normal	aneurysm	<0.00009
	1,632 (870 & 3,530)	5,079 (2,690 & 12,447)	
ACoA aneurysm	yes	no	NS
	4,551 (2,458 & 10,009)	5,102 (2,716 & 14,241)	
troponin-I at admission	normal	high	<0.0000001
	3,018 (1,348 & 5,781)	6,402 (4,010 & 20,407)	
vasospasm treatment	no	yes	NS
	4,401 (2,200 & 10,336)	5,102 (2,355 & 13,909)	
neurological deficit	no	yes	<0.0002
	2,873 (1,246 & 5,652)	5,132 (2,812 & 14,241)	
any infection	no	yes	<0.003
	3,630 (1,345 & 6,216)	5,588 (2,758 & 12,783)	
aneurysm treatment	clip	coil	NS
	4,517 (2,368 & 14,241)	5,058 (2,758 & 12,352)	
outcome	favorable	poor	<0.00001
	3,657 (1,347 & 6,600)	6,308 (3,628 & 21,525)	

*Table 4.* Parameters indicating a more severe SAH such as more blood detected by CT, worse clinical condition at admission, and the presence of an aneurysm had significantly larger values of AUC NT-proBNP. This finding is also true for female patients, patients with any infection, and patients with neurological deficits/deterioration. ACoA = anterior communicating artery; NS = not significant.

The AUC for NT-proBNP was larger when variables indicated a more severe SAH. These variables were more blood detected on CT images, worse clinical condition at admission, presence of an aneurysm, high plasma troponin-I at admission, neurological deficits either at admission or during the course of the disease, and presence of any infection. The AUC for NT-proBNP was also larger among women and patients with poor outcome. The AUC for NT-proBNP did not differ between surgical and endovascular treatment of aneurysms or between groups that did or did not receive treatment for vasospasm. The AUC for NT-proBNP did not differ according to whether aneurysms were on the anterior communicating artery or in other locations. Using Spearman's

rank-order correlation coefficient, age was correlated with AUC for NT-proBNP; correlation coefficient was 0.41. Using the generalized model and including variables significantly correlated with NT-proBNP in univariate analysis, the best predicting model of large AUC NT-proBNP was the combination of the following variables: female sex, high plasma troponin-I, presence of an aneurysm, neurological deficits, and increasing age. Using Glasgow Outcome Scale dichotomized to poor/favorable as the outcome parameter indicated that the best predictive model consisted of the variables of age, clinical condition at admission, and amount of blood on the first CT scan. The AUC for NT-proBNP was included in the second best model, but AUC for NT-proBNP was not an independent predictor for clinical outcome.

The time course for serum NT-proBNP values is shown in Figure 7. Concentrations were low at time of admission and increased during the next few days. The values peaked on Day 3 for patients with aneurysms and on Day 2 for those with normal angiography results. The change was significant for patients with aneurysms ( $p < 0.025$ ), but not for those with normal angiography results.



*Figure 7.* Daily median concentrations (and 25th and 75th percentiles) of serum NT-proBNP for 138 patients with aneurysms and for 18 patients with normal angiographic results. Among patients with aneurysms, serum NT-proBNP changed significantly over time ( $p < 0.025$ ).

## Paper IV

In total, 124 measurements were performed during the first 7 days from onset of SAH. As indicated by the graph (Figure 8), measured values increased in the later part of the first week. When comparing day 2-3 to day 5-6, there was a difference between the groups,  $p=0.017$ .

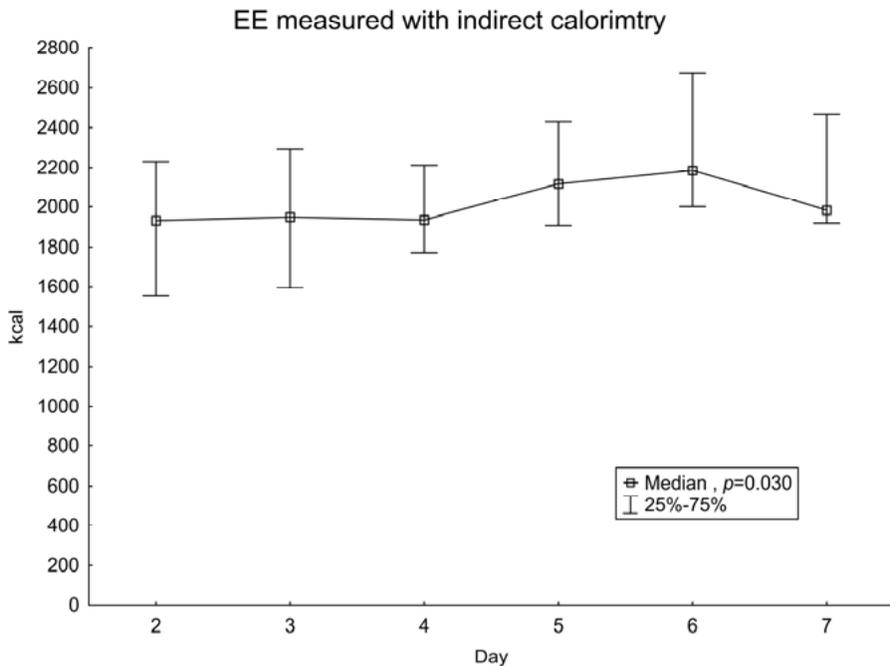


Figure 8. Daily EE measured with indirect calorimetry. Significance testing over time with the Skillings-Mack test.

### Measured values related to predictive equations

For each patient and IC measurement, the ratio of IC to the expected value was calculated using the H-B equation (Figure 9) and the PSU-98 equation (Figure 10).

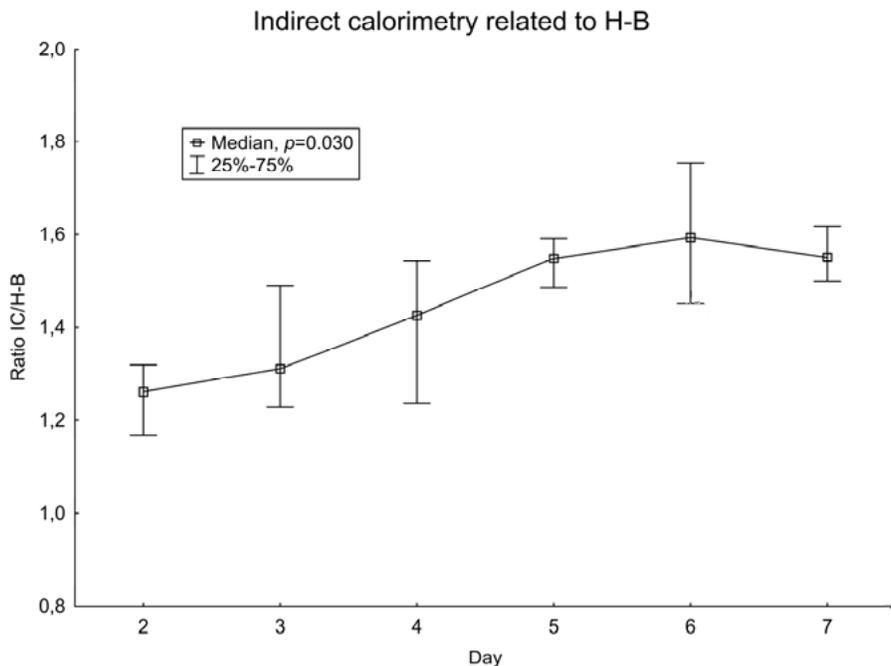


Figure 9. EE measured with indirect calorimetry related to the Harris-Benedict equation. Significance testing over time with the Skillings-Mack test.

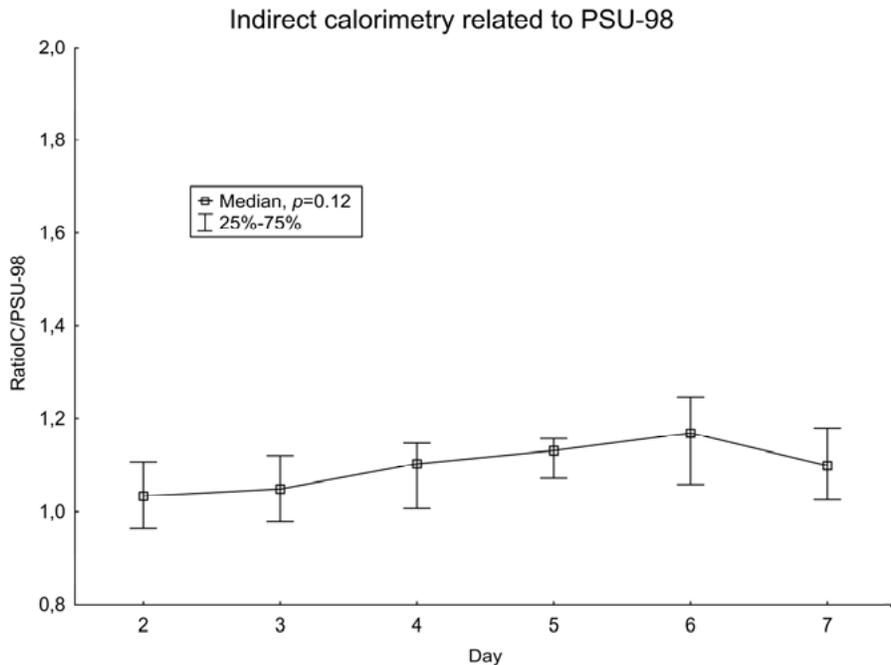


Figure 10. EE measured with indirect calorimetry related to the Penn State 1998 equation. Significance testing over time with the Skillings-Mack test.

The ratio of IC to the H-B equation increased during the first week after SAH, peaking at day 6 when the measured value was around 60% higher than expected. There was a significant difference between days 2-3 and days 5-6,  $p=0.020$ . The ratio of measured IC to the PSU-98 equation also increased in a similar manner. The variations were, however, smaller than with the H-B equation. On the day with the peak value, day 6, the measured value was less than 20% higher than expected. There was not a significant difference between days 2-3 and days 5-6,  $p=0.15$ .

The EE as measured with IC was also related to the patient's body weight (Figure 11).

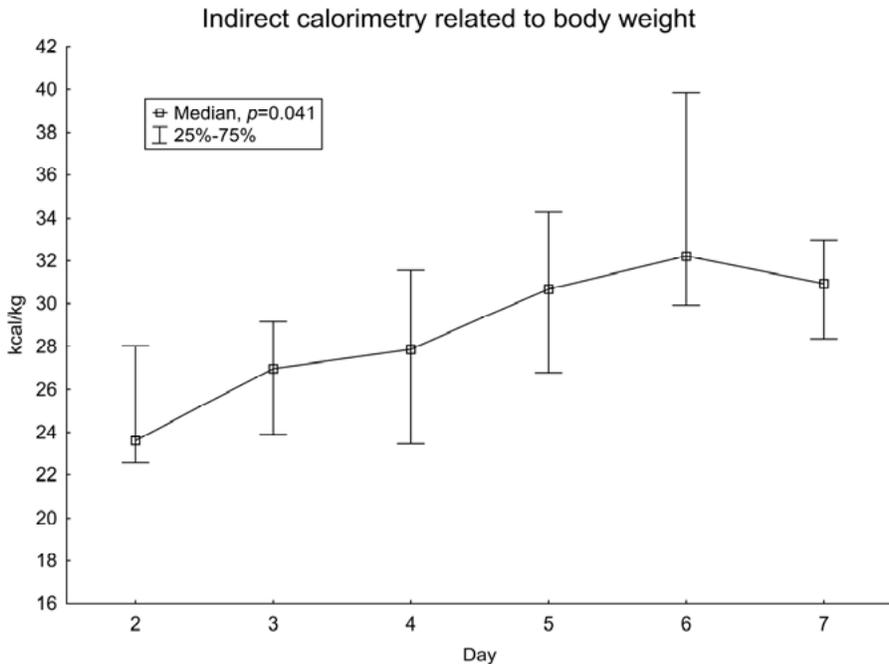
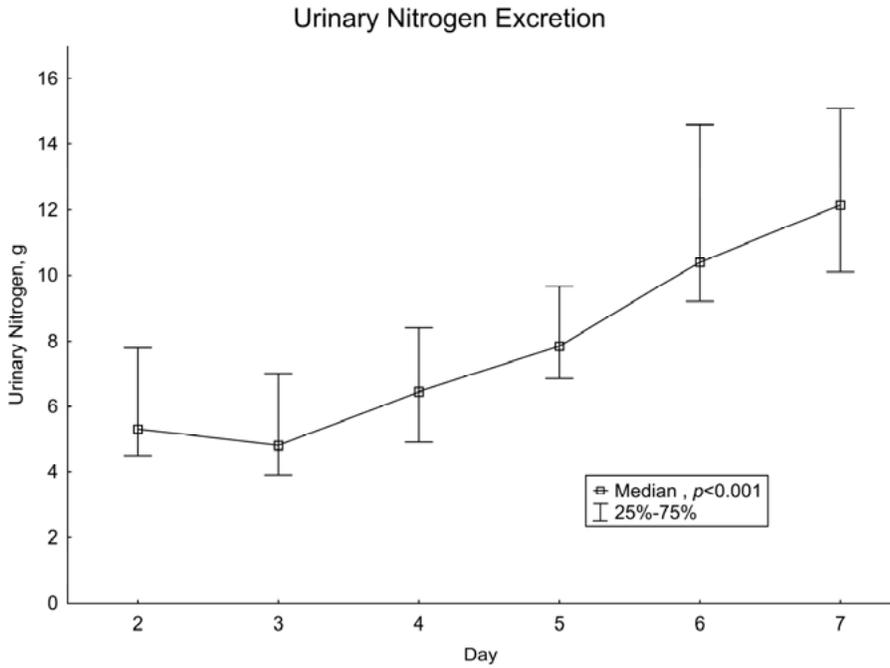


Figure 11. EE measured with indirect calorimetry related to body weight. Significance testing over time with the Skillings-Mack test.

### Urinary nitrogen excretion

Urinary nitrogen is presented in Figure 12. From initially low values on the second and third day after SAH, there was a distinct significant increase throughout the first week.



*Figure 12.* Daily nitrogen excretion in urine. Significance testing over time with the Skillings-Mack test.

# Discussion

## The ultra-early response in SAH

The events occurring at time of aneurysm rupture and shortly thereafter are difficult to study in patients. In paper I, we wanted to study cerebral metabolism and hemodynamics at the time of aneurysm rupture.

In patients, cerebral microdialysis and measurements of ICP and CPP can in a few cases be initiated when the patient presents at the hospital and has been diagnosed with subarachnoid hemorrhage, typically at least one hour after the actual time of aneurysm rupture. In general, however, patients are transferred to a neurosurgical unit in a secondary hospital and there is a considerable delay before neurointensive care with adequate monitoring is provided.

The same type of problem exists in studying stress-related hormones in the very early phase in SAH. To be able to study cerebral and systemic responses in acute SAH, an animal model is needed.

## Porcine animal model of SAH

We developed an animal model in the pig with focus on the very early events after SAH. We consider the pig to be easy to monitor and study using the same equipment as in a clinical situation. Specific for our model is that we tried to mimic the conditions at an aneurysm rupture by injecting blood in the basal cisterns until the cerebral perfusion pressure was 0, creating a transient global brain ischemia. In order to mimic conditions when the aneurysm ruptures, an important issue is the creation of the transient global ischemia. In order to do this in a reliable way, ICP and CPP need to be monitored. This has been described for animal models using rabbits<sup>56</sup> or rats<sup>55,74</sup>. We verified the ictal global ischemia by recording a transient negative CPP. In our protocol we decided to keep CPP 0 for approximately one minute. It is common that patients are admitted in a fairly good clinical condition after a brief period of unconsciousness, presumably caused by transient brain ischemia at time of aneurysm rupture. When the blood injection in our model stopped, ICP and CPP gradually and spontaneously returned to pre-injury levels spontaneously. During the measurements following SAH, ICP slowly increased. This elevation of ICP may be interpreted as the development of hydrocephalus due to the SAH, as frequently seen in patients.

## Cerebral metabolism in experimental SAH

The transient ischemia in the animal model was also evaluated by intracerebral microdialysis for energy related biomarkers. In our model, there was an immediate transient ischemic microdialysis pattern with low glucose, high lactate and slightly decreased pyruvate, causing a markedly elevated lactate/pyruvate ratio. This energy metabolic biomarker pattern of cerebral ischemia has been validated in a number of studies, using e.g. positron emission tomography<sup>72,73</sup>. The short-lasting ischemia was followed by a period of elevated lactate and pyruvate and peaking at 15-25 minutes (20-30 minutes without compensation for dead space) after SAH in conjunction with normalized glucose. The peaks in lactate and pyruvate were not simultaneous, pyruvate peaking 5 minutes after lactate. This pattern may reflect a post-ischemic hyperemia allowing glucose and oxygen return with an ensuing hyperglycolytic state to produce energy for restoring ionic perturbation following the ischemic event. From about 45 minutes after SAH and throughout the remaining monitoring time, all analyzed microdialysis energy-related biomarkers were normalized.

Previous studies have demonstrated alterations in the cerebral energy metabolism after SAH<sup>74-76</sup>. However, in these studies, since microdialysis collection was performed at intervals of 15-30 minutes, the delay in the pyruvate peak compared to lactate was not detected. A delayed pattern of hyperglycolysis has been observed in SAH patients and associated with favorable outcome, although those measurements were not done in the ultra-early phase<sup>77,78</sup>. One problem with microdialysis is that it provides a focal measurement. However, in the present model we assumed that the change in CPP following the intervention produced global energy metabolic perturbation, presumably making our microdialysis data representative.

Another important matter in order to simulate the patient situation is the distribution of the blood. In the few published studies on SAH models in pigs<sup>54,79-82</sup>, blood was injected in the cisterna magna. Prechiasmatic blood location, as in the present model, may be important, because the proximity to the hypothalamus and pituitary systems. Prechiasmatic blood deposit has only been used in a small number of rat studies<sup>75,82,83</sup>.

The findings of cerebral microdialysis and ICP/ CPP demonstrate that the pig model described in this study seems to be a usable SAH model for studies of ultra-early events after SAH.

## Endocrine stress response after SAH

In paper II, we investigated the endocrine stress response immediately after onset of the disease. We used the animal model described in paper I and measured ACTH, cortisol and aldosterone in blood. To examine the sympathetic stress response, we analyzed catecholamines in urine and chromogranin-A in plasma. Chromogranin-A is co-released with catecholamines and a marker of sympathetic activity<sup>84,85</sup>.

We found evidence of an endocrine stress response after experimental SAH, involving both the HPA axis and the sympathetic nervous system. There is evidence in the literature that the stress response in patients with SAH involves the endocrine systems described here, but also other components, including brain natriuretic peptide (BNP)<sup>17</sup>. The stress response in patients is most likely essential to survive the challenges of life-threatening disease, but the complicated mechanisms in the stress response may also be involved in many complications of SAH, such as hypovolemia, cerebral vasospasm and delayed ischemic neurological deficits (DIND).

### Hypothalamic-pituitary-adrenal axis response

The rise of ACTH and cortisol seem to be very rapid after onset of disease. Both ACTH and cortisol showed elevated concentrations in the first measurement 15 minutes after SAH induction, and peaked at the measurement 75 minutes after SAH. In the last measurement, 135 minutes after SAH, ACTH and cortisol decreased compared to the peak value. This indicates that the initial peak of these two hormones may be very early. The dynamics of aldosterone was very similar to ACTH and cortisol, which implicates that this rapid and early rise of aldosterone was most likely stimulated by ACTH, rather than by other mechanisms.

It has previously been well established that activation of the HPA axis occurs in patients with SAH, but these measurements were not done in the ultra-early phase of the disease. One interesting fact in our study is that concentrations of all analyzed hormones in blood of the HPA axis decreased in the last measurement. This raises a suspicion that the peak of these stress hormones could be very early and difficult to catch in patients because of the delay from onset of disease to blood sampling.

### Sympathetic stress response

Many samples of catecholamines and their metabolites in urine were below detection level, not allowing for statistical analysis. However, adrenalin, met-adrenalin, methoxy-tyramine and vanillylmandelic acid (VMA) were found in some cases, showing an increasing trend at 75 minutes after SAH. Met-adren-

alin and met-noradrenalin are degradation products of adrenalin and noradrenalin, respectively. These two metabolites are further degraded to VMA and excreted in urine. Methoxy-tyramine is a degradation product of another catecholamine, dopamine. The findings of catecholamines and their metabolites support that there was an early sympathetic stress response following the induction of SAH.

In our study, plasma chromogranin-A showed an increasing trend and thereafter decreased. The measurements at 75 and 135 minutes after SAH were lower than baseline values at 10 minutes before SAH. This finding may be connected with the urinary metabolites of catecholamines that also were higher at baseline, possibly because the experiment started too soon after surgical preparation.

## NT-proBNP

Increased BNP has been shown to be involved in a number of complications frequently seen some days after SAH. The aim in paper III was to determine whether high levels of BNP during the first four days could be predicted at admission based on parameters describing the severity of the disease, including troponin-I as a sign of pre-hospital cardiac injury.

The finding that NT-proBNP values increased over the first days after admission is in line with earlier results<sup>17,86</sup>. We found that 51% of the patients had elevated troponin-I at admission, indicating pre-hospital cardiac injury. The fact that serum levels of NT-proBNP were highest after 3 days in patients with SAH and aneurysms argues that the initial cardiac impact is not solely responsible for the increased levels.

In our study, factors indicating a more severe disease predicted higher BNP load. Whether BNP load is causing worse clinical condition or is a result of it has to be further studied.

We found that patients with poor outcome had significantly larger AUC NT-proBNP. In the generalized model, AUC NT-proBNP was included in the second best model for predicting clinical outcome, but was not an independent predictor. This is partly due to the fact that BNP load is related to the severity of the disease. Another possibly contributing explanation is that complications related to high BNP load, such as hyponatremia, fluid imbalance and clinical vasospasm are treated during neurointensive care.

## Energy expenditure in SAH

Patients with SAH often have impaired consciousness and are unable to regulate nutritional intake themselves. Providing an adequate amount of energy is one factor that may affect the outcome. We performed a study with repeated measurements using IC during the first week after SAH (Paper IV).

The basal metabolic rate varies with multiple factors such as age, body weight, height, gender and systemic inflammatory state. In order to be able to compare measured EE in this group of patients, we used the ratio of measured EE to the H-B equation. The predicted basal metabolic rate with the H-B equation is constant during the first week, since it is based on constant or slow-changing factors. This means that the calculated ratio can be used not only to compare EE within patients, but also between. The H-B equation underestimated metabolic demand in this patient group. Since the H-B equation was developed for prediction of the basal metabolic rate in healthy individuals, it is not surprising that the equation does not apply without modification on patients with severe illness.

We performed the same type of calculation with the PSU-98 equation. This is an equations used to predict energy need in an ICU setting. It is based on the H-B equation, but also includes information about respiration and body temperature to compensate the increased metabolism caused by the systemic inflammatory response. Our results indicates that the PSU-98 equation may underestimate EE in patients with SAH. However, the difference between predicted and calculated EE was smaller than when using H-B equation. Also, the increase in EE that we found during the course of the disease was compensated for by the PSU-98 equation since a significant difference could not be found between the early days compared to later. This suggests that the PSU-98 equation is a better alternative than the H-B equation for this patient group, although there were large variations between patients.

Another simple approach to calculate the daily need of energy for a patient is using the equation 25 kcal per kg body weight. With the patients in this study, using the 25 kcal/kg equation underestimated EE, especially in the later part of the first week.

Urinary nitrogen excretion can be used as an indicator of protein turnover. Our measurements showed low nitrogen excretion the first days after onset of disease. Thereafter, there seemed to be a linear rise in nitrogen excretion. A similar pattern have been demonstrated in plasma measurements in previous studies<sup>61</sup>. One likely interpretation is a shift in protein metabolism towards catabolism. Whether the possible catabolic state is only due to the disease itself is unclear. The increase in EE during the acute phase after SAH is almost parallel to nitrogen excretion and raises the suspicion that the proteolytic state could be enhanced by underfeeding. In this study, we did not collect detailed information about nitrogen intake, which means we cannot rule out high protein intake as a factor influencing nitrogen excretion. However, the measurements of EE or nitrogen excretion did not influence the type of nutrition or amount of energy provided to the patients. One factor possibly contributing to the low values the first day is the fact that enteral nutrition in our unit is not provided the first day after onset of disease, thereby causing low protein metabolism.

Measured EE increased gradually during the first 6 days and there was a difference in EE between days 2-3 compared to days 5-6. An increase in metabolic demand could be expected after such an event as intracranial aneurysm rupture and the subsequent triggering of a stress response. In our study, however, EE had a dynamic response and there were substantial variations both within and between patients. Matching provided amount of energy to the actual energy expenditure could be beneficial for the patients and possibly improve outcome.

# Conclusions

The porcine animal model of acute SAH used in paper I has features resembling spontaneous SAH and seems suited for use in studies of early cerebral and systemic changes in SAH. The metabolic cerebral pattern in ultra-early SAH suggests transient substrate failure followed by hypermetabolism.

The endocrine stress response in experimental SAH, studied in paper II, was found to start within 15 minutes in the HPA axis with early peaks of ACTH, cortisol and aldosterone. There were indications of early activation of the sympathetic nervous system, but small number of cases and methodological issues made interpretation difficult.

In paper III, measurements of NT-proBNP showed a temporal course with increasing values the first 4 days after SAH. NT-proBNP was related to factors indicating a more severe SAH. The best subset of variables predicting high NT-proBNP load were female sex, elevated troponin-I at admission, high age, angiographic finding of an aneurysm, and worse clinical condition on admission. The findings suggest the initial injury in aneurysmal SAH is an important factor in predicting high NT-proBNP load during the acute stage of the disease.

Energy expenditure in patients with aneurysmal SAH, studied in paper IV, increased gradually during the first week after SAH. There were considerable variations within and between patients. Estimations of energy demand using predictive equations is difficult and measurements of EE with indirect calorimetry would help providing an adequate amount of energy.

## Summary in Swedish – sammanfattning på svenska

Subarachnoidalblödning är en allvarlig sjukdom med risk för svår hjärnskada eller död hos den drabbade patienten. Den vanligaste orsaken är ett brutet aneurysm på ett av de stora blodkärlen i hjärnan. När ett aneurysm brister läcker blod från den arteriella cirkulationen till subarachnoidalrummet, det intrakraniella trycket stiger och hjärnan utsätts för syrebrist av varierande längd och svårighetsgrad. Denna dramatiska händelse leder till ett stressvar från kroppen, avsett att återställa normal blodcirkulation i hjärnan och förbereda kroppen på sjukdom. Stressvaret innefattar bland annat aktivering av hormonaxeln hypotalamus-hypofys-binjurebark (HPA-axeln) vilket resulterar i ökade nivåer av kortisol i blodet. Detta hormon är viktigt för att kroppen ska kunna hantera sjukdom och annan stress. Även det sympatiska autonoma nervsystemet aktiveras, vilket bland annat resulterar i att blodtrycket stiger, tarmfunktionen minskar och kroppen försetts i beredskap att kunna svara snabbt på prövning.

Andra typer av reaktioner har också konstaterats i det tidiga förloppet hos patienter med subarachnoidalblödning. Dessa innefattar ökade halter av BNP (brain natriuretic hormone) i blodet, ett hormon som bland annat påverkar kroppens vätske- och saltbalans. Påverkan på både hjärnans och kroppens energiomsättning har påvisats hos patienter med subarachnoidalblödning.

Alla dessa reaktioner är viktiga för att hjärnans funktioner ska upprätthållas och för att kroppen ska kunna hantera svår sjukdom, men det finns också risk att kroppens reaktioner på svår stress i sig själva leder till förändringar som försämrar hjärnans förutsättningar till återhämtning efter subarachnoidalblödning. Studierna som utförts inom ramen för denna avhandling syftar till att beskriva den hormonella responsen samt hjärnans och kroppens energiomsättning tidigt i förloppet efter subarachnoidalblödning.

Händelser som inträffar precis vid insjuknandet i subarachnoidalblödning och tiden direkt därefter kan inte studeras på patienter, eftersom de då inte befinner sig på sjukhus. För studier av detta slag behövs djurmodeller. De flesta djurmodeller framtagna för studier av subarachnoidalblödning är inte inriktade på det tidiga förloppet. Det finns också anledning att misstänka att hjärnans känslighet för syrebrist kan variera mellan olika typer av djur. En ny djurmodell i gris användes i delarbete I och II för att undersöka hjärnans energiomsättning

i samband med insjuknandet. I djurmodellen studerades även aktiveringen av HPA-axeln med analys av adrenokortikotropt hormon (ACTH), kortisol och aldosteron samt tecken på aktivering av det sympatiska autonoma nervsystemet. Hjärnans energiomsättning i insjuknandeögonblicket studerades med mikrodialys.

I djurmodellen noterades kraftig stegring av det intrakraniella trycket och artärtrycket vid induktionen av subarachnoidalblödningen. Vidare sågs ett mönster av energi-relaterade substanser i hjärnan som talar för en period med syrebrist och efterföljande ökad energiomsättning. Kraftig och mycket snabb aktivering påvisades av HPA-axeln. Det fanns även tecken talande för aktivering av det sympatiska autonoma nervsystemet. Resultaten talar för att djurmodellen är lämplig för studier av tidiga förändringar vid subarachnoidalblödning. Det hormonella svaret efter subarachnoidalblödning tycks ske mycket snabbt. Vid den sista mätningen, 135 minuter efter blödningen, hade nivåerna av ACTH, kortisol och aldosteron börjat sjunka. Detta skulle kunna innebära att den maximala koncentrationen av dessa hormoner kan vara svår att uppmäta hos patienter eftersom det ofta hunnit gå flera timmar innan patienten nått sjukhus och diagnostiserats med subarachnoidalblödning.

I delarbete III undersöktes relationen mellan subarachnoidalblödning och den N-terminala delen av prohormonet för BNP (NT-proBNP). NT-proBNP anses vara ett bättre mått på total BNP-utsöndring eftersom det har längre halveringstid än BNP och inga kända biologiska effekter. Etthundrafemtiosex patienter med subarachnoidalblödning inkluderades. NT-proBNP de första 4 dagarna efter insjuknandet relaterades till faktorer beskrivande sjukdomens svårighetsgrad, hjärtmuskelpåverkan i form av förhöjt troponin-I samt patientens återhämtning efter 6 månader. Nivåerna av NT-proBNP steg de första dagarna efter insjuknandet. Högre nivåer av NT-proBNP kunde relateras till faktorer förenliga med en svårare form av subarachnoidalblödning såsom större blödning, sänkt medvetandegrad, förhöjt troponin-I, neurologiska bortfallssymptom och infektion under vårdförloppet. Högre NT-proBNP påvisades även hos kvinnor och hos patienter med dålig återhämtning. De variabler som bäst kunde prediktera högt NT-proBNP var kvinnligt kön, förhöjt troponin-I, förekomst av ett aneurysm, neurologiska bortfallssymptom samt hög ålder. Resultaten talar för att den initiala skadan efter subarachnoidalblödning är av stor betydelse för nivåerna av NT-proBNP i det tidiga sjukdomsförloppet.

Kroppens energiomsättning efter subarachnoidalblödning studerades i delarbete IV. Mätningar med indirekt kalorimetri, en metod där syrgasförbrukningen och koldioxidproduktionen mäts och används för att beräkna energiomsättningen, utfördes dagligen på 32 patienter den första veckan efter insjuknandet. Resultaten jämfördes med tre prediktiva formler framtagna för uppskattning av energiomsättningen. Utsöndringen av kväve i urinen undersöktes

som ett mått på nedbrytning av protein. Det fanns en succesiv ökning av energiomsättningen under mätperioden. Jämfört med prediktiva formler var den uppmätta energiomsättningen generellt stegrad även om stor variation sågs mellan och inom patienter. Kväveutsöndringen i urin ökade kraftigt under mätperioden, vilket kan tyda på ökad nedbrytning av protein. Resultaten talar för att prediktiva formler är svåra att använda för uppskatta energiomsättningen för patienter med subarachnoidalblödning och att mätningar av faktisk energiomsättning bör utföras oftare.

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