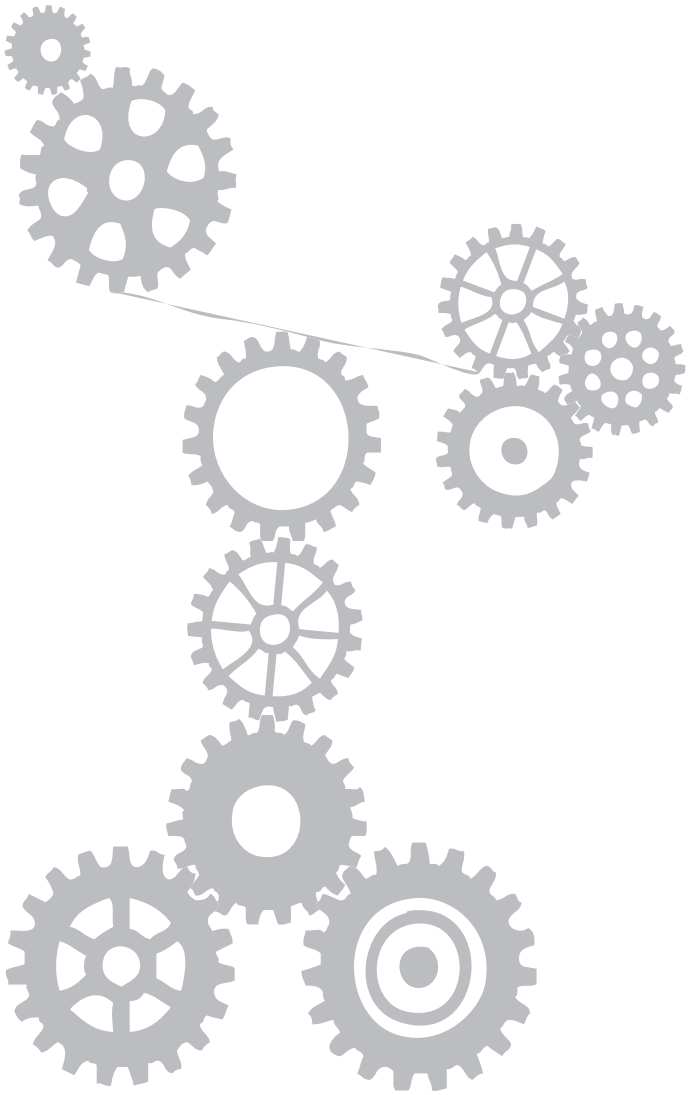
Chapter 1

Introduction and aims of the thesis



THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The hypothalamic-pituitary-adrenal (HPA) axis functions through a complex set of interactions between the hypothalamus, the pituitary and the adrenal glands. It is our central stress response system which is activated during physically or mentally challenging situations. Activation of the system causes the hypothalamus to secrete corticotrophin releasing hormone (CRH). When CRH binds to CRH receptors on the anterior pituitary gland, adrenocorticotropic hormone (ACTH) is released by the pituitary. ACTH stimulates the secretion of cortisol by the adrenal cortex. Cortisol, in turn, exerts inhibitory effects to the hypothalamus and the pituitary via a negative feedback loop (Figure 1). Cortisol secretion shows strong diurnal variation with the lowest levels at around midnight, an initiation of the rise at approximately 02.00–03.00 h and peak values in the morning directly after waking. Thereafter, levels slowly decrease throughout the day until the nadir at midnight.

Besides being activated during stressful situations, the HPA axis is also vital for supporting normal physiological functioning. Cortisol serves several functions in the body like the regulation of blood glucose, suppression of the immune system and assistance in fat, protein and carbohydrate metabolism.

ADRENAL INSUFFICIENCY

Patients with adrenal insufficiency (AI) are characterized by the loss of endogenous cortisol production. This lack of cortisol production can be caused by loss of function of the adrenal gland itself, in which case it is called primary AI (PAI) (Figure 1). This form of AI is most frequently caused by autoimmune adrenalitis (Addison's disease) or by a disturbed function of one of the enzymes involved in cortisol synthesis (congenital adrenal hyperplasia).[1,2] AI can also be caused by impairment of the pituitary (secondary AI (SAI)) or hypothalamus (tertiary AI), resulting in a deficiency of ACTH or CRH, respectively, and subsequently a lack of stimulation of the adrenal cortex to produce cortisol. Pituitary tumors or treatment of these pituitary tumors, by means of pituitary surgery or radiotherapy, are the most frequent causes of SAI.[3] Pituitary tumors can be classified as functioning or non-functioning, depending on their hormonal activity. Non-functioning adenomas have no clinical or biochemical features of excessive hormonal secretion, whereas functional pituitary adenomas are characterized by excessive pituitary hormonal secretion. Other pituitary region tumors, such as craniopharyngiomas, meningeomas, germinomas, intrasellar or suprasellar metastases as well as traumatic brain injury can also cause SAI.[4] The first choice of treatment of pituitary (region) tumors is surgical resection of the tumor, usually via

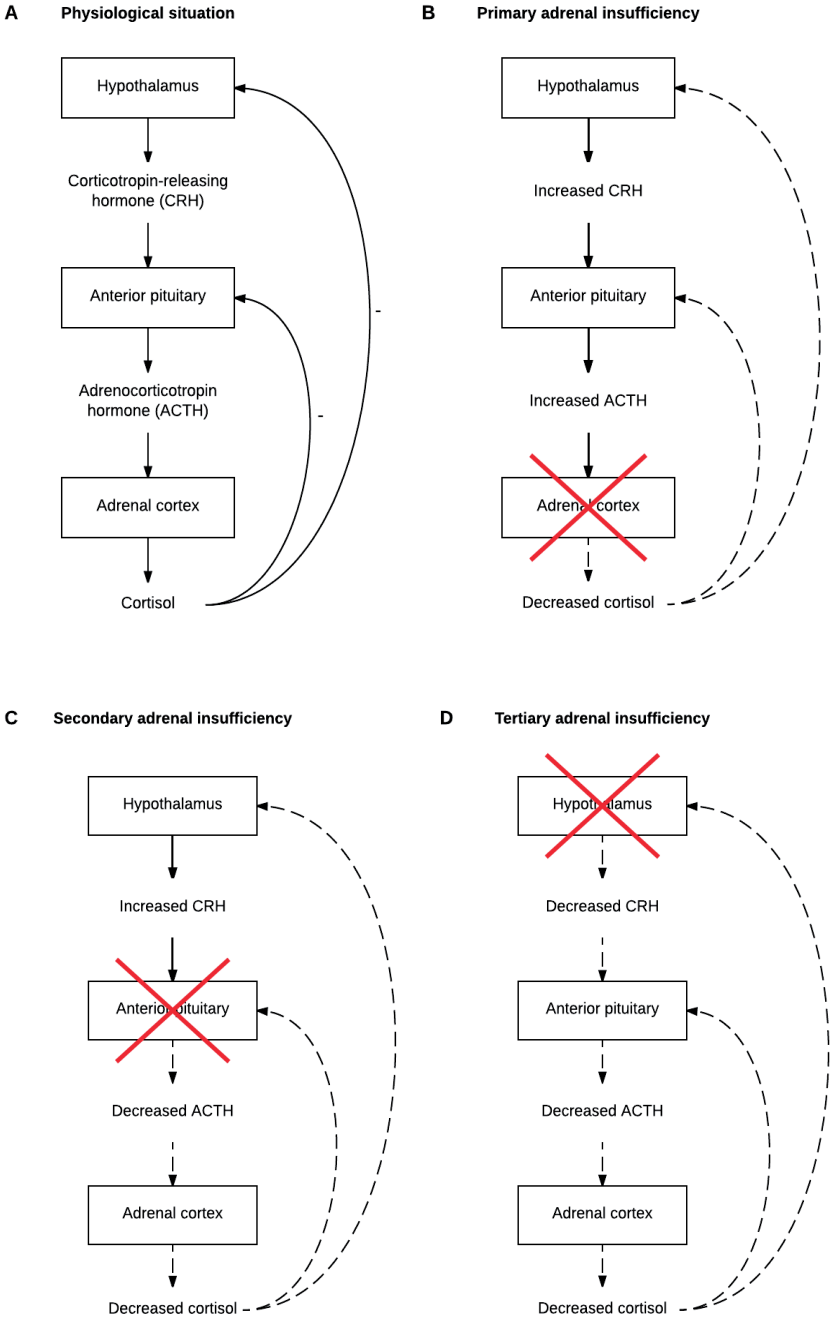


Figure 1. The hypothalamus-pituitary-adrenal axis during A) the normal physiological situation, B) with primary adrenal insufficiency, C) with secondary adrenal insufficiency and D) with tertiary adrenal insufficiency.

the transsphenoidal route. It is a minimally invasive technique in which the tumor is approached through the nose and sphenoid sinus. Less commonly, when the tumor is large or is not accessible through the transsphenoidal route, transcranial surgery is performed, in which a section of the skull is removed in order to access the brain. In some cases additional radiotherapy is applied, for instance when the tumor is not fully resected, when the tumor is recurring or in case of secreting adenomas where hormonal control cannot be achieved after surgery and medical therapy.

SAI is a rare disease, it has an estimated prevalence of 150–280 per million.[3,5–7] Patients with untreated SAI present with weight loss, anorexia or loss of appetite, generalized fatigue, loss of energy, reduced muscle strength, and increased irritability.[4] However, presenting signs and symptoms are often subtle and unspecific, impeding the diagnosis which often leads to a delay in diagnosis.

Diagnosis

The diagnosis of SAI is based on the finding of low early morning cortisol levels. Furthermore, in case of indeterminate cortisol values, various stimulation tests are available to assess the integrity of the HPA axis. For instance, the ACTH stimulation test, also known as the short synacthen test, has been advocated by several investigators in cases with a suspected disease duration of at least one month. This test can be done at any time of the day. Serum cortisol levels are measured at baseline and 30 or 60 minutes after the administration of 250 µg ACTH. Cortisol responses below 500–550 nmol/L (specific cut-off values can vary, depending on the assay used for the measurement of cortisol) are indicative of AI. However, when performed within 4 weeks after pituitary surgery, the adrenal glands are not yet atrophied and thus may still respond to ACTH, wrongly indicating intact HPA axis.

Therefore, the insulin tolerance test is the gold standard for the diagnosis of SAI. In this test, a hypoglycemic state is provoked by the administration of 0.1 units of insulin/kg body weight. Hypoglycemia is a powerful stressor that stimulates the HPA axis.[8] Cortisol concentrations in response to the insulin tolerance test below 500 nmol/L are considered to indicate AI. However, the test is cumbersome, expensive and contraindicated in patients with a history of seizures or cardiovascular diseases.[2,9,10]

TREATMENT OF SAI

Glucocorticoid substitution treatment

Until corticosteroid replacement became available, AI was a deadly disease. Edward Kendall was the first to isolate cortisosterone in 1936 and two years later Leonard Simpson first used synthetic deoxycorticosterone acetate in the treatment of Addison's

disease with success.[11] However, it was only after the synthesis of cortisone that glucocorticoids (GCs) became widely available,[12] for which Edward Kendall, Philip Hench and Tadeus Reichstein received the Nobel Prize in Physiology or Medicine in 1950.

Patients with AI are treated with GCs, of which oral hydrocortisone (HC) is the most commonly used preparation. Traditionally it was recommended to administer two-thirds (20 mg) of the substitution dose in the morning and one-third (10 mg) in the evening. This dose was based on cortisol production rate estimates in healthy individuals of 12–15 mg/m²/day. However, using stable isotope tracers the daily cortisol production rate is currently estimated to be approximately 6–10 mg/m²/day,[13,14] corresponding to total daily doses of 15–20 mg/day. Body weight was found to be an important factor in the clearance of HC and therefore a weight-adjusted morning dose of 0.12 mg/kg body weight is recommended by some investigators.[15] Furthermore, thrice-daily dosing resulted in more stable and physiological cortisol levels throughout the day compared to twice-daily dosing.[16] However, uniform guidelines are lacking, resulting in a wide variety of substitution regimens with different doses and number of daily doses used in clinical practice.[17]

Next to the lack of consensus about the dose and dose frequency, agreement about how to objectively monitor the adequacy of current GC substitution therapy is also absent. Blood sampling is informative only when knowing the time of HC administration and time of blood sampling. Some clinicians use cortisol day curves,[18] but they are inconvenient for the patient and time-consuming. Moreover, day curves turned out to be unable to discriminate between well-substituted and under- or over-substituted patients.[19]

Urinary free cortisol levels have been used as an overall indicator of the adequacy of cortisol substitution therapy.[18] However, urinary free cortisol levels are influenced by corticosteroid-binding-globulin (CBG) binding capacity. CBG saturates rapid after oral HC ingestion, at approximately total cortisol concentrations of 450–550 nmol/L,[20,21] which leads to increases of cortisol in urine. As a result, normal ranges for healthy individuals cannot be used in the assessment of urinary cortisol excretion during GC substitution. Furthermore, daily fluctuations in cortisol are missed due to the nature of this measure.[22]

Salivary cortisol has been used as an alternative, non-invasive method for monitoring substitution therapy. It has several advantages compared to serum cortisol day curves, as it is inexpensive, easy to perform, and can be collected at home. However, correlations between salivary cortisol and serum cortisol vary.[22–25] Furthermore, in remains unclear to what extext salivary cortisol reflects tissue cortisol levels.[25–27]

In practice, clinical assessment of symptoms potentially suggestive of over- or under-treatment is often used. Muscle and joint pain, reduced strength, nausea, fatigue and lack of energy are symptoms suggestive of under-replacement, whereas weight

gain, new onset abdominal obesity, sleeplessness, hypertension and diabetes may indicate over-replacement.[19] Under-treatment bears the risk of insufficient cortisol supply in the case of severe stress risking an adrenal crisis,[4] whereas chronic exposure to high cortisol levels is associated with increased mortality,[28,29] increased risk for cardiovascular diseases,[30,31] osteoporosis,[32] reduced quality of life (QoL) [33] and cognitive impairment.[34]

Hydrocortisone treatment and cognition

The brain is a major target area for GCs.[35] GCs can easily pass the blood-brain barrier and they exert their effect via corticosteroid receptors. There are two types of corticosteroid receptors: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). GRs are widely expressed throughout the brain with highest densities in regions such as the prefrontal cortex and limbic areas including the hippocampus, amygdala, thalamus and hypothalamus.[36] Data on mapping of MRs in the brain is less consistent, but high MR density is found in the hippocampus.[37] GCs bind to MRs with a 6–10 fold greater affinity than to GRs.[38] This differential affinity results in differences in occupation of the two receptors under different conditions. At basal cortisol levels, predominantly MRs are occupied. GRs will be activated additionally to MRs only when cortisol levels are high, e.g. at the circadian peak or during stress.[39]

An association between GCs and cognitive functioning is well described. Both in animals and in humans, there is convincing evidence of an inverted "U"-shape relation between plasma cortisol levels and cognitive functioning.[36,40] This means that very low and very high cortisol levels impair information processing and cognition.[40,41] Particularly the hippocampus is a major target area for GCs, due to its high density of GRs and MRs. Hippocampal functioning is therefore likely to be affected by GC levels. It is generally accepted that the hippocampus plays an important role in reactivity to novel situations and is essential for learning and memory.[42]

In healthy individuals, several studies reported decreased memory performance in association with higher cortisol levels.[34,43–51] The impairing effects of GC excess seem to be most pronounced in declarative memory as studies have shown that increases in endogenous cortisol levels due to stressful conditions induce impairment in declarative memory, but not in non-declarative memory.[34,44] Another study showed that the administration of 25 mg of cortisone to healthy subjects before retention of a previously learned list of nouns impaired delayed recall of those words.[43] Immediate recall and recognition were not impaired, suggesting that cortisol impaired retrieval specifically. In addition, declarative verbal memory performance was decreased after administration of HC doses resembling cortisol levels during major stress in healthy subject.[52]

The negative side effects of cortisol excess on cognition have also been shown in several patient groups. Patients that were exposed to excessive levels of cortisol, for

instance due to Cushing's syndrome, showed deficits in memory, visual and spatial information processing, reasoning, language performance, verbal learning and attention.[53–56] On the other hand, patients with hypocortisolism also reported impairments in cognitive functioning.[57–60] Compared to healthy matched controls, patients with Addison's disease performed worse on memory and executive functioning tasks,[57] selective attention,[58] episodic memory and speed of processing,[59] and verbal learning.[60]

Besides being present in the hippocampus, GRs are also widely expressed in the prefrontal cortex, a brain structure known to be involved in executive functioning. Executive functions are a set of cognitive processes, including attentional control, inhibitory control, working memory, cognitive flexibility/set-shifting, reasoning and planning. So far, only a few studies focused on the influence of cortisol on prefrontal cortex-dependent cognitive functioning and results are inconclusive. McCormick and colleagues[61] demonstrated a sex-dependent relationship in healthy subjects between salivary cortisol levels and the number of perseverative errors, as a measure of cognitive flexibility and set-shifting. In this task, participants had to sort cards according to a certain classification rule (for instance color or shape) and feedback was provided after each trial whether the classification was right or wrong. After several trials, the rules of classification changed and participants had to shift to a new method of classification. Perseverative errors were the trials in which the participants gave the same response as in the previous trial even though they received feedback that the response was not correct. In women, higher cortisol levels were associated with more perseverative errors, and thus less cognitive flexibility and reduced set-shifting, whereas in men higher cortisol levels were associated with more cognitive flexibility. In contrast, Newcomer and colleagues[52] were not able to demonstrate an effect of HC administration on the performance on another test of executive functioning (verbal fluency test) in healthy subjects.

Besides being a key area for executive functioning, the prefrontal cortex is also known to be involved in social cognition and attention.[62,63] Social cognition refers to how people process, store and apply information about other people and social situations. To our knowledge there are no studies examining the effect of cortisol on social cognition. With regard to the effects of cortisol on attention, results are also inconclusive. A study showed that, compared to a placebo, the administration of 80 mg prednisone/day for 5 days to healthy subjects, leading to increased levels of cortisol, led to less salient imprinting of meaningful stimuli and may impair selective attention.[64] In contrast, another study did not find impairments in attentional functioning in patients with Addison's disease,[59] neither in healthy subjects after dexamethasone treatment[48] or after HC treatment.[52]

In conclusion, these results suggest that cognitive functions that are mediated by the hippocampus and the prefrontal cortex are affected by cortisol levels. However, most

of the studies performed are cross-sectional studies and included healthy individuals. It is therefore likely that the intact negative feedback mechanism in healthy individuals influenced the results. No studies have been performed so far assessing the effect of the administration of different doses of HC treatment on memory, executive functioning, attention and social cognition for a substantial period of time in patients treated for SAI.

Hydrocortisone treatment and quality of life

Patients who receive GC substitution often report an impaired QoL compared to healthy controls,[33,65,66] which results in a high number of patients being unable to work and receiving disablement pension.[66] The reason for this impairment is likely to be multifactorial and, among others, inappropriate GC treatment might play a role. The pharmacokinetic properties of currently available GC treatment results in over- or under-substitution during certain periods of the day. Indeed, retrospective analyses revealed that there is a relationship between the daily GC dose and QoL.[33,65,66] Hahner and colleagues[66] showed a reduced subjective health status in patients with chronic AI. Patients with SAI exhibited a slightly more impaired subjective health status, particularly in the domains physical functioning and bodily pain, compared to patients with PAI, probably due to common concomitant endocrine disorders that accompany SAI. Higher doses were associated with a more profound impairment of subjective health status. Ragnarsson and colleagues[33] confirmed these findings by showing that higher HC equivalent doses were associated with more severely impaired QoL in hypopituitary patients. However, there was no difference in QoL between ACTH sufficient and ACTH insufficient patients, indicating that other factors also influence QoL. Due to the cross-sectional nature of the above mentioned studies, it remains unclear whether the impaired QoL was a result of the higher GC dose taken, or that a higher dose was prescribed in order to improve the comprised QoL.

To disentangle cause and effect, QoL in relation to GC dose was assessed within a few randomized controlled trials. Wichers and colleagues[67] performed a small randomized double-blind crossover study and found no differences in QoL between the three doses (15, 20 or 30 mg HC/day) administered. Furthermore, in another randomized open label crossover study no difference in QoL scores between the three doses (15, 20 and 30 mg HC/day) was found; irrespective of the dose, patients reported significantly lower levels of energy compared to healthy controls.[68] In another randomized double-blind crossover study patients showed improvements in physical functioning on the lowest HC dose administered (15 mg HC/day) compared to the other regimens (20 mg HC/day or 5 mg prednisone/day).[69] However, even though QoL improved on the lowest dose regimen, it remained impaired compared to healthy controls. Furthermore, in

accordance with the study by Ragnarsson,[33] QoL did not differ from ACTH-sufficient patients.

It can thus be concluded that GC substitution treatment appears to have an effect on QoL. However, in the abovementioned controlled trials overall sample sizes were small, treatment periods were short and in some studies timing of the dosing was changed together with the total daily dose, introducing an extra potential influencing factor. Furthermore, results are conflicting and the exact relationship between GC dose and QoL remains inconclusive.

Hydrocortisone treatment and cardiovascular risk factors

Patients with SAI show an increased mortality rate, predominantly caused by cardiovascular and cerebrovascular diseases.[7,28] Inadequate substitution therapy with GCs might contribute to this increased risk for cardiovascular diseases. In order to prevent low levels of cortisol as a consequence of the short half-life of currently available immediate release tablets, patients are administered doses resulting in supraphysiological cortisol concentrations after intake. Higher doses of GC substitution treatment are associated with increased severity of cardiovascular risk factors. A study in the Scottish population showed that patients receiving exogenous GCs of doses equivalent to more than 30 mg HC/day had increased rates of all cardiovascular diseases, including myocardial infarction, heart failure and cerebrovascular disease.[30] Cardiovascular risk in patients exposed to low dose GCs was similar to the risk in patients not receiving exogenous GCs.[30] Furthermore, a large Scandinavian study in 2424 patients with hypopituitarism also demonstrated that higher doses of HC are associated with increased cardiovascular risk.[17] Hypopituitary patients requiring GC replacement therapy had higher waist circumference, hemoglobin A1c, total cholesterol and triglycerides than ACTH sufficient patients (i.e. those not requiring GC treatment). Only those receiving doses of 20 mg/day or more showed an unfavorable metabolic profile. Moreover, all new cases of diabetes, stroke and myocardial infarction occurred in the GC treated group.[17]

High blood pressure is known to be an independent risk factor for cardiovascular diseases and hypertension is responsible for approximately 50% of deaths from stroke or cardiovascular disease.[70] A few controlled studies have investigated the effect of GC dose on blood pressure. Dunne and colleagues[71] studied whether a reduction of the GC replacement dose would improve cardiovascular parameters in 13 hypopituitary patients. A reduction of the dose from 30 mg HC/day to 15 mg HC/day did not alter blood pressure values. Furthermore, several other cardiovascular function parameters were comparable between patients and a matched control group. Another group studied whether an increase in GC dose would alter blood pressure in 17 SAI patients.[72] After

increasing the HC equivalent dose from < 20 mg/day to 30 mg/day for 7 days, blood pressure did not change.

Thus, even though there is a clear relationship between GCs and cardiovascular risk factors, the exact effect of GC dose on risk factors and the mechanisms underlying this relationship have not yet been fully defined.

Pharmacokinetics of hydrocortisone

The pharmacokinetic properties of oral HC make it difficult to adequately mimic the physiological circadian rhythm of cortisol secretion. Oral HC is well absorbed, bioavailability is reported to be 96 ± 20%, indicating complete oral absorption.[73] Peak levels in plasma are reached at approximately 1.2 h after ingestion and the terminal half-life is around 1.8 h.[22,73] As mentioned before, a fixed dose of 20 mg in the morning and 10 mg in the evening was initially suggested as the standard substitution therapy. However, Mah and colleagues[15] showed that body weight was an important factor in the clearance of HC. Therefore, a weight-adjusted morning dose of 0.12 mg HC/kg body weight was recommended as this reduced variability in the maximum cortisol concentration, reduced variability in area under the curve and reduced overexposure to less than 5%.[15]

More than 90% of circulating cortisol is bound, predominantly to CBG (approximately 70%) with high affinity and low capacity and to a lesser extent to albumin (approximately 20%) with low affinity and high capacity.[74] This leaves approximately 2–12% of free cortisol in the circulation, dependent on the total cortisol concentration. Free cortisol is considered the biologically active part of cortisol and can bind to MRs and GRs. CBG acts as a reservoir for circulating cortisol.

Several drugs influence cortisol metabolism through altered activity of cytochrome P450 3A4 (CYP3A4). Drugs that induce CYP3A4 activity, such as barbiturates, carbamazepine, phenytoin, rifampicin, lead to increased metabolism and hence decreased cortisol levels, whereas CYP3A4 inhibitors such as arepitant, ketoconazole, ritonavir, and diltiazem reduce metabolism and thus increase cortisol levels.[75,76]

A high inter-individual variability in pharmacokinetic parameters is reported in patients receiving HC substitution therapy.[22,77] Due to the pharmacokinetic properties of currently available immediate release tablets, patients are over- or under-treated during certain periods of the day.[77] Insight into the effect of dose adjustments on pharmacokinetic parameters of HC could provide an indication for evaluation and adjustment of HC substitution therapy.

CONSIDERATIONS AND AIMS FOR THIS THESIS

Due to the lack of high-quality data underlying current recommendations about treatment of SAI, a randomized controlled trial investigating the effects of HC substitution dose in patients with SAI was desirable. This study was designed in 2011–2012 and conducted in 2012–2013. Two different doses of HC within the physiological range and used in clinical practice were compared. Thrice daily, weight-adjusted dosing before food intake, with a morning dose of 0.12 mg HC/kg body weight (with corresponding total daily doses of 0.2–0.3 mg HC/kg body weight), was found by other investigators to reduce inter-patient variability in maximum cortisol concentrations and this scheme was chosen as base for HC substitution in our study.[15] However, much higher doses are also used in clinical practice,[17] and therefore we compared this dose of 0.2–0.3 mg HC/kg body weight/day to the double amount of it (0.4–0.6 mg HC/kg body weight/day). To reduce inter-subject variability, a crossover design was applied in which patients were their own controls.

In this study we focussed on the clinical outcome measures cognitive functioning, QoL, somatosensory functioning, blood pressure and regulating hormones, and pharmacokinetic parameters. As described above, all these variables are known to be influenced by cortisol levels and are important parameters in the quality of the substitution therapy.

The aim of this thesis is to assess the pathophysiologic effects of these two doses of cortisol, and to add evidence for recommendations regarding GC substitution therapy in SAI. As described above, we initiated a randomized double-blind crossover study (the Supreme Cort study, Clinicaltrials.gov identifier: NCT01546922). This thesis describes the results of our study on the effects of two different physiological doses of HC with regard to psychological outcome measures (cognition and QoL) as well as several somatic outcome measures (somatosensory functioning, blood pressure and regulating hormones, and pharmacokinetic parameters).

OUTLINE OF THIS THESIS

This thesis is divided into two parts. The first part focuses on psychological outcome measures, the second part describes somatic outcome measures.

The first part consists of the **Chapters 2**, **3,** and **4** and describes the effect of HC substitution dose on psychological measures. In **Chapter 2** we aimed to evaluate whether a lower dose of HC would be beneficial for cognitive functioning. The cognitive domains memory, attention, executive functioning and social cognition were

studied. These domains rely on the integrity of brain structures known to be influenced by cortisol due to the high density of GRs in these areas.

In **Chapter 3** we aimed to evaluate the effect of the two different doses of HC on several aspects of health-related QoL. To this end we used validated questionnaires assessing QoL at the end of each treatment period as well as the daily assessment of somatic complaints, depression, and anxiety by means of diaries.

In **Chapter 4** we aimed to assess the mediating role of cortisol levels in the relationship between stress and pain. An individual approach was used in the analysis of a relationship between perceived stress, as measured with an anxiety questionnaire, and pain, and the mediating role of low cortisol concentrations therein.

The second part consists of **Chapters 5, 6,** and **7** and describes the effect of HC substitution dose on somatic outcomes. In **Chapter 5** we aimed to investigate whether somatosensory functioning would be affected by treatment with two different doses of HC. Detection and pain thresholds were established using mechanical stimuli.

In **Chapter 6** we aimed at assessing the effect of HC dose on blood pressure and regulating hormones. High doses of GCs are associated with increased cardiovascular risk factors including blood pressure. However, the mechanisms underlying this relationship remain inconclusive. Elaborate laboratory measurements enabled us to also explore the underlying mechanisms.

In **Chapter 7** we aimed at parameterizing a pharmacokinetic population model of HC in patients with SAI. Furthermore, we compared pharmacokinetic properties of HC on the two doses of HC for plasma total cortisol, plasma free cortisol and salivary cortisol.

Chapter 8 provides the general discussion of the main findings in this thesis and addresses future perspectives. In **Chapter 9** a summary is given.

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Part 1

The effect of hydrocortisone treatment on psychological outcome measures in patients with secondary adrenal insufficiency