Clinical consequences of endogenous and exogenous glucocorticoid excess

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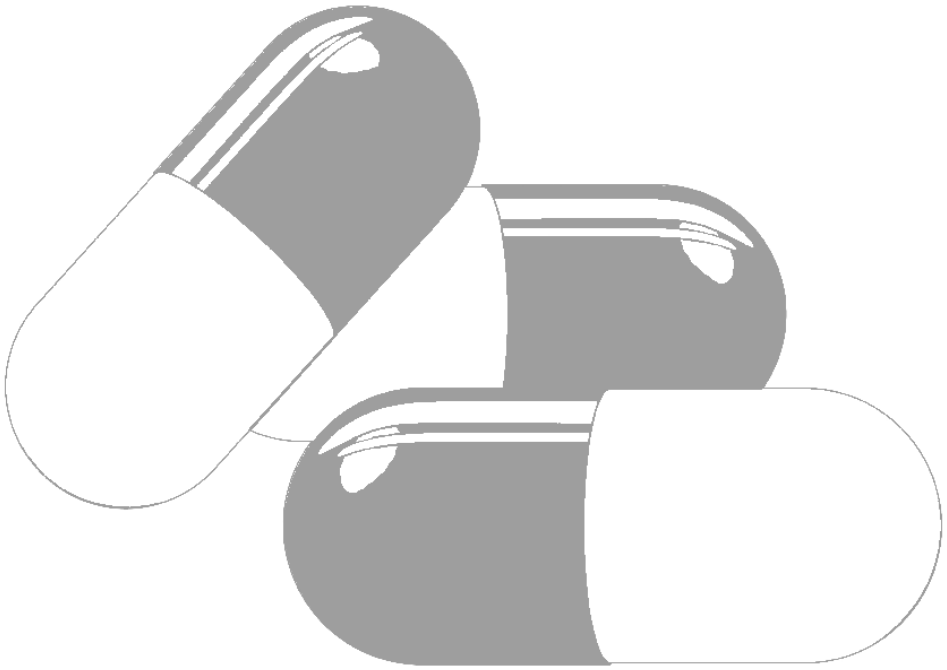
Chapter 1

General introduction and outline of the thesis



Introduction

Cortisol is a steroid hormone, which is produced in the cortex of the adrenal gland. Cortisol was first discovered by Edward Kendall, Tadeus Reichstein, and Philip Hench in the 1930s (1). After collecting 1,000 kg of adrenals from cattle, they extracted 25 g of active substance, in which they discovered 29 different steroids. Twenty-four of these were still undiscovered previously, including cortisol. In 1950, they shared the 'Nobel prize in physiology or medicine' for their discoveries relating to the hormones of the adrenal cortex, their structure, and biological effects (1).

The biological effects of cortisol are derived from the evolutionary perspective of stress response control, and consequently, these effects are widespread throughout the human body. These include immunosuppressive functions, e.g. reduction of an inflammatory response, and prevention of an allergic reaction, as well as the modern use of cortisol in the prevention of rejection of organ transplants (2, 3). In addition, cortisol co-regulates metabolic functions, e.g. control of blood pressure, glucose metabolism, bone mineral density, and lipid metabolism (4, 5). Finally, cortisol controls psychological functions, e.g. regulation of mood, well-being, and cognitive function (6). Therefore, cortisol is released in response both to physical and mental stress (7, 8).

In this chapter, the physiological and pathophysiological role of cortisol is explored, starting with the production and regulation of cortisol in healthy individuals. Subsequently, disruptions in the regulation of cortisol are discussed, including both states of cortisol excess (hypercortisolism) as well as cortisol deficiency (hypocortisolism), which is also known as adrenal insufficiency. Underlying causes, clinical features, and treatment options are presented.

The hypothalamus-pituitary-adrenal axis

Cortisol concentrations in the human body are controlled by the hypothalamus-pituitary-adrenal (HPA) axis (Figure 1). The central circadian oscillator (pacemaker), which is located in the hypothalamic suprachiasmatic nucleus in the brain, regulates this HPA-axis under basal, non-stressed conditions. This pacemaker ensures a daily rhythm in the production of cortisol (the so-called circadian rhythm), which is externally synchronized by the light-dark cycle as its most important input. The pacemaker mainly influences the hypothalamus, driving the release of corticotropin-releasing hormone (CRH) from the hypothalamic paraventricular nucleus. CRH stimulates the release of adrenocorticotropic hormone (ACTH) from the corticotroph

cells in the anterior pituitary gland. CRH also stimulates the production of new ACTH from its precursor POMC in the anterior pituitary, which is secreted after the initial fast release of pre-stored ACTH. ACTH is released from the anterior pituitary gland in around 40 pulses per 24 hours, with the highest amount of ACTH released in the morning around the time of waking. ACTH induces the release of cortisol from the adrenal cortex. Lowest concentrations of cortisol in the human body are found around midnight, and highest concentrations are found in the morning after the highest ACTH peak (9).

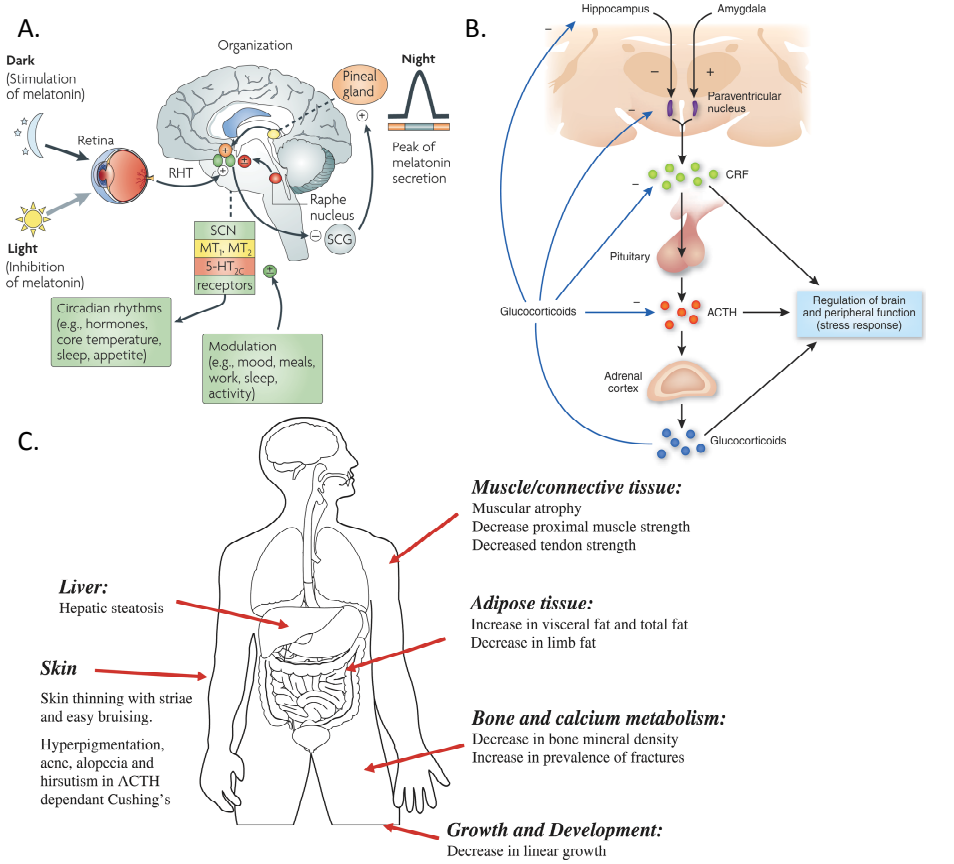


Figure 1: The hypothalamus-pituitary-adrenal (HPA) axis is regulated through the light-dark cycle via the retinohypothalamic tract (RHT), which in case of light inhibits the activity of the superior cervical ganglia (SCG). When dark, the SCG stimulates production of melatonin. Melatonin activates the suprachiasmatic nucleus (SCN) by the melatonin 1 and 2 receptors (MT1 and MT2), which in turn influences circadian rhythms including the HPA-axis. The SCN is modulated through daily behaviors and through serotonin from the raphe nucleus which acts at the serotonin receptor (5-HT2C) (A). The HPA-axis regulates production and release of glucocorticoids by excitatory control of the amygdala and inhibitory control of the hippocampus. The paraventricular nucleus in the hypothalamus produces corticotropin-releasing hormone (CRH), also known as corticotropin-releasing factor (CRF), which stimulates ACTH production and release from the pituitary, which in turn stimulates the adrenal cortex to synthesize and release glucocorticoids. Glucocorticoids inhibit the HPA-axis by negative feedback at various levels (B). Glucocorticoids have several metabolic influences in various tissues of the human body (C). Adapted from de Bodinat *et al.*, Hyman, and Fernandez-Rodriguez *et al.* (4, 11, 12).

Cortisol controls the abovementioned immunosuppressive, metabolic, and psychological functions by binding to the glucocorticoid receptor and also to the mineralocorticoid receptor. In addition, cortisol regulates its own secretion by a negative feedback mechanism through glucocorticoid and mineralocorticoid receptors in the brain and in the anterior pituitary gland (10).

Factors influencing cortisol concentrations

Circulating cortisol concentrations are lower in premenopausal women than in men of corresponding age, but there is no difference in cortisol concentrations between postmenopausal women and men older than fifty years. Therefore, there appears to be no sex difference, but there seems to be an influence of female hormones on cortisol concentrations. In older age, the morning cortisol peak occurs later than in younger age (13, 14). Food consumption rapidly increases concentrations of cortisol, mainly during the daytime (15). Differences in cortisol concentrations between different ethnic groups in relation to differences in social activity and living habits, suggest that these habits influence the synchronization of daily cortisol rhythms. E.g. in Chinese people, the cortisol peak occurs two hours earlier than in Caucasians, which may be related to their habits of early awakening and going to sleep early (16).

The morning cortisol peak after awakening is also known as the cortisol awakening response. This response seems to be influenced by both waking up as well as by daylight, as a smaller cortisol awakening response is seen after waking up in the afternoon than in the morning, and no cortisol awakening response is seen in the evening (17). Altered habits, such as lack of sleep, changes in meal pattern, and light-dark transitions, as may occur during travelling (jet lag) or shift work, affect the circadian rhythm in cortisol production, with complete inversion of cortisol rhythm when sleeping twelve hours out of phase with the usual sleep pattern (18). Circadian misalignment, by sleeping and eating at unusual times, reduces glucose tolerance in shift workers, increasing the risk of type 2 diabetes mellitus (19).

In reaction to unforeseen changes in the environment called ‘stressors’, which can be both external (e.g. temperature changes, food deprivation, physical trauma, infection) and internal (e.g. psychological stress), the stress system is activated. This stress system senses environmental changes through sensory organs and adjusts the central nervous system and peripheral organ activity to improve the chance of survival through restoration of internal homeostasis. The stress system includes both activation of the autonomic nervous system and the HPA-axis, and therefore leads to increased production of catecholamines and cortisol, which in turn increases metabolism (e.g. glucose concentrations), as needed to cope with the stressor at hand (the ‘fight-or-flight response’) (20).

Measurement of hormone concentrations and testing the HPA-axis

Approximately 95% of circulating cortisol is transported bound to cortisol-binding globulin (CBG). The remaining 5% is unbound, also called 'free cortisol', and is therefore available for executing the previously mentioned functions of cortisol by binding to the glucocorticoid and mineralocorticoid receptors (21). Cortisol bound to CBG can be released, depending on the saturation of the CBG (the percentage of CBG binding cortisol) and the affinity with which cortisol is bound to CBG. Cortisol-binding affinity is reduced locally in case of local inflammation and systemically in case of fever, leading to increased cortisol release from CBG (21).

Nowadays, cortisol can be measured by immunoassay or mass spectrometry in serum, saliva, urine, and hair. Serum total cortisol is usually measured in the morning to provide the peak cortisol concentration (22). Salivary cortisol on the other hand is collected at midnight, to capture the physiologically lowest cortisol concentration (23). Urinary free cortisol is usually measured in 24 h urine samples to provide the mean cortisol excretion (24). Hair cortisol can be examined to determine long-term cortisol exposure, which is until now only performed in research settings (25). Serum ACTH concentrations are measured by immunoassay, and they are often measured accompanying the morning serum total cortisol (26). Both salivary and hair cortisol reflect free cortisol measurements.

As cortisol concentrations vary during the day, single cortisol measurements provide limited information and are generally considered inadequate in determining normal functioning of the HPA-axis. Therefore, dynamic stimulation as well as suppression tests have been developed. In general, a rise in plasma glucocorticoids inhibits the ACTH secretion from the anterior pituitary gland, thereby suppressing the activity of the HPA-axis, whereas a fall in plasma glucocorticoids stimulates ACTH secretion from the anterior pituitary gland, thereby stimulating the activity of the HPA-axis. HPA-axis stimulation tests aim to maximize cortisol production from the adrenal cortex, whereas HPA-axis suppression tests aim to minimize cortisol production.

The most commonly used stimulation test in clinical practice is the ACTH stimulation test, which is used to test the maximum potential function of the adrenal glands when insufficient functioning is clinically suspected. During the ACTH stimulation test, usually a high dose (250 μg), or alternatively a low dose (0.5-1.0 μg), of ACTH is administered, and cortisol is measured after 30 (and sometimes also after 60) minutes and compared to the baseline cortisol measurement. Another stimulation test is the CRH test, in which 100 μg of CRH is administered, and cortisol and ACTH are measured for maximum increase compared to baseline (27). The insulin tolerance (stress) test can be used to induce the physiological stress response by administering insulin to induce hypoglycemia, leading to a rapid increase in catecholamines and

cortisol concentrations. However, as this is a potentially hazardous test in selected patients and uncomfortable for the patient, it is less commonly used (28). Finally, the overnight metyrapone stimulation test can be used to test the HPA-axis, in which patients are given a midnight metyrapone dose to inhibit the conversion of 11-deoxycortisol to cortisol, resulting in increased ACTH secretion due to loss of negative feedback in case of intact hypothalamic-pituitary function (29).

The low dose (1 mg) and high dose (8 mg) overnight dexamethasone suppression tests are available to test whether endogenous cortisol production can be suppressed. Because dexamethasone binds to the glucocorticoid receptor, both CRH and ACTH production and release are inhibited, and therefore also cortisol production is inhibited. Dexamethasone does not interfere with the assay used to measure cortisol concentrations. The high dose overnight dexamethasone suppression test is more potent in suppressing cortisol concentrations than the low dose test, but does not result in meaningful higher discriminatory power and is therefore not often necessary in the screening process of Cushing's syndrome (27).

Endogenous hypercortisolism

Cortisol overproduction by the human body itself is called endogenous hypercortisolism. The combination of clinical symptoms accompanying endogenous hypercortisolism is now known as Cushing's syndrome, and was first described in 1912 by Harvey Cushing (30):

"A syndrome of painful obesity, hypertrichosis, and amenorrhoea, with over-development of secondary sexual characteristics accompanying a low grade of hydrocephalus and increased cerebral tension. Pituitary, adrenal, pineal or ovary?"

An excess concentration of glucocorticoids induces specific metabolic changes and thereby alter body composition, including fat maldistribution, muscle wasting, insulin resistance, dyslipidemia, and hypercoagulability. Furthermore, risk of osteoporosis, hypertension, and neuropsychiatric disorders is increased by hypercortisolism (4, 31). Symptoms and signs associated with Cushing's syndrome include weight gain (with central obesity, rounded 'moon' face, and increased dorsal and supraclavicular fat pads called 'buffalo hump'), proximal muscle weakness, menstrual irregularities, loss of libido, depression, hirsutism, and thinness and fragility of the skin (with acne, purple striae, hyperpigmentation, and easy bruising) (Figure 2) (4, 32). Mortality is high in untreated severe Cushing's syndrome, with an estimated five-year survival rate of only 50% (33).

Endogenous hypercortisolism can be caused by a variety of etiologies (Table 1). The majority of these etiologies are ACTH-dependent, meaning that ACTH secretion is increased along with cortisol, and a minority of these etiologies are ACTH-independent, meaning that cortisol concentrations are increased without increase in ACTH concentrations (32). Cushing's disease is the most common underlying cause of endogenous hypercortisolism, with an incidence of 1.2-1.7 patients per million persons each year (35), which accounts for approximately 70% of cases of endogenous hypercortisolism (36). Cushing's disease is caused by an ACTH-secreting pituitary adenoma, which stimulates cortisol production by the cortex of the adrenal gland, and disrupts the negative feedback mechanism. Ectopic Cushing's syndrome results from a non-pituitary ACTH-producing source, which is a rare cause of ACTH-dependent Cushing's syndrome and occurs in approximately 5% of cases (36). ACTH-independent Cushing's syndrome is caused by an adrenal adenoma or carcinoma, which produces cortisol and thereby inhibits ACTH production by the pituitary gland, with an incidence of 0.2-0.6 patients per million persons each year (35). Adrenal Cushing's syndrome accounts for approximately 25% of cases of endogenous hypercortisolism (36). Other rare causes for Cushing's syndrome are ectopic CRH secretion, bilateral primary pigmented nodular adrenal hyperplasia, the ectopic actions of gastric-inhibitory peptide or catecholamines, and other adrenal-dependent processes associated with adrenocortical hyperfunction such as McCune-Albright syndrome and Carney's complex (32). These will not be further discussed in this thesis.

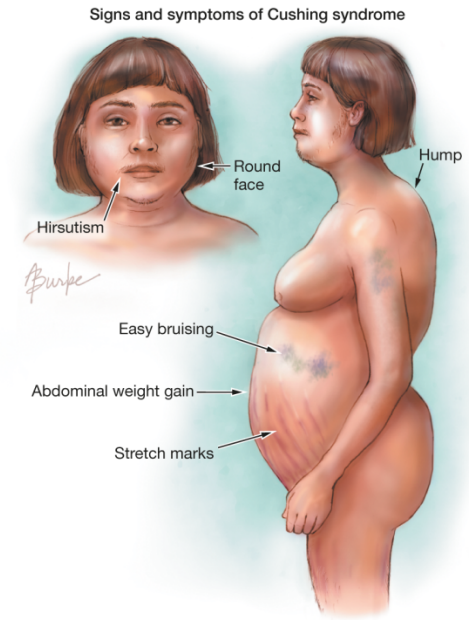


Figure 2: Signs and symptoms in Cushing's syndrome, derived from Pluta *et al.* (34).

Besides Cushing's syndrome, several other diseases are associated with hypercortisolism, formerly known as 'pseudo-Cushing states', now called 'non-neoplastic hypercortisolism'. These states need to be considered when diagnosing Cushing's syndrome, also because these patients show increased cortisol concentrations, as well as varying degrees of symptoms associated with Cushing's syndrome. Non-neoplastic hypercortisolism is related to excessive alcohol intake, depression and other psychiatric diseases, obesity, type 2 diabetes mellitus, insulin resistance, polycystic ovary syndrome, chronic kidney disease, anorexia nervosa, intense chronic exercise, and multiple sclerosis (37).

Treatment of Cushing's syndrome

Treatment of Cushing's syndrome is first aimed at reducing the disease burden of symptoms associated with hypercortisolism by normalizing cortisol secretion, or at least, reducing circulating cortisol concentrations. Further aims are restoring the metabolic changes and reducing the risk of complications, such as hypertension and diabetes mellitus, as well as reducing the increased mortality risk associated with untreated Cushing's syndrome. The ideal treatment leads to the highest percentage of patients in remission after treatment (effectiveness), with the lowest complication rate, and the lowest rate of disease recurrence (safety).

For Cushing's disease, the first-choice treatment method is transsphenoidal pituitary surgery. In 1907, Hermann Schloffer was the first to perform transsphenoidal pituitary surgery (38). Around 1970, this technique was re-discovered and improved due to the use of the operating microscope. This microscope allows selective removal of the ACTH-producing adenoma instead of complete hypophysectomy, as was performed early in the 20th century (39). Starting from 1992, the endoscope became available for transsphenoidal pituitary surgery, which can be used either separately or in combination with the microscope (40). Potential complications of transsphenoidal pituitary surgery are meningitis, cerebrospinal fluid leakage, bleeding, syndrome of inappropriate antidiuretic hormone release (SIADH), diabetes insipidus, and anterior pituitary deficiencies. Repeat transsphenoidal surgery can be performed in case of persistent or recurrent disease after first surgery. Radiotherapy is recommended for patients with an invasive pituitary adenoma, and can be used in case of persistent or recurrent disease. Although radiotherapy is generally highly effective, a potential complication of radiotherapy is hypopituitarism, and it usually takes several months before the first beneficial effects of radiotherapy are evident (41).

First-choice treatment for ectopic Cushing's syndrome is removal of the ACTH-producing tumor. Adrenalectomy is the first-choice treatment in adrenal Cushing's syndrome if one adrenal gland is involved, removing the adrenal gland with the cortisol-producing adenoma, or carcinoma. Bilateral adrenalectomy can also be

Table 1: Overview depicting the different types of hypercortisolism.

	Cushing's disease	**Adrenal Cushing's syndrome**	**Ectopic Cushing's syndrome**	**Pseudo-Cushing states**	**Exogenous hypercortisolism**
Cortisol	High	High	High	High	High
ACTH	High	Low	High	High	Low
Cause	Pituitary adenoma	Adrenal adenoma or carcinoma	Ectopic ACTH-producing tumor	Other diseases, e.g. excessive alcohol intake, depression, obesity, chronic kidney disease	Use of corticosteroids
Treatment	1: Transsphenoidal pituitary surgery 2: Radiotherapy, bilateral adrenalectomy and/or cortisol lowering medication	1: Adrenalectomy 2: Cortisol lowering medication	1: Ectopic tumor resection 2: Cortisol lowering medication	Treatment of the underlying disease	Withdrawal of corticosteroids

ACTH=adrenocorticotropic hormone

performed in Cushing's disease or ectopic Cushing's syndrome when a contra-indication for the first-choice surgical procedure exists (e.g. unidentified tumor, metastatic ectopic tumor), or in case of persistent or recurrent disease after first surgery. Bilateral adrenalectomy has the disadvantage of causing primary adrenal insufficiency, necessitating life-long hydro-cortisone and fludrocortisone replacement therapy. Medical treatment aimed at reducing cortisol concentrations (steroido-genesis inhibitors) is recommended as second-line treatment in patients with Cushing's disease when surgery is unsuccessful, as primary treatment in patients with ectopic Cushing's syndrome with unidentified or metastatic tumor, and as adjunctive therapy to reduce cortisol concentrations in adrenocortical carci-noma. Potential side effects vary per medical agent (41). A complete summary of cortisol-lowering drugs tested in Cushing's disease is provided by Ciato *et al.*, including site and mechanism of action and development stage (42).

Criteria for diagnosis and remission of Cushing's syndrome

Cushing's syndrome is diagnosed based on both clinical grounds as well as bio-chemical test results. Biochemical criteria are dependent on the assay used. Therefore, reference values should be determined per laboratory for all used biochemical parameters. The following biochemical tests are considered relevant for establishing the diagnosis of Cushing's syndrome: 24 h free urinary cortisol excretion, midnight salivary cortisol, and the 1 mg overnight dexamethasone suppression test (43). To differentiate

between ACTH-dependent and ACTH-independent Cushing's syndrome, ACTH concentrations are measured. In case of ACTH-dependent Cushing's syndrome, pituitary imaging by magnetic resonance imaging (MRI) can identify a pituitary adenoma in the majority of cases. Computed tomography (CT) can be performed if a contraindication for MRI exists, but its discriminatory power to detect intrasellar pathology is very limited. If pituitary imaging remains inconclusive, bilateral simultaneous sampling of the inferior petrosal sinuses (IPSS) can be performed to confirm the pituitary origin of ACTH overproduction. If IPSS is negative, additional imaging of the thorax and abdomen are performed to identify a potential ectopic source of ACTH secretion. In case of ACTH-independent Cushing's syndrome, adrenal imaging by MRI or CT is used to identify an adrenal adenoma or carcinoma (44).

Remission status is determined based on clinical and biochemical criteria three months after surgery. Patients are considered in remission if there is dependence on hydrocortisone replacement, or hydrocortisone independence without any biochemical signs of hypercortisolism, in combination with regression of clinical signs (41). Patients are considered to have persistent Cushing's syndrome if there is absence of remission after the first operation and before a second intervention for hypercortisolism is performed. Patients have recurrent disease if, after a period of remission, there is re-occurrence of clinical signs as well as biochemical recurrence of the hypercortisolism as measured by the aforementioned tests.

Exogenous hypercortisolism

When cortisol is administered as a medical agent, it is called hydrocortisone (1). A variety of synthetic corticosteroids, which act similar to hydrocortisone, has been developed, which can be administered in various ways, e.g. orally, via inhalation, intranasally, topical application (e.g. skin, eye), intravenously, intra-muscularly, and intra-articularly. Each has different characteristics, which results in a different choice of agent per disease with varying treatment schedules (45).

Hypercortisolism due to the administration of corticosteroids as medication is called exogenous hypercortisolism, which can lead to iatrogenic Cushing's syndrome. Corticosteroids are used in the treatment of inflammatory diseases (e.g. asthma, rheumatic diseases), malignancies, and after organ transplantation, in order to prevent an inflammatory response (2, 46). Both oral and inhalation corticosteroids are used by approximately 1% of the adult population at any moment (46, 47). Potential side effects of corticosteroid treatment are all symptoms listed for endogenous hypercortisolism because corticosteroids bind to the same receptors as cortisol. Additionally, use of corticosteroids as medical treatment can suppress the

HPA-axis by binding to the glucocorticoid and mineralocorticoid receptors in the hypothalamus and the anterior pituitary gland, lowering ACTH concentrations and thereby suppressing adrenal function (45).

Iatrogenic Cushing's syndrome can only be treated by reducing the dose of corticosteroids. However, due to the potential suppression of the adrenal function during the state of hypercortisolism, corticosteroid dose reductions should be executed with caution to prevent adrenal insufficiency. Additionally, patients with both exogenous and endogenous hypercortisolism may experience corticosteroid withdrawal syndrome to a variable degree when corticosteroid doses are tapered too quickly. Patients with corticosteroid withdrawal syndrome may experience a range of symptoms that are difficult to interpret, e.g. fatigue, mood changes, diffuse aches, and weakness, resulting from a decrease in cortisol concentrations, even though adrenal function is sufficient (48).

Adrenal insufficiency

The state of hypocortisolism is known as adrenal insufficiency, because the adrenal cortex is incapable of producing adequate cortisol concentrations. In primary adrenal insufficiency, this is due to a disease of the adrenal gland itself, whereas in secondary adrenal insufficiency lack of ACTH production leads to insufficient cortisol production. The most common cause of adrenal insufficiency is exogenous corticosteroid use. Secondary adrenal insufficiency, resulting from e.g. congenital hypopituitarism, a pituitary tumor, or pituitary surgery, is the next most common cause of adrenal insufficiency, with a prevalence of 1 in 3,000 persons. The most common cause of primary adrenal insufficiency is Addison's disease, due to autoimmune adrenalitis, leading to destruction of the adrenal cortex. Other causes of primary adrenal insufficiency are congenital adrenal hyperplasia (of which 21-hydroxylase deficiency is the most common form), or bilateral adrenalectomy. All-cause primary adrenal insufficiency is a rare condition with a prevalence of 1 in 8,000 persons, of which 85% is caused by an autoimmune disease (49, 50). Patients with Cushing's syndrome using cortisol-lowering medication can also experience adrenal insufficiency if cortisol concentrations are suppressed excessively by the cortisol-lowering medication without concurrent hydrocortisone replacement.

Patients with adrenal insufficiency generally present with fatigue, weight loss, postural dizziness, anorexia and/or abdominal discomfort. Hyperpigmentation of the skin can be present to a variable degree in primary adrenal insufficiency, as well as low blood pressure with increased postural drop, and failure to thrive in children. Furthermore, laboratory testing can show hyponatremia, hyperkalemia, and

uncommonly hypoglycemia and hypercalcemia (51). Adrenal insufficiency is diagnosed if cortisol remains under the test-specific cut-off value after a stimulation test using ACTH, CRH, insulin-induced hypoglycemia, or metyrapone. As most symptoms are nonspecific, diagnosis is often delayed. Mortality is increased in both treated and untreated patients with adrenal insufficiency (50).

The most feared complication of adrenal insufficiency is a potentially life-threatening situation called adrenal crisis, which represents an acute and severe state of glucocorticoid deficiency. An adrenal crisis can occur after any situation with increased demand for cortisol, e.g. any illness or psychological stress. There is no consensus on the definition of adrenal crisis, and various definitions are used in both clinical and research settings. The following definition may be most useful for clinical practice: a combination of 1) Major impairment of general health with at least two of the following signs and symptoms: hypotension, nausea or vomiting, severe fatigue, fever, somnolence, hyponatremia or hyperkalemia, hypoglycemia, and 2) Clinical improvement following parenteral glucocorticoid administration (52). Importantly, patients with adrenal crisis can have many other nonspecific symptoms, e.g. weight loss, dizziness, abdominal pain, fever, back and leg cramps, which can complicate a correct and timely diagnosis of adrenal crisis.

Patients with adrenal insufficiency are treated with supraphysiological glucocorticoid replacement to control symptoms of adrenal insufficiency and to prevent occurrence of an adrenal crisis. On the other hand, overtreatment with glucocorticoids should be avoided, to prevent symptoms of hypercortisolism. Typically, patients with adrenal insufficiency receive 15-25 mg of hydrocortisone daily, divided into a larger dose in the morning and two smaller doses at lunch and dinner time, to represent the normal circadian cortisol secretion (49). An adrenal crisis is treated by an immediate bolus injection of 100 mg of hydrocortisone intravenously or intramuscularly, followed by continuous infusion of 200 mg hydrocortisone per 24 hours intravenously. Furthermore, rehydration with a rapid intravenous administration of 1 L of isotonic saline should take place within the first hour (53).

To prevent (recurrent) adrenal crisis, an endocrinologist and a specialist endocrine nurse should periodically evaluate all patients with adrenal insufficiency, at least once every 6-12 months. Patients should be educated about the risk of adrenal crisis, including recognition of symptoms of adrenal crisis, and about correct dose adjustment of glucocorticoid replacement. As a rule, the glucocorticoid replacement dose should be doubled during illness with fever that requires bed rest or antibiotics. Furthermore, glucocorticoids should be administered intravenously or intramuscularly during longer periods of vomiting or diarrhea, during preparation for colonoscopy, and in case of acute trauma or surgery. The patient with adrenal insufficiency and

partner or parents should know how to (self-)administer intramuscular hydrocortisone, and they should be in possession of a hydrocortisone emergency injection kit. Finally, patients with adrenal insufficiency should carry a steroid emergency card (Figure 3) (53).

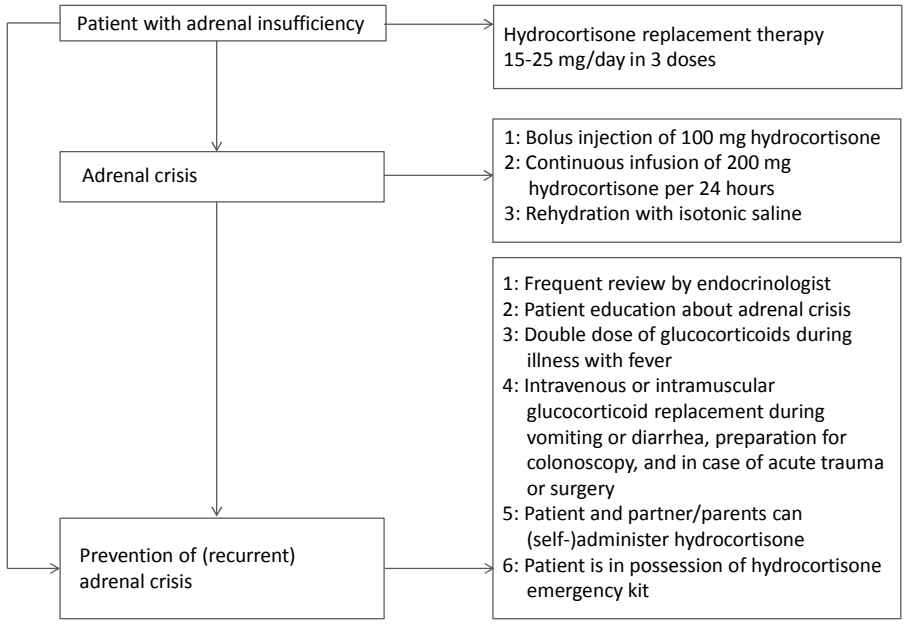


Figure 3: Management of patients with adrenal insufficiency.

Outline of the thesis

Endogenous Cushing's syndrome is rare, but hypercortisolism due to use of exogenous corticosteroids is not. Severe complications due to exogenous hypercortisolism are underestimated and not well-recognized in clinical practice, including mortality and adrenal insufficiency. Endogenous Cushing's syndrome on the other hand causes high morbidity and mortality rates, and therefore treatment of this rare syndrome should be optimized. Furthermore, Cushing's syndrome can serve as a model for long-term exposure to stress, which also leads to high concentrations of cortisol. Research on the topic of Cushing's disease before and after treatment will therefore inform us about potential consequences of long-term exposure to stress, both during periods of stress as well as after abrogation of the stress-inducing situation.

Part I: Complications of corticosteroid use

In part I of this thesis, the complications of corticosteroid use will be explored. Adrenal insufficiency is a well-known complication of corticosteroids use. However, it is unknown in which patients, or under which circumstances, this complication occurs. In **Chapter 2** and in **Appendix I**, the literature is systematically reviewed and, by use of meta-analysis, the proportion of patients with adrenal insufficiency after use of corticosteroids is estimated per route of administration, underlying disease, treatment dose, and treatment duration, to identify patients with high risk of adrenal insufficiency. Mortality may be increased in patients using corticosteroids as medication. However, both corticosteroid use, and mortality, depend on underlying disease. Therefore, in **Chapter 3**, we examined all patients with incident perforated diverticular disease in Denmark, and compared mortality rates between patients that used corticosteroids and patients that did not use corticosteroids to find if use of corticosteroids causes excessive mortality in patients with perforated diverticular disease.

Part II: Treatment outcome in Cushing's syndrome

For the first-choice treatment in Cushing's disease, selective transsphenoidal adenomectomy, two surgical techniques are in use: microscopy and endoscopy. Until now, only few small cohort studies have compared both techniques, so insufficient evidence exists to recommend use of one technique over the other. In **Chapter 4**, we compare both techniques in the large Leiden cohort of patients treated at our referral center, aiming to find differences in surgical outcome, e.g. remission rate and recurrence rate, and in complication rates, e.g. cerebrospinal fluid leakage and anterior pituitary deficiencies. This subject is further investigated in **Chapter 5**, where we perform a systematic review of the literature regarding microscopic and endoscopic transsphenoidal surgery. We use meta-analysis to find differences between both techniques that indicate a preference for either technique based on surgical outcome or complication rates. Medical treatment aimed at reducing cortisol concentrations is used regularly in patients with contraindications to surgery or in case of persistent or recurrent disease. To explore effectiveness and potential side effects of this treatment method, we systematically reviewed the literature and performed meta-analyses per cortisol-lowering drug in **Chapter 6**.

Part III: Clinical outcome in Cushing's syndrome

Cushing's disease occurs more often in females than in males, and males are thought to be at higher risk for ectopic Cushing's syndrome than females. In **Chapter 7**, we study a cohort of patients from Leiden and Berlin to determine whether there are differences between both sexes regarding clinical presentation, diagnostic strategy, and treatment outcome in ACTH-dependent Cushing's syndrome. A potential serious complication of treatment of Cushing's syndrome is adrenal crisis due to inadequately

treated adrenal insufficiency. However, it is unknown how often this complication occurs, and which patients may be at increased risk of developing adrenal crisis after treatment for Cushing's syndrome. We determine the incidence of adrenal crisis in a population of successfully treated patients with Cushing's syndrome from Leiden and Berlin and explore patient characteristics for potential risk factors for adrenal crisis in **Chapter 8**. Hypercortisolism also negatively affects cognition and quality of life, which may even be present in the long-term. In **Chapter 9**, we perform a systematic review of the literature and we determine by meta-analysis if patients improve in quality of life and cognitive functioning after treatment. We also determine if patients normalize regarding quality of life and cognitive functioning by comparing their outcomes to scores from a healthy control population.

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