# Glucocorticoids – outcome in patients with glucocorticoid deficiency and Cushing's syndrome

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All direct quotations in the book are from Harvey Cushing's paper: "The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism)"

*“Physical examination showed an undersized, kyphotic young woman of most extraordinary appearance. Her round face was dusky and cyanosed and there was an abnormal growth of hair. Her abdominous body had the appearance of a full-term pregnancy.”*

**CONTENTS**

# GLUCOCORTICOIDS – OUTCOME IN PATIENTS WITH GLUCOCORTICOID DEFICIENCY AND CUSHING'S SYNDROME

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## ABSTRACT

Glucocorticoids (GCs) are steroid hormones that have a major impact on human metabolism and are essential for life. Chronic GC overexposure, called Cushing's syndrome, is characterized by central obesity, muscle atrophy, osteoporosis, hypertension, impaired glucose tolerance and neurocognitive impairment. Cushing's syndrome can be caused by increased endogenous GC production or arise due to pharmacological GC treatment.

This thesis is based on four studies, including four different patient populations, aimed at investigating outcomes in patients with Cushing's syndrome and patients receiving current standard GC replacement therapy for adrenal insufficiency. In a large study of patients with hypopituitarism it was demonstrated that GC replacement therapy was independently associated with reduced bone mineral density in women with adrenal insufficiency receiving an average daily hydrocortisone dose of approximately 20 mg. In another study of adult patients, treated for Cushing's disease during childhood, final adult height was compromised in the majority of the patients and the prevalence of hypertension was high. In a study of patients in long-term remission after successful treatment for Cushing's syndrome, numerous domains of cognitive function were impaired at long-term follow-up in comparison to healthy individuals. Finally it was demonstrated that short-term treatment with growth hormone and testosterone increases skeletal muscle mass in men on chronic low dose GC treatment.

In conclusion, this thesis demonstrates that long-term GC exposure has various long-term adverse health related consequences for patients receiving GC replacement therapy and in patients in long-term remission from Cushing's syndrome. Furthermore, anabolic treatment with growth hormone and testosterone, has the potential to improve GC induced muscle wasting.

**Key words:** Cardiovascular risk, bone mineral density, paediatric, final height, cognitive function, fatigue, attentional network test, body composition, sarcopaenia, growth hormone

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## LIST OF PAPERS

**This thesis is based on the works contained in the following papers, which are referred to in the text by their roman numerals:**

I. Oskar Ragnarsson, Helena Filipsson-Nyström, Gudmundur Johannsson. Glucocorticoid replacement therapy is independently associated with reduced bone mineral density in women with hypopituitarism. *Clin Endo 2012; 76: 246-52.*

II. Oskar Ragnarsson, Charlotte Höybye, Peter J Jönsson, Ulla Feldt-Rasmussen, Gudmundur Johannsson, Beverly MK Biller, Maria Kołtowska-Häggström. Co-morbidity and cardiovascular risk factors in adult growth hormone deficiency following treatment for Cushing's disease or non-functioning pituitary adenomas during childhood. *Eur J Endocrinol 2012; 166: 593-600.*

III. Oskar Ragnarsson, Peter Berglund, Derek N. Eder, Gudmundur Johannsson. Long-term cognitive impairments and attentional deficits in patients with Cushing's disease and cortisol producing adrenal adenoma in remission *Submitted manuscript.*

IV. Oskar Ragnarsson, Morton G. Burt, Ken K. Y. Ho, Gudmundur Johannsson. Effect of short-term growth hormone and testosterone treatment on body composition and glucose homeostasis in men receiving chronic glucocorticoid therapy. *Manuscript.*

## ABBREVIATIONS

| | |
|---|---|
| 11β-HSD | 11β-hydroxysteroid dehydrogenase |
| ACTH | Adrenocorticotropic hormone |
| ADX | Adrenalectomy |
| ANT | Attentional network test |
| ASMM | Appendicular skeletal muscle mass |
| BCM | Body cell mass |
| BMD | Bone mineral density |
| BMI | Body mass index |
| CD | Cushing's disease |
| CO-CD | Childhood onset Cushing's disease |
| CO-Idio | Childhood onset idiopathic hypopituitarism |
| CO-NFPA | Childhood onset non-functioning pituitary adenoma |
| CPAA | Cortisol producing adrenal adenoma |
| CPRS-A | Comprehensive psychopathological rating scale |
| CRH | Corticotrophin-releasing hormone |
| CS | Cushing's syndrome |
| CT | Computed tomography |
| CV | Coefficient of variation |
| DXA | Dual-energy x-ray absorptiometry |
| ECW | Extracellular water |
| FIS | Fatigue impact scale |
| GC | Glucocorticoid |
| GH | Growth hormone |
| GHD | Growth hormone deficiency |
| HOMA | Homeostatic model assessment |
| HPA | Hypothalamus-pituitary-adrenal |
| IGF-I | Insulin-like growth factor I |
| IGI | Insulinogenic index |
| IR | Insulin resistance |
| ISI | Insulin sensitivity index |
| LBM | Lean body mass |
| MRI | Magnetic resonance imaging |
| NFPA | Non-functioning pituitary adenoma |
| OGTT | Oral glucose tolerance test |
| PIIIP | Procollagen III peptide |
| RTX | Radiation therapy |
| SDS | Standard deviation score |
| SF-36 | Short form 36 |
| TSS | Transsphenoidal pituitary surgery |
| QoL | Quality of life |

# INTRODUCTION

## History

“The Pituitary body and its disorders”, written by the American surgeon Harvey Williams Cushing, was published 100 years ago (1). In the book, Cushing described various clinical states caused by diseases of the pituitary gland. In the last part of the book he presented a separate group of patients with what he called polyglandular syndrome. One of the patients was a 23-year-old woman with "the most peculiar appearance". Her abdomen was huge and the face large and round with hypertrichosis and hyperpigmentation. She had gained weight and suffered from muscle weakness, back pain, irregular menstruation and elevated blood pressure. Even though Cushing did not know the aetiology of this disorder, one of his hypotheses was that a disease of the pituitary gland might be the cause. More than 20 years later Cushing reported on a further 11 patients (2). Besides having similar clinical features as in the original patient, he had also noted that at autopsy, some of these patients had a small pituitary basophilic adenoma. He concluded that this might be the cause of the clinical syndrome and encouraged pathologists to scrutinize the pituitary gland in patients having similar characteristics. The same year that Cushing wrote his paper, another similar case was reported where the authors, being well aware of Cushing’s paper, named the clinical state “ Cushing’s syndrome” (CS) (3).

It did not become generally accepted that CS could be caused by pituitary adenoma, as well as adrenal adenoma, until 10 years later when Dr. Fuller Albright stated that CS was caused by “hyperadrenocorticism” with excess production of a “sugar-hormone”, today called glucocorticoids (GCs) (4). He declared that the clinical state, irrespective of aetiology, should be called CS and when caused by pituitary adenoma it should be called Cushing’s disease (CD).

The history of GC therapy begins as early as 1896 when Sir William Osler used extract from porcine adrenal glands to treat patients with adrenal insufficiency (5). The use of GCs became widespread first when cortisone was isolated and used to treat patients with rheumatoid arthritis in the late 1940’s. For this major scientific breakthrough the inventors were rewarded with the Nobel Prize in 1950. Shortly thereafter, GC's became commercially available and have since then been used for treatment of various diseases due to their anti-inflammatory and immunosuppressive properties as well as for replacement treatment in patients with adrenal insufficiency.

## Glucocorticoids

GCs are steroid hormones whose effect is mediated through the GC receptor, found in all tissues of the body (6). Cortisol, produced in the adrenal cortex, is the main GC in humans. GCs are also produced synthetically for use in various medical disorders. GCs have a major impact on protein, fat, carbohydrate and bone metabolism, as well as affecting the cardiovascular, immune and the central nervous systems, and are essential for life.

The production of cortisol is regulated by the hypothalamus and pituitary gland (6). By producing corticotropin-releasing hormone (CRH), the hypothalamus stimulates the pituitary gland to produce adrenocorticotropic hormone (ACTH), which in turn affects the adrenal cortex that responds by increasing the production and secretion of cortisol. Under normal physiological conditions, the activity of the hypothalamus-pituitary-adrenal (HPA) axis is under negative feedback control by cortisol itself; *i.e.* in the presence of high cortisol concentration the activity of the axis decreases, and during states accompanied by low cortisol concentrations (*e.g.* primary adrenal insufficiency) secretion of CRH and ACTH are increased. Other characteristic features of the HPA-axis are the diurnal and highly pulsatile (ultradian) secretion of cortisol; cortisol is secreted in pulses with a periodicity of approximately one hour, with high concentrations early in the morning which thereafter gradually decrease and become low late in the evening (7). The HPA axis is also activated by physical and psychological stress in order to prepare the body to react appropriately to the stressor.

**Cushing's syndrome – General aspects**

CS is a clinical state caused by chronic overexposure of GCs (8). The typical features of CS are weight gain, central obesity, muscle and skin atrophy, osteoporosis, hypertension, impaired glucose tolerance, dyslipidemia, fatigue, depression and cognitive impairment (9). The most common cause of CS is pharmacological GC treatment (exogenous CS).

Endogenous CS is considered to be an uncommon condition with an incidence of between 1.8-2.4 patients per million/year. This incidence rate is mainly based on two studies, a nationwide Danish survey (10) and a study from New Zealand (11), supported by two other smaller studies (12, 13). The median age at diagnosis is 40 years with female to male ratio of 3:1 (10, 11). The most common cause of endogenous CS is CD (ACTH producing pituitary adenoma), seen in approximately 70% of patients with CS. Cortisol producing adrenal adenoma (CPAA) and ectopic ACTH producing tumours are less common, each accounting for approximately 10-15% of cases. All these disorders present with similar clinical features.

Mild endogenous hypercortisolism in patients with incidentally discovered adrenal mass (subclinical hypercortisolism) is more common than the classic CS. Adrenal incidentalomas are present in more than 4% of healthy adults (14). Between 5 to 30% of those may have subclinical hypercortisolism (15) which is especially common in patients with concomitant osteoporosis (16, 17) or obese patients with type 2 diabetes mellitus (18-20). The true prevalence of subclinical hypercortisolism is however a matter of debate since it is highly dependent on the diagnostic criteria used.

Shortly after the introduction of GCs it became evident that patients who were administrated high doses for a long time could develop iatrogenic CS (also called exogenous CS). In a study from the United Kingdom, 0.9% of the population are receiving GC treatment at any given time point, with highest use (2.5%) in patients between 70 and 79 years of age (21). Another study reported that 1.4% of patients older than 55 years are receiving long-term GC treatment

(>3 months) (22) and a recent study reported an increasing prescription rate (23). Since it is mainly the elderly who receive GC treatment, the risk for osteoporosis and sarcopaenia is of major concern. Various treatment alternatives are available for GC induced osteoporosis (24). However, no therapy is available to treat GC induced sarcopaenia.

**Diagnosis of Cushing's syndrome**

The diagnosis of CS is one of the most challenging topics in the field of clinical endocrinology (25). Especially difficult is distinguishing patients with CS who have modestly elevated cortisol levels from patients with other diseases that may have similar clinical and laboratory findings. Simple obesity, depression, anxiety, high alcohol intake and polycystic ovary syndrome are examples of such disorders and when accompanied by hypercortisolism are called pseudo CS.

The diagnosis of CS is based on the establishment of hypercortisolism and the absence of the two fundamental features of a normal functioning HPA axis; *i.e.* absent diurnal cortisol variation and inability to suppress cortisol production after administration of exogenous GCs. The diagnostic tests that are recommended to confirm or rule out the presence of CS are measurement of urinary free cortisol, serum or salivary cortisol at midnight and/or serum cortisol concentration after administration of dexamethasone (*i.e.* dexamethasone suppression test) (26). However, none of the tests available today to diagnose CS have 100% sensitivity and specificity; thus the definitive diagnosis is often not confirmed until postoperatively when histopathological diagnosis is available and the clinical course indicates remission - characterized by low cortisol concentrations and symptoms consistent with adrenal insufficiency.

After the diagnosis of CS has been confirmed, the next step is to determine the aetiology through measurement of ACTH in plasma and radiological examinations. Patients with CPAA have low ACTH concentrations (ACTH independent CS) and detectable adrenal adenoma on computed tomography (CT) in almost all cases. Patients with high ACTH levels (ACTH dependent CS) have either CD or ectopic ACTH producing tumours. Distinguishing between these two forms can be complicated, especially when no pituitary tumour is detected by magnetic resonance imaging (MRI). However, the introduction of inferior petrosal sinus sampling as a diagnostic tool has led to almost all cases of ACTH dependent CS being correctly diagnosed (27).

**Treatment and outcome of Cushing's syndrome**

The first line treatment for patients with CD is transsphenoidal pituitary surgery (TSS) and unilateral adrenalectomy (ADX) for CPAA (28). Although TSS, as a treatment for pituitary tumours, was first performed more than 100 years ago (29), its use first became widespread for patients with CD in the 1980s. Before that time, patients were either treated with pituitary radiation therapy (RTX) or bilateral ADX.

Unilateral ADX is curative in almost all patients with CPAA and permanent hormone deficiency is rare. Conversely, pituitary hormone deficiencies are common after TSS, with a range between 13 and 81%. Determining factors are the size of the tumour, the surgical approach and the experience of the surgeon (30-33). Furthermore, 14-29% of patients with CD are not cured after TSS, and additionally 9-25% of patients relapse at long-term follow-up after an initially successful treatment (34-36). Second-line treatments, such as additional neurosurgical intervention, RTX and/or bilateral ADX, may then become necessary which further increases the risk for compromised pituitary function.

**Glucocorticoid replacement therapy**

Without GC replacement therapy, patients with adrenal insufficiency do not survive. Before GC treatment became available, the great majority of patients died within a year of diagnosis (37). Today, many chemically different GCs are available, each having its specific pharmacokinetic profile. The two most commonly used GCs for replacement therapy are hydrocortisone and cortisone acetate. Synthetically produced hydrocortisone has the same chemical structure and mode of action as cortisol. Cortisone acetate is a synthetic analogue that is converted to cortisol in the liver. Both have a relatively short half-life which makes them suitable for replacement therapy. Prednisolone, dexamethasone and betamethasone are more potent but have longer half-lives and are therefore rarely used for replacement therapy. However, they have lower mineralocorticoid effects that make them attractive for treatment of anti-inflammatory and immunosuppressive disorders where high doses are frequently needed. Based on previous *in vitro* studies on the anti-inflammatory response to GCs, the equivalent dose of 20 mg hydrocortisone is 25 mg cortisone acetate, 5 mg prednisolone, 0.75 mg dexamethasone and betamethasone, (38, 39).

Historically, patients with adrenal insufficiency received higher GC doses than they do today. A common regimen was 20 mg of hydrocortisone in the morning and 10 mg in the evening (40). It has since been shown that the endogenous cortisol production is approximately 10 $mg/m^2$ per day, corresponding to 15-20 mg of hydrocortisone (41). Therefore, the doses frequently used for GC replacement therapy may be too high. In fact, an adverse metabolic profile (42), lower bone mineral density (BMD) (43) and impaired quality of life (QoL) (44, 45) have all been associated with GC doses during replacement therapy. Reducing the total daily hydrocortisone dose has led to weight reduction, improved metabolic profiles and QoL without decreased lean body mass (LBM) (46).

**Effects of glucocorticoids on bone**

*"A marked osteoporosis of the skeleton was found, it being easily possible to cut the vertebral bodies with a knife, the spongy part of the bone having largely disappeared"*

Decreased BMD, osteoporosis and osteoporotic fractures are important features of both endogenous (47, 48) and exogenous CS (49) as well as subclinical hypercortisolism (50, 51). In patients with CS in remission, duration of postoperative GC replacement therapy and duration of active disease are both associated with low BMD (52). Patients with GC excess

have a decreased number of osteoblasts in the skeletal tissue, an increased rate of osteocyte apoptosis, and consequently, reduced bone formation (24). Other factors such as decreased uptake of calcium from the intestines, increased bone resorption by osteoclasts, hypogonadotropic hypogonadism and decreased growth hormone (GH) secretion (both common during the state of hypercortisolism) may further contribute to the negative effect on bone quality (53).

Studies on bone metabolism in patients with primary adrenal insufficiency on conventional GC replacement therapy present conflicting results (54), either reporting normal (55, 56) or reduced BMD (57), decreased BMD in males only (43) or females only (58). Most of these studies are limited by the small number of patients studied. Patients with pituitary insufficiency also have reduced BMD and an increased fracture rate (59-62). Among many potential explanations for these observations is the use of supraphysiological GC doses, inadequate sex hormone replacement therapy and untreated GH deficiency (GHD). In one study, GC replacement therapy was independently associated with reduced BMD (63) while another study did not find an increased fracture risk (60). Whether there is a gender specific or dose related association has not been studied.

**Effects of glucocorticoids on body composition**

*"The obesity of the trunk was in marked contrast to the somewhat thin extremities"*

Weight gain and obesity are among the most commonly occurring features of CS. In a recent European study on 481 patients with newly diagnosed CS, 81% had experienced weight gain and the mean body mass index (BMI) was 30.7 $kg/m^2$ (9). Body compositional changes in CS are characterized by an increased fat mass, mainly in the visceral compartment, and a reduction in LBM (64-67).

The effect of remission on body composition has been studied prospectively in few studies. These all show reduction in adipose tissue following treatment (65, 68-71) while LBM is unchanged (65, 69-71) or even reduced after treatment (68). In a recent study, body composition, measured with DXA in 37 patients in remission for mean time of 11 years (range 0.7-22), was compared with controls and patients with active CS (72). Seven patients (19%) had GHD and two were receiving GH replacement. Patients in remission had increased total and truncal fat mass compared to controls while LBM was not significantly different between the groups.

GC excess has a catabolic effect on protein metabolism. Decreased protein synthesis and increased protein breakdown consequently cause skeletal muscle atrophy (sarcopaenia) and weakness (70, 73). The suggested main mechanisms involved are decreased transport of amino acids into muscle cells, decreased local production of insulin-like growth factor I (IGF-I) and increased myostatin levels (74). To date, no specific treatment is available to prevent or reverse GC-induced muscle wasting. Such treatment would be valuable since sarcopaenia is associated with substantial morbidity and increased mortality (75).

**Effects of glucocorticoids on cardiovascular risk factors**

*"The heart was enlarged and the aorta atheromatous"*

The majority of patients with active CS have the metabolic syndrome, *i.e.* central obesity, hypertension, dyslipidemia and impaired glucose tolerance or diabetes mellitus (76). Although significant improvement is reported after cure, especially after treatment for CPAA, a substantial number of patients still have an adverse cardiovascular risk profile (76). Indeed, 27% of patients treated for CD have atherosclerotic plaques at 5 years of follow-up, compared with only 3% of gender, age and BMI-matched controls (77).

Patients with subclinical hypercortisolism have higher blood pressure, triglycerides and glucose concentrations after oral glucose tolerance test (OGTT) compared to patients with non-functioning adrenal incidentalomas (78, 79) and healthy individuals (80). Furthermore, in GC replaced patients with hypopituitarism, receiving a mean hydrocortisone dose of 24 mg, a dose-related increase in BMI, triglycerides, and total- and low-density lipoprotein cholesterol concentrations was demonstrated (42). In another study, ACTH insufficient patients had higher triglycerides and cholesterol concentrations than ACTH sufficient patients (81). Based on these data, it is likely that even subtle GC overexposure may have adverse effects on cardiovascular risk factors.

**Effects of glucocorticoids on quality of life, cognitive function and psychopathology**

*"He found himself without energy, easily fatigued, unable to concentrate his mind on his work, and fits of unnatural irritability alternated with periods of depression"*

Patients with active CS have markedly impaired QoL, both in comparison to healthy individuals (9, 82) and patients with other pituitary tumours (83). At long-term follow-up, QoL improves, but is still worse than in controls (82, 84, 85). Furthermore, fatigue, which is one of the most common and distressing symptoms in patients with CS, has been reported by 41-85% of patients at long term follow-up after treatment (82).

Patients with active CS also have substantial psychiatric problems (86). Up to 80% have generalized anxiety and 70% have major depression (87). Although not as common, manic or hypomanic symptoms may occur and be among the early manifestations of the disease (88). In prospective studies, psychiatric abnormalities are reported to improve after treatment (89, 90). In one study, 33 patients with CS were examined before and at 3, 6 and 12 months after correction of hypercortisolism (90). Before cure, two-thirds had significant psychopathology that was predominantly in the form of atypical depressive disorder. After cure, overall psychopathology decreased significantly to 54% at 3 months, 36% at 6 months and 24% at 12 months. Even at long-term follow-up, patients with a mean duration of remission of 11 years showed an increased prevalence of psychopathology (91).

Cognitive function is also negatively affected in patients with CS. In an early study on 35 patients with active CS, diffuse bilateral cerebral dysfunction was found in two-thirds of patients (92). In another study on 23 patients with CS, 66% had difficulty in concentration and 83% had memory impairments (93). A significant correlation has been found between the degree of hypercortisolism, the extent of cognitive impairment and decreased hippocampal volume (94). Improvement in cognitive function (95, 96) and morphological brain changes (97, 98) following treatment have been reported. However, the number of patients in these studies was limited and the follow-up times were short.

**Effects of glucocorticoids on mortality**

"*The average duration of the disease from onset to death is slightly over five years"*

Untreated patients with CS have a grave prognosis with an estimated 5 year survival of only 50% (2, 99). Despite treatment, mortality seems to be increased, both in comparison to the normal population (10-12, 100) and to patients with non-functioning pituitary adenomas (NFPA) (101). However, patients with persistent disease have a much worse prognosis than patients in remission. In fact, it has been questioned whether mortality is actually increased in the latter group (100).

Patients with hypopituitarism have increased cardiovascular morbidity and mortality (102, 103). The aetiology of increased mortality in hypopituitarism is probably multi-factorial; the unfavourable metabolic profile due to GHD (104), the quality of gonadal replacement, the underlying cause of hypopituitarism (105) and pituitary radiotherapy may all be important factors (106). The relative risk of death in patients with primary adrenal insufficiency treated with currently available GC replacement therapy is 2-fold higher than in the background population (107, 108). Patients with acromegaly and ACTH insufficiency who receive a daily hydrocortisone dose of more than 25 mg per day have increased mortality (109). Hence, although not sufficiently investigated, there are indirect indications that GC replacement may to some extent influence mortality in patients with adrenal insufficiency.

**Cushing's syndrome in children and adolescents**

*"In her sixth year she suddenly began to put on flesh and became disproportionately adipose, gaining about 34 kg"*

CS is rare in children and adolescents (110, 111). In a recent epidemiological study from New Zealand, only 4% of patients with CS were younger than 16 years of age (11). Symptoms and signs in children and adolescents with CS differ from those in adults. In addition to typical Cushingoid features, decreased growth rate, inappropriate weight gain and pubertal delay are among early manifestations (110, 112). The gender distribution in the paediatric population, in contrast to adults, is reported as equal (111), boys are diagnosed at younger age than girls (113).

Long-term consequences after cure of paediatric CS have been reported in a few studies. Final height and BMD are compromised (114-117). Anterior pituitary function is also affected in a

substantial number of patients (118, 119); especially in those treated with pituitary RTX (120). In children with CS, a decline in cognitive function has been reported following cure (121). This is in disagreement with findings in adults where an improvement is seen. QoL in paediatric CS patients is also known to be impaired. One year postoperatively improvement is seen, though residual impairment remains (122). Metabolic status after cure is poorly studied. Only one study has addressed blood pressure and demonstrated that 16% of patients had systolic and 4% had diastolic hypertension 1 year postoperatively (123). So far, there are no studies on glucose and lipid metabolism.

## AIMS OF THIS THESIS

The overall aim of this thesis was to study consequences of long-term GC overexposure. The more specific aims were:

- To study the cardiovascular risk profile and bone metabolism in ACTH insufficient hypopituitary patients receiving GC replacement therapy (*Paper I*)

- To determine the long-term outcome of adult patients treated for childhood onset CD (CO-CD) (*Paper II*)

- To evaluate cognitive function in patients with CS in long-term remission (*Paper III*)

- To investigate whether short-term treatment with GH and testosterone exert anabolic effects on protein metabolism in patients on chronic pharmacological GC treatment (*Paper IV*)

## SUBJECTS AND STUDY DESIGN

### *Paper I* – Hypopituitary patients

This was a cross-sectional, single-centre study of 365 consecutive adult hypopituitary patients diagnosed with GHD between 1990 and 2006 at the Sahlgrenska University Hospital. Fifty-six percent (n = 204) were ACTH insufficient and received treatment with GCs. The most common underlying causes of hypopituitarism were NFPA (n = 145), idiopathic pituitary insufficiency (n = 46), prolactinoma (n = 35) and craniopharyngioma (n = 33). Data from physical and laboratory examinations and anthropometric, body composition and BMD measurements were analyzed before any GH replacement was commenced.

*Considerations on patient selection and study design in Paper I*

The ideal setting to evaluate the effects of GCs on bone metabolism and cardiovascular risk factors would be to study two groups of patients similar in all aspects except for the GC dependency. Ideally, the patients would be free from other diseases known to affect the outcome variables of interest. In *Paper* I, neither was fulfilled. The two groups, ACTH sufficient and insufficient patients, were not comparable concerning several variables such as aetiology, hormone deficiencies and weight. Being aware of this, data were analysed using multivariate analysis to account for potential confounders in order to minimise the risk of over-interpretation.

Patients with CD and acromegaly were not excluded from the analysis in *Paper I*. Both diseases can independently affect BMD, body composition and cardiovascular risk factors. However, patients with these diagnoses were few and their numbers did not differ between ACTH insufficient and sufficient patients. It is therefore not likely that exclusion of these patients would have affected the results substantially.

### *Paper II* – Adult patients with childhood onset Cushing's disease

This was a retrospective study of prospectively collected data – a large pharmaco-epidemiological surveillance study on GH therapy in adults with GHD obtained from Pfizer's International Metabolic Database (KIMS) (124). Patients who were younger than 18 years when diagnosed with CO-CD or childhood onset NFPA (CO-NFPA) and enrolled in KIMS between 1994 and 2009, were identified and compared to a control group of ACTH sufficient patients with childhood onset idiopathic hypopituitarism (CO-Idio). They were matched for gender and age at pituitary disease onset. Two analyses were performed:

a) A cross-sectional study on background characteristics, anthropometry and co-morbidity in all patients, irrespective of previous GH treatment. In this portion of the analysis, 47 patients with CO-CD and 62 with CO-NFPA were identified and compared to 100 matched ACTH sufficient patients with CO-Idio.
*b)* A longitudinal study in which the effect of GH treatment on the metabolic risk profile was analysed in a cohort with data available from visits at baseline and after one year on GH. Patients who had never received treatment with GH (true-naïve) or received none during the 6 months prior to entry into KIMS (semi-naïve) were studied. Data on

17 patients with CO-CD, 24 with CO-NFPA and 55 with CO-Idio were analyzed in this portion of the analysis.

*Considerations on patient populations and study design – Paper II*

KIMS is the largest database of hypopituitary adults with GHD. In November 2006, 11,374 GH treated patients were registered (124). The size of the database becomes important when studying uncommon diseases such as CD in children and adolescents where randomized controlled trials are difficult and unlikely to be conducted. There are, however, limitations with all databases. The main limitations in *Paper II* concerns completeness of data and potential selection bias. In our study, data on some variables, such as presence of visual field defects and ophthalmoplegia, were missing in a substantial number of cases. However, "key data variables" such as underlying aetiology, GH status and medical history were reported in all, or almost all, patients - in agreement with previous quality control of data collection in KIMS (124). Another important thing to point out is a potential selection bias since all patients in the KIMS database per definition have GHD. Data from the database can therefore not be extrapolated to patients with CO-CD and CO-NFPA in general. Finally, the lack of a normal control group is also a limitation. Comparison of results from a patient group of interest is therefore either restricted to another patient group from same database or to normative data from previous studies.

### *Paper III* – Patients with Cushing's syndrome

This was a cross-sectional, case-controlled study on 55 patients (50 women and 5 men) previously treated for CD (n=43) or CPAA (n=12) and 55 controls matched for age, gender and educational level. Neuropsychological and computer-based neurocognitive testing were performed. The HPA axis status was evaluated with overnight dexamethasone suppression test, CRH test and measurements of 24-h urinary free cortisol. In patients with a clinical status indicating GHD, an insulin tolerance test was performed.

*Considerations on patient populations and study design – Paper III*

Patients in *Paper III*, as in the ordinary clinical setting, comprised a heterogeneous group of patients in terms of age, hormonal status and previous treatment. Only 20% of patients with CD were hormonally intact and one-third of patients had required two or more treatment modalities to attain remission. As CS is a relatively rare syndrome, single-centre studies will always be limited by the size of the cohorts. Even though our study included a relatively large number of patients compared to previous studies; the number of patients in some of the various subgroup analyses was small, accompanied by risk of over-interpretation of the data. Furthermore, only 5 men participated in the study, which makes it impossible to draw any conclusion on potential gender differences. Interestingly, 6 of 9 patients who declined to participate were men.

Three patients were diagnosed with GHD in connection with participation in the study. Additionally, one of 22 patients previously diagnosed with GHD, was not receiving GH replacement therapy. Seventeen patients had never been tested for GHD and did not have

subjective or objective findings that prompted testing. QoL is negatively affected by GHD and improves with GH replacement (125). Cognitive function is also impaired in patients with GHD (126). Two prospective placebo-controlled trials have been conducted to investigate the effects of GH treatment on cognitive function. One of these showed no beneficial effects (127) while the other showed beneficial effects on attention but not on verbal memory or non-verbal intelligence (128). The inclusion of the 4 GHD patients in the analysis in *Paper III* is therefore not likely to have had a significant impact on the results.

### *Paper IV* – Patients on chronic pharmacological glucocorticoid therapy

This was a prospective, open-label, randomized, crossover study comparing the effect of GH for two weeks, testosterone for two weeks and the combination of GH and testosterone for two weeks, on protein metabolism in men on chronic GC treatment. Each treatment period was followed by a two-week wash-out period. GH was administrated subcutaneously every evening at a dose of 0.8 mg and testosterone was given transdermally at a dose of 50 mg daily in the morning. Measurements were performed at baseline and at the end of each treatment period. Twelve men with polymyalgia rheumatica were studied. They had all received treatment with prednisolone (mean ± SD dose was 5.8 ± 1.5 mg; range 5 – 8.75 mg) for more than one year, were in remission for at least 6 months and had a stable prednisolone dose in the three months prior to inclusion.

#### *Considerations on patient populations and study design – Paper IV*

*Paper IV* was a proof-of-concept trial. In a previous study, with comparable design, whole body protein metabolism in elderly women receiving long-term GC treatment was measured with the leucine turnover technique (129). Using this technique, the effects of GH and dehydroepiandrostenedione on protein synthesis, breakdown and irreversible oxidation were assessed. While changes in protein metabolism happen quite rapidly after administration of anabolic hormones, the effects on body composition evolve more slowly. The short treatment periods in *Paper IV* are therefore a limitation. Measurement of whole body protein metabolism and/or longer treatment periods would have given the study a greater impact.

# METHODS

## Body composition and bone mineral density

In *Papers I and IV*, body composition was measured using DXA (Lunar DPX-L, Lunar Corporation, Madison, WI, USA). A phantom (BONA SIDE, Ltd 313, West Beltline HWY, Madison, WI, USA) was used for calibration throughout the study periods. In *Paper I*, data on total LBM, total fat mass, central abdominal fat and BMD in the lumbar spine and proximal femur neck were analyzed. In *Paper IV*, the same body compositional parameters were analyzed, except that bone mineral content was analyzed instead of BMD, and appendicular skeletal muscle mass (ASMM) was calculated from lean mass in the arms and legs (130). In addition, in *Paper IV*, BCM was calculated in accordance with the four compartment model by subtracting extracellular water (ECW) from LBM, where ECW was measured using the bromide-dilution technique (131).

DXA is a non-invasive and widely used technique to evaluate BMD and body composition. Measurements with DXA are based on the assumption that the body consists of three compartments that can be distinguished by their different X-ray attenuations. These are bone mineral, fat and fat-free tissues (132). DXA is the most commonly used technique for estimation of BMD, where high precision, low X-ray exposure, accessibility and short scanning time are among properties that makes it an attractive alternative in comparison to other techniques. The classification of osteopenia and osteoporosis according to different BMD levels, as recommended by the World Health Organisation, is based on measurements with DXA.

The main body compositional variable of interest in *Paper I*, in addition to BMD, was visceral fat mass which has been associated with increased cardiovascular risk (133). DXA however, does not separate subcutaneous fat from visceral fat and it has been questioned if abdominal fat mass measured with DXA offers any advantage over anthropometric measurements (134). Both CT and MRI give better information on regional body composition, including separation of abdominal fat mass into visceral and subcutaneous fat, and would have been a more appropriate approach. However, both these techniques are time-consuming and expensive which limit their widespread use in clinical research.

In *Paper IV*, one of the main outcome variables was ASMM measured with DXA. Of major concern was the potential confounding effect of changes in ECW accompanied by treatment with GH and testosterone (135). We attempted to account for changes in ECW by using a four compartment model and calculating changes in BCM. Assessing BCM from measures of potassium 40, since it eliminates the influence of changes in total ECW, might have given more reliable information. Measuring muscle mass with CT or MRI may also have had advantages over measurements with DXA.

**Questionnaires**

In *Paper III*, validated Swedish versions of the fatigue impact scale (FIS) (136) and the Short form-36 (SF-36) (137) were used to evaluate fatigue and QoL, respectively. FIS is a questionnaire composed of 40 questions for evaluation of the impact of fatigue on three subscales: Physical fatigue, cognitive fatigue and social fatigue. The subjects are asked to rate the extent to which fatigue had caused problems for them during the past month in relation to sample statements on a five level scale. Zero indicated no problem and 4 a severe problem, yielding a maximum score of 160. The comprehensive psychopathological rating scale (CPRS-A) (138) was used to evaluate depression and anxiety.

The FIS is well validated and has been used for assessment of fatigue in several disorders including multiple sclerosis, stroke, brain injury and chronic liver diseases (139, 140). Similarly, the SF-36 has been extensively used for evaluation of QoL and general well-being, including studies on patients with CS in remission (82, 84). It has, however, been questioned how well general questionnaires, such as SF-36, represent QoL in specific patient groups such as CS. A new disease-generated questionnaire has therefore recently been designed (141). It may have been more appropriate to use such a disease specific questionnaire in *Paper III*, but the approved version in Swedish was not available at the time when the study was designed.

**Cognitive function**

In *Paper* III, 7 standardized neuropsychological tests were used to evaluate cognitive function. The trail making tests A and B were administered to measure visual scanning, divided attention and motor speed. Digit symbol-coding was used to assess information processing speed. Digit span and spatial span tests were used to evaluate auditory and visual attention and working memory. Other tests were used to measure verbal fluency and reading speed. Furthermore, sustained attention (vigilance) and the three main aspects of attention (alerting, spatial orienting and executive control) were assessed by two computer-based neurocognitive tests.

The goal with cognitive testing should be to give a comprehensive picture of cognitive status using validated tests. The neuropsychological tests used in *Paper III* are all validated and widely used. However, there were no tests included that specifically assessed hippocampal function, *i.e.* short-term memory and learning, and this is a limitation. The reason for the decision was that hippocampal function has been thoroughly studied before in patients with CS and we were especially interested in studying extrahippocampal domains of cognition such as attention. For this purpose, the Attentional Network Test (ANT) was used to evaluate the three main components of attention - *i.e.* alerting, orienting and executive control. Each component is modulated by different neurotransmitters and found in three distinct neuroanatomical networks (142). Functional MRI has shown that alerting is associated with strong thalamic involvement and activation of anterior and posterior cortical sites, orienting with activated parietal sites and frontal eye fields and executive control with activation of the anterior cingulate along with several other brain areas (142). Including the ANT in the test

battery was therefore thought to improve current understanding of potential consequences of GC excess on the central nervous system.

### Evaluation of the HPA-axis

In *Paper III*, the 1 mg overnight dexamethasone suppression test was performed in all patients, except in patients operated with bilateral ADX, to confirm remission of CS. S-cortisol concentration ≤ 50 nmol/l was considered an adequate suppression. The CRH test was performed to evaluate the HPA-axis. After an overnight fast, a bolus of 100 µg CRH was injected intravenously and blood samples for analysis of s-cortisol were collected 15 and 5 minutes before and 15, 30, 45 and 60 minutes after the injection. In patients with clinical status indicating GHD an insulin tolerance test was performed.

The 1 mg overnight dexamethasone suppression test is one of three recommended tests for initial evaluation of patients with suspected CS (26). With a cut-off value for serum cortisol set to below 50 nmol/l, as used in *Paper III*, the sensitivity and specificity rates are 95 and 80%, respectively. If the diagnostic threshold is increased to 140 nmol/l, specificity is increased to 95%. Two patients had increased s-cortisol levels after the dexamethasone suppression test according to the more stringent criteria; 120 and 141 nmol/l, respectively. However, neither patient had any symptoms or signs of hypercortisolism and their urinary free cortisol values were normal, and were therefore not excluded from the analysis.

In *Paper II* all patients with CO-CD were considered in remission. This assumption relies on the information found in the KIMS database, including adverse events reports, and not on formal biochemical testing.

The gold standard for testing the HPA-axis is the insulin tolerance test. The test is, however, cumbersome and not without risk. In *Paper III* we used the CRH test to make an overall assessment of the HPA-axis function in patients as compared to controls. Maximal s-cortisol response to CRH has been found to correlate well with the insulin tolerance test (143), and to provide more reliable results than both the low and high dose synacthen tests (144). However, there are also reports that indicate a low sensitivity of the CRH test (145), and its use for diagnosing secondary adrenal insufficiency has therefore been questioned (40).

### Serum Procollagen III peptide

In *Paper* IV, serum Procollagen III peptide (PIIIP) was measured using the immunoradiometric two-step sandwich method (See original paper). Changes in PIIIP concentrations have been shown to be a good predictor of increases in ASMM, LBM and muscle strength (146).

### Glucose metabolism

In *Paper I* and *IV*, Insulin Resistance (IR) was calculated according to the HOmeostatic Model Assessment (HOMA) from fasting plasma insulin and glucose concentration where the output of the model is calibrated to give a normal IR of 1 (147). In *Paper IV*, estimates of the

Insulin Sensitivity Index (ISI) and β-cell function [InsulinoGenic Index (IGI)] (based on the OGTT) were calculated as previously described (148, 149).

HOMA1-IR, ISI and IGI are all indirect simplified measures of glucose metabolism. The gold standards for estimation of insulin secretion capacity and insulin resistance are the hyperglycaemic clamp and hyperinsulinaemic clamp (also called euglycaemic clamp), respectively. Comparisons between HOMA1-IR, ISI and IGI versus the clamp techniques have been performed and have correlated well (148, 150).

**Statistical analysis**

In *Papers I-IV,* paired samples t-tests or Wilcoxon signed rank tests were used for paired variables while unpaired t-tests, Mann-Whitney U-tests or Wilcoxon rank sum tests were used for independent variables, as appropriate. For proportions, Pearson Chi-square or Fishers exact tests were used. In *Papers I and III,* multiple linear and logistic regression analyses with backward elimination were used in an attempt to correct for important potential confounders.

## RESULTS

### *Paper I – Hypopituitary patients*

The mean hydrocortisone dose for ACTH insufficient patients was 20.5 ± 5.8 mg per day: 32 patients had <20 mg, 138 had 20 mg and, 34 had >20 mg (range 5-50 mg). The mean hydrocortisone dose per kg body weight was on average 15% higher in women (0.28 mg/kg) than in men (0.24 mg/kg: $P = 0.009$).

ACTH insufficiency was independently associated with lower fasting glucose (B -0.83: $P < 0.001$) but not with blood pressure, lipids, haemoglobin A1c, HOMA-IR, central fat mass, LBM or presence of the metabolic syndrome.

In women, ACTH insufficiency was independently associated with decreased BMD in the lumbar spine ($P = 0.002$) and femural neck ($P = 0.006$; Figure 1) as well as with osteopaenia (Odds Ratio = 5.8, 95% CI = 1.8-18.9, $P = 0.004$). BMD did not differ between ACTH insufficient and ACTH sufficient men.

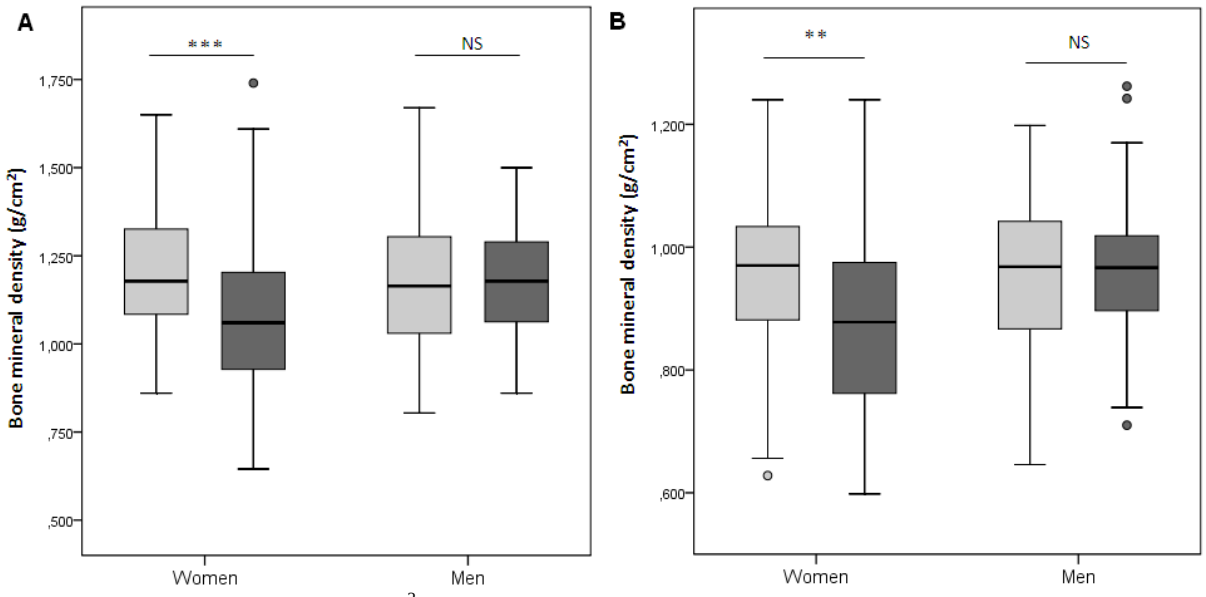


**Figure 1.** Bone mineral density (g/cm$^2$) in the A) lumbar spine and B) proximal femural neck in ACTH sufficient (light boxes) and ACTH insufficient (dark boxes) women and men. **=$P$<0.01, ***=$P$<0.001, NS = No significant difference. Reprinted with permission from Blackwell Publishing Ltd.

### *Paper II – Adult patients with childhood onset Cushing's disease*

The mean age at diagnosis, gender distribution and age at baseline did not differ between patients diagnosed with CO-CD, CO-NFPA or CO-Idio (Table 1). Patients with CO-CD had less severe hypopituitarism compared to CO-NFPA; 41% of CO-CD had 3 or 4 additional pituitary hormone deficiencies compared to 78% of CO-NFPA ($P < 0.001$).

**Table 1:** Background characteristics and anthropometry at baseline in growth hormone deficient adults treated for Childhood Onset-Cushings Disease (CO-CD), CO-Non-Functioning Pituitary Adenoma (CO-NFPA) and CO-Idiopathic hypopituitarism (CO-Idio).

| | **CO-CD (n = 47)** | **CO-NFPA (n = 62)** | **CO-Idio (n = 100)** |
|---|---|---|---|
| **Male/Female (N)** | 22/25 | 32/30 | 50/50 |
| **Age at KIMS start (yr)** | 29.7 ± 9.2 | 30.7 ± 10.5 | 29.0 ± 9.9 |
| **Age at diagnosis of pituitary disease (yr)** | 13.9 ± 2.6 | 13.3 ± 4.0 | 13.8 ± 2.4 |
| **Treatment (%)** | | | |
| Surgery | 51 | 38 | 0 |
| Radiotherapy | 13 | 6 | 0 |
| Surgery and radiotherapy | 32 | 38 | 0 |
| **Height (cm)** | 162 ± 9 | 167 ± 12[#] | 161 ± 9[§§] |
| **Weight (kg)** | 75 ± 23 | 75 ± 21 | 68 ± 20 |
| **BMI (kg/cm$^2$)** | 28.8 ± 10.5 | 26.6 ± 5.9 | 26.2 ± 7.4 |

Data are presented as mean ± S.D. Significant differences between CO-CD and CO-NFPA [#] $P$ = <0.05, significant differences between CO-NFPA and CO-Idio [§§] $P$ = <0.01.

Patients with CO-CD were shorter compared to CO-NFPA but not compared to CO-Idio (Table 1). More patients with CO-CD had height SDS lower than 0 compared to patients with CO-NFPA (Figure 2). Weight and BMI did not differ between the groups (Table 1). Patients with CO-CD who had never received treatment with GH before entering KIMS (true-naïve; n = 16) showed a tendency towards shorter stature and higher BMI compared to semi- and non-naïve patients.

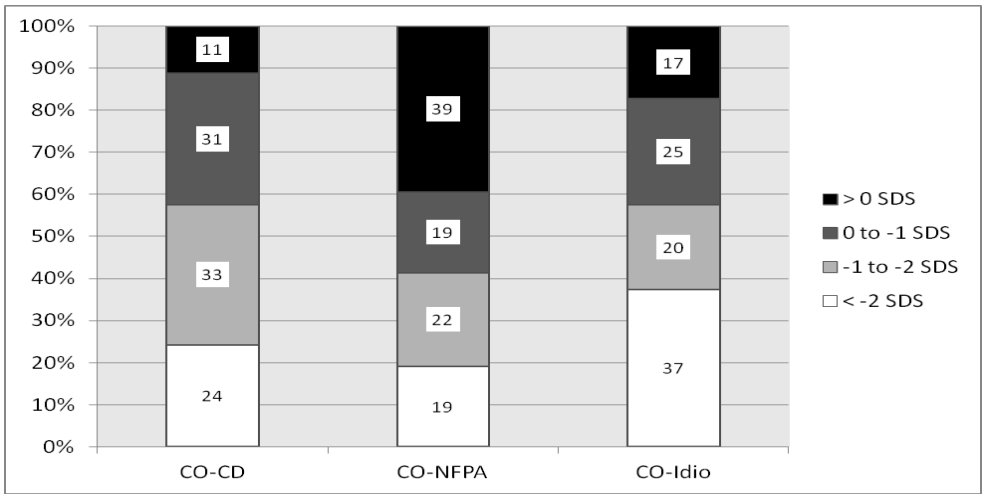


**Figure 2. Height standard deviation score (SDS) in adult patients treated for Childhood Onset Cushing's Disease (CO-CD), CO-Non-Functioning Pituitary Adenoma (CO-NFPA) and CO-Idiopathic hypopituitarism (CO-Idio).** Eighty-nine percent of patients with CO-CD had height SDS lower than 0 compared to 61% of patients with CO-NFPA ($P$ = 0.002) and 83% of CO-Idio (vs. CO-CD; $P$ = 0.349; vs. CO-NFPA; $P$ = 0.002). Twenty-four percent of patients with CO-CD had height SDS lower than -2 compared to 19% of patients with CO-NFPA ($P$ = 0.527) and 37% of CO-Idio (vs. CO-CD; $P$ = 0.127; vs. CO-NFPA; $P$ = 0.016). Reprinted with permission from the European Society of Endocrinology.

Twenty-three percent of patients with CO-CD and 14% of CO-NFPA had hypertension (*P* = 0.220). The prevalence of hypertension was lower in CO-Idio (9%) compared to CO-CD (*P* = 0.018). The prevalence of dyslipidaemia did not differ between the groups and only two patients had type 2 diabetes mellitus.

### *Paper III – Patients with Cushing's syndrome*

The median time in remission was 13 years (interquartile range 5-19). Patients had higher scores on all three subscales of the FIS, indicating greater burdens of fatigue and had higher scores on the CPRS-A subscales for depression and anxiety compared to controls. In a regression analysis adjusted for these confounders, patients performed worse on digit symbol-coding, digit span tests, verbal fluency tests and reading speed tests; indicating impairments in processing speed, auditory attention, working memory, verbal fluency and reading speed (Figure 3).

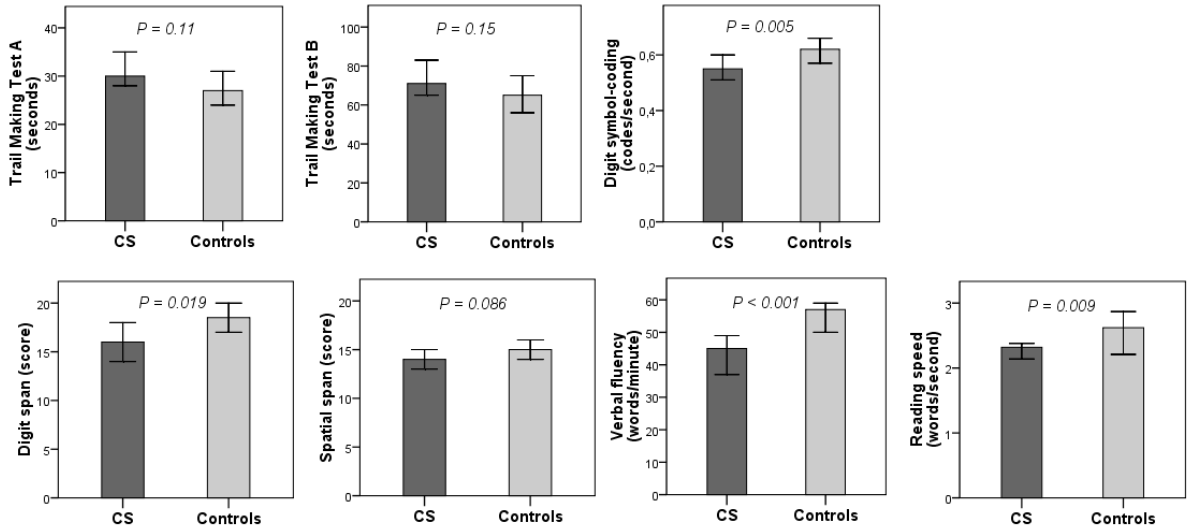


**Figure 3.** Bar chart showing results on a) trail making test A, b) trail making test B (visual scanning, divided attention and motor speed), c) digit symbol coding test (speed processing) d) digit span test (auditory attention and working memory), e) spatial span test (visual attention and working memory), f) verbal fluency test, and g) reading speed test, in patients with Cushing's Syndrome (CS) in remission and healthy controls, matched for age, gender and educational level. *P*-values are obtained from multiple regression analyses adjusted for fatigue and affective disorder. Error bars represent 95% confidence intervals.

On the ANT, alerting and orienting effects were weaker in patients compared to controls (Figure 4). There were no apparent group differences in conflict (executive control).

No overall differences in neuropsychological or neurocognitive performance were seen between a) CD and CPAA, b) hormone insufficient and sufficient patients, c) patients on GC replacement treatment and patients with intact HPA axis, and d) patients who had received RXT or not.

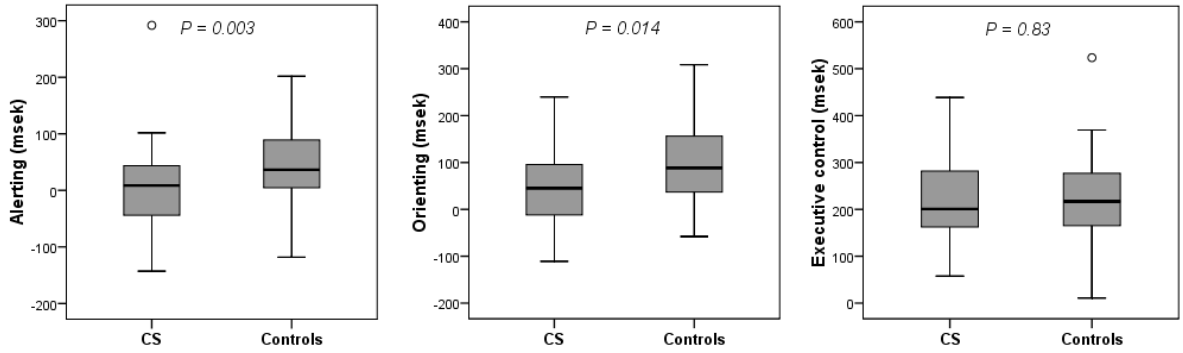


**Figure 4.** Box plots showing results from the Attentional Network Test on a) alerting, b) orienting and c) executive control in patients with Cushing's Syndrome in remission (CS) and healthy controls, matched for age, gender and educational level. The *P*-values are obtained by multiple linear regression analysis, after adjustment for fatigue and affective disorder.

### *Paper IV – Patients on chronic pharmacological glucocorticoid therapy*

LBM increased significantly after treatment with GH and GH + testosterone. The increase in LBM was greater after GH + testosterone than with testosterone alone (Figure 5A). ASMM increased significantly after all three treatment periods with more marked increases after combination treatment than with GH and testosterone alone (Figure 5B). S-PIIIP increased after GH + testosterone. A similar trend was seen for GH and testosterone alone. The increase from baseline was more marked after GH + testosterone than with testosterone alone (Figure 5C).

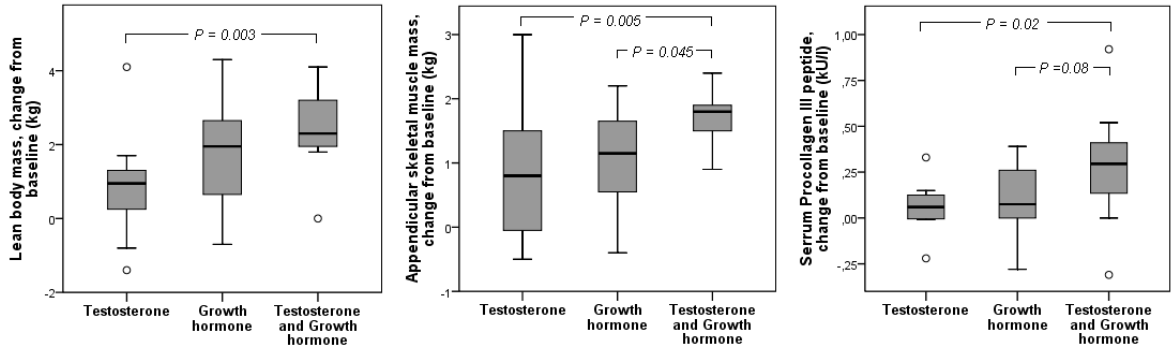


**Figure 5.** Box plots showing change in A) lean body mass B) appendicular skeletal muscle mass and C) serum procollagen III peptide during treatment with growth hormone for two weeks, testosterone for two weeks and combination with both for two weeks.

Fasting plasma glucose concentration increased after GH and GH + testosterone but not during testosterone alone. No patient recorded a fasting plasma glucose concentration above 6.1 mmol/L at any visit. The 2-h plasma glucose after the OGTT was increased after GH + testosterone, but was not significantly different after GH or testosterone alone. Two patients recorded a 2-h plasma glucose concentration above 11.1 mmol/L after treatment with GH + testosterone.

# GENERAL DISCUSSION

In this thesis different aspects of long-term consequences of GC exposure have been explored. This topic is important, not only for patients with a rare disorder such as CS, but also for patients receiving pharmacological and replacement treatment with GC. This thesis has demonstrated:

- That GC replacement in hypopituitary women is associated with low BMD, despite administration of relatively low doses.
- That adult hypopituitary patients, diagnosed with CD during childhood, have compromised final height and a high prevalence of hypertension at long-term follow-up.
- That patients with CS in long-term remission have impaired cognitive function independently of concomitant fatigue, depression, anxiety and underlying type of CS.
- That anabolic treatment with GH and testosterone has the potential to reverse skeletal muscle atrophy in men on long-term GC treatment.

## Glucocorticoid replacement in hypopituitary patients

During the last few years, knowledge of the potential risk of GC over replacement in patients with adrenal insufficiency has emerged (42, 57). A major impact on this development was the discovery that normal cortisol production corresponds to 15-20 mg of hydrocortisone per day (41). In *Paper I,* the effect of GC replacement therapy on cardiovascular risk factors and BMD in a large cohort of hypopituitary patients was studied. Approximately half of the patients had secondary adrenal insufficiency treated with a mean hydrocortisone dose of 20 mg per day. No differences in blood pressure, blood lipids, IR or abdominal fat mass were detected between patients on GC replacement and ACTH sufficient patients. In a previous large international study on 2424 GH deficient patients with hypopituitarism, GC replacement had a dose-related association with increase in BMI, triglycerides, total- and low-density lipoprotein cholesterol concentrations (42). The mean hydrocortisone dose in that study (24 mg) was higher than in *Paper I,* and more than one-third had more than 30 mg per day. Interestingly, patients with hydrocortisone doses less than 20 mg did not differ from ACTH sufficient patients. Patients receiving between 20 and 30 mg per day were pooled together in the statistical analysis that makes it difficult to draw any further conclusions on which doses are considered safe. This and the higher mean hydrocortisone dose as well as the statistical power of this large study possibly explain the different results compared to the results in *Paper I.* In conclusion, of the available data at the present time, GC replacement with hydrocortisone doses of no more than 20 mg per day seems to be safe regarding the cardiovascular risk profile.

In *Paper I,* GC treatment was associated with decreased BMD in woman, an association that was independent of body weight, treatment with sex hormones, and serum concentrations of fT4 and IGF-I. Again, the mean hydrocortisone dose in women was 20 mg - a dose that was approximately 15% higher than in men when adjusted for weight. The influence of GCs on bone health in patients with pituitary insufficiency has been studied in two large studies from

the KIMS database where GC replacement therapy was found to be associated with reduced BMD in one study (63), but not with increased fracture risk in another (60). However, the GC doses were not stated in these papers and whether there was a difference between men and women was not analysed.

The results from *Paper I* are interesting in comparison to the two largest studies previously published on patients with primary adrenal insufficiency. In a study on cohorts of 292 patients from Norway and New Zealand, receiving a relatively high mean hydrocortisone dose of 32 mg per day, BMD was lower compared to a reference population and the weight adjusted GC dose was associated with Z-scores at the femoral neck, total hip and total body (57). On the contrary, in a study from Germany were the mean daily hydrocortisone dose was considerably lower (21 mg in women and 24 mg in men), BMD was within the normal reference range (55). However, the Z-scores at most sites in that study were lower than zero and the study lacked a control group. In the light of the results from *Paper I*, it is likely that even modestly supraphysiological GC doses can have negative effects on bone metabolism. Since GC exposure is dependent on body weight (151), females in particular may suffer an adverse outcome of the current replacement regimen. It is still unclear which doses can be considered safe and further studies on this topic are needed.

Not only the doses, but also the dosing scheme may be important regarding outcome in patients with adrenal insufficiency - *i.e.* when and how many times per day GCs are administrated. Today, no consensus exists. However, the cortisol concentration profile during a thrice daily regime has been shown to mimic the physiological profile better than a twice daily regime (152). Similarly, improved QoL has been observed in patients switched from twice to thrice daily regimes (153). Nevertheless, with the conventional GC replacement therapies available today, the diurnal and ultradian patterns of cortisol secretion will never perfectly match the normal physiological profile.

Recently, new hydrocortisone tablet formulations (154, 155) and continuous hydrocortisone pumps (156) have been developed in an attempt to better mimic the physiological cortisol profile. In comparison to a thrice daily regime, hydrocortisone dual-release tablets administrated once daily resulted in lower hydrocortisone exposure and an improved metabolic profile (155). The long-term effects of these new treatment alternatives remain to be examined.

**Consequences of childhood onset Cushing's disease**

Growth retardation is one of the cardinal features of paediatric CD (114-117). In *Paper II*, 89% of the adults treated for CO-CD had height SDS below 0 (*i.e.* the median average height in the normal population) and one-fourth had SDS below -2. Patients with CO-CD had shorter stature compared to CO-NFPA despite more severe hypopituitarism in the latter group. This supports the concept that the hypercortisolism associated with CD during childhood is most likely a key mechanism behind their short stature. Patients with CO-Idio were also included in the analysis as a control group to evaluate the potential effect of tumour treatment and GC

exposure (*i.e.* a control group that has not been operated on, or received RTX and never been exposed to elevated GC levels or GC replacement). If hypercortisolism and pituitary tumour treatment result in additive negative effects on final height, then patients with CO-CD would have had the most compromised final height, followed by CO-NFPA and then CO-Idio. However, final stature was equally compromised in ACTH sufficient CO-Idio and CO-CD patients, arguing against the aforementioned concept. Nevertheless, one may question the suitability of including the CO-Idio group for this purpose. In contrast to patients with CD who come to medical attention due to various symptoms and signs, the main complaint in patients with CO-Idio is just short stature. It is therefore likely that patients with CO-Idio have more severely affected GH secretion, supported by the fact that their IGF-I concentrations at baseline were significantly lower compared to both CO-CD and CO-NFPA.

One-fourth of patients with CO-CD had hypertension, which was more prevalent than in patients with CO-Idio. Even though healthy controls were not included in the study, the prevalence of hypertension is higher than expected for individuals with a mean age of 30 years. In a population based study from USA, the prevalence of hypertension (defined as systolic blood pressure >140 and/or diastolic blood pressure >90) in 30-39 year-old non-Hispanic white men was 12% and in women, 6%. (157).

The prevalence of diabetes mellitus, stroke and coronary artery disease was low in all three groups. In a recent study of 160 patients with adult-onset GHD who were previously treated for CD, the prevalence of cardiovascular and cerebrovascular disease was greater compared to 879 patients treated for NFPA. The prevalence of diabetes mellitus and the metabolic syndrome did not differ between groups (158). The discrepancy between these two studies can probably be explained by a lower number of patients in our study and that patients in the adult-onset study were older (the mean age in the CD group was 38 years and in the NFPA group it was 48). Nevertheless, physicians taking care of patients who have been treated for CS should be aware of the increased cardiovascular risk at long-term follow-up (76, 77), regardless of age at diagnosis.

**Growth hormone treatment in children with Cushing's disease**

Endocrine regulation of the growth plate is complex. GCs, thyroid hormones, oestrogens, androgens and GH all directly affect the growth plate chondrocytes (159). The growth retardation observed in patients with CS is therefore not only a consequence of hypercortisolism but can also be caused by impaired GH and sex hormone secretion as GC excess *per se* inhibits both. In adults (160, 161) and children (162) with CS, GH secretion is compromised for long time after remission has been achieved. Evaluation of GH status in adults is therefore usually not performed earlier than 1 or 2 years after successful treatment. In children on the other hand, no consensus exists regarding when GH evaluation should be performed.

Short adult stature in the general population is associated with a reduction in QoL (163). In short-stature children, GH therapy may improve behaviour and self-esteem (164, 165).

Therefore, for patients with CS, some recommend early evaluation and commencement of GH administration in order to accelerate linear growth and ensure optimal height achievement after treatment (166). Others have a more conservative approach (123). In *Paper II*, although not statistically significant, height tended to be lower in patients who had never received GH treatment before entry compared to those who had. In a previous study, early diagnosis and treatment of GHD resulted in majority of patients achieving final height within the target range (116). This and the results in *Paper II* therefore support early intervention regarding GH status. It should be emphasized that individual judgment is needed and other factors such as age, pubertal stage and treatment of other hormone deficiencies are also important.

**Non-functioning pituitary adenoma is rare in childhood**

The main intention of *Paper II* was to study long-term sequelae in patients with CO-CD. We realized early on in planning that studies involving patients with CO-NFPA were few and it would be equally important to publish results from them as well. Pituitary neoplasms are rare in children and adolescents. Of these, the most commonly occurring are craniopharyngiomas (167), and prolactinomas (112, 168). NFPA is exceptionally rare in children and adolescents. In a series of children requiring surgical treatment for pituitary adenomas, 4-6% had NFPA (169, 170). Long-term follow-up studies have not been published previously. In *Paper II*, pituitary insufficiency was common in the CO-CD group, but thyrotropin and gonadotropin deficiencies as well as diabetes insipidus were even more common in patients with CO-NFPA. Patients with CO-NFPA also had a higher frequency of reported visual field defects than the CO-CD group, which is most likely due to a larger tumour size in CO-NFPA patients. Although final height was not as strikingly compromised as in CO-CD, 19% of CO-NFPA patients had height SDS below -2, when the expected number in the general population would be 2.5%. It is therefore clear that patients diagnosed with paediatric NFPA can have compromised health at long-term follow-up.

**Impaired cognitive function after long-term remission in Cushing's syndrome**

Whether patients with CS in remission have impaired cognitive function at long-term follow-up has not been studied until recently when two studies have addressed this issue. In a study from the Netherlands, cognitive function in 74 patients with CD in remission was compared to 74 healthy individuals and 54 patients previously treated for NFPA (171). The mean duration of remission ranged between 1 and 51 years. Cognitive testing, which mainly focused on memory and executive functioning, showed worse performance in patients with CD compared with both control groups. Even global cognitive functioning, evaluated using the Mini Mental State Examination, was significantly worse in CD patients. In another recent study, verbal and visual memory were found to be impaired after a mean biochemical cure time of 7 years (172). Interestingly, brain gray matter volumes, evaluated using MRI, were decreased in patients with CS, indicating brain atrophy though no significant difference in hippocampal volume was seen. This has, until now, been the only available data indicating long-term cognitive impairment in CD. Whether this also holds true for patients with CPAA and if the cognitive dysfunction can be explained by concomitant fatigue and psychiatric disorders has not been explored previously.

In *Paper III*, various domains of cognitive function were compromised in patients with CS after long-term remission. Speed processing, auditory attention and working memory, verbal fluency, reading speed, spatial orienting and alerting were all negatively affected in comparison to controls. The results from *Paper III* demonstrate three important findings. Firstly, cognitive function is not only temporarily affected at short-term follow-up, but seems to be a permanent consequence of previous hypercortisolism. Secondly, patients with CS in remission have impairment in various domains of cognitive function and not only in hippocampal function which has until now received greatest attention (171, 172). Thirdly, cognitive dysfunction cannot be explained by concomitant affective disorder or chronic fatigue, both common and important potential confounders in patients with CS.

The reason for the neurocognitive impairment in patients with CS is not clearly understood. Potential explanations include concomitant neuropsychiatric problems such as anxiety and depression, noted in up to 24% of patients one year after curative treatment (90), inadequate replacement therapy of postoperative hormone deficiencies commonly seen after TSS (30-33), and consequences of pituitary RTX, administrated in one-third of patients in *Paper III*. In *Paper III*, none of these potential confounders were found to have a major impact on cognitive performance. The number of patients in the subgroup analyses was, however, small and the results should be interpreted with caution. Nevertheless, our results in addition to two other recently published papers (171, 172), suggest that long-term exposure to excess GC plays a causative role in the long-term cognitive consequences of CS.

GCs have a great impact on the central nervous system through its binding to the GC receptor which is expressed widely in the brain (173). GCs also bind to mineralocorticoid receptors that are found in more restricted areas such as the hippocampus, amygdala and pre-frontal cortex - collectively referred to as the limbic system. The limbic system is of great importance for cognitive function. The hippocampus is the main centre for memory and learning. The amygdala is the principal emotional centre while the prefrontal cortex is involved in behavioural inhibition, decision-making, executive function and working memory (174). The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1, an enzyme that converts the inactive metabolite cortisone to the active compound cortisol, is also widely distributed in the brain. On the other hand, 11β-HSD type 2, with the opposite function, is only scarcely expressed. During states of GC excess, the protective role of 11β-HSD type 2 is therefore absent, and areas that express GC and mineralocorticoid receptors abundantly, such as the limbic system, may therefore be especially vulnerable. In rodents, short term GC administration leads to atrophy of dendrites in hippocampal neurons, changes that are reversible when GCs are withdrawn. Longer exposure to high GC levels causes hippocampal degeneration, partly due to loss of neurons (175). These observations support that the cognitive dysfunction observed in *Paper III* are caused by irreversible changes in the central nervous system.

It is surprising how differently the postoperative course evolves in patients with CS. Some patients can discontinue their GC replacement and experience recovery relatively shortly after surgery while for others it can take years. Unfortunately, there are also patients that after

many years in remission are still suffering from the distressing sensation of chronic fatigue and impaired general well-being. The reason for this discrepancy is unknown. Possibilities that have been suggested include postoperative hormone deficiencies, untreated GHD, inadequate GC replacement therapy and previous pituitary RTX. Neither hormone deficiency nor RTX were found to affect the cognitive outcome in *Paper III* which is consistent with a previous study from the Netherlands (171).

Another possible reason may be the duration of active CS and the degree of hypercortisolism - *i.e.* patients who have had a longer duration of, or more extensive, hypercortisolaemia may have worse outcomes. Exploring this topic is methodologically difficult for number of reasons. Firstly, endogenous CS is a rare disorder and designing a sufficiently powered study for this reason would be difficult. Secondly, it is difficult to estimate the duration of time with active disease. This will always be done retrospectively and since the symptoms and signs of CS usually develop insidiously, the exact time-point of debut is difficult to estimate. In addition, there is a risk that patients with the greatest suffering overestimate their time with active CS. Thirdly, the evaluation of the extent of hypercortisolaemia is difficult. Both urinary free cortisol and serum cortisol can vary from one time to another and are, moreover, generally not good predictors of GC exposure in the tissues. An ideal method would be a measurement of tissue response to GC, a method that currently does not exist. In *Paper III*, longer duration of uncontrolled CS was associated with worse performance in 3 of 7 neuropsychological tests. For the reasons mentioned above, these findings should be interpreted with caution.

Recently, the possible role of different GC receptor sensitivity has been discussed as a possible factor that can affect various components of CS. Indeed, specific polymorphisms in the GC receptor are associated with altered sensitivity, *e.g.* BclI and N363S polymorphisms have increased sensitivity while ER22/23EK and A3669G have decreased sensitivity (176). In patients with active CS, BclI has been found to be associated with reduced BMD (177) and A3669G with a decreased risk of developing diabetes mellitus (178). Whether GC receptor polymorphisms have relation to postoperative outcome has not been studied, but is an interesting concept that could improve the current knowledge on this important topic.

**Anabolic treatment of GC induced sarcopaenia**

Sarcopaenia and muscle weakness in the elderly is associated with increased morbidity and mortality (75). The economic burden secondary to sarcopaenia has been estimated to be comparable to the costs for treatment of osteoporosis (179). In contrast to osteoporosis, there is no specific treatment available to prevent or reverse GC induced muscle atrophy.

GH and testosterone are anabolic hormones. GH increases LBM when administrated to patients with GHD (180) and healthy elderly men (181-183). Similarly, testosterone administration increases LBM in men with acquired hypogonadism (184), and increases protein synthesis, ASMM and muscle strength in the aging male (182, 183, 185). GH treatment has also been shown to prevent GC induced protein catabolism in healthy subjects

(186, 187) and patients on long-term GC treatment (188). Likewise, testosterone treatment increases LBM and muscle strength in men on long term GC therapy (189, 190). Amongst the important transmitters involved in muscle cell protein turnover are IGF-I (anabolic effect) and myostatin (catabolic effect). Both GH (191) and testosterone (192, 193) are known to reduce myostatin levels by a mechanism that may, at least partially, involve an increase in myostatin propeptide, a potent myostatin inhibitor (194). GH administration increases intramuscular IGF-I mRNA expression (195, 196). Increased IGF-I mRNA expression after administration of androgens is also reported in some studies (197, 198) but to be unchanged in other (195).

In *Paper IV*, short term treatment with GH and testosterone increased ASMM in men on chronic GC treatment. When GH and testosterone were administrated in combination, serum levels of PIIIP increased significantly and a greater increase in muscle mass was seen, compared to treatment with either hormone alone. Anabolic treatment with GH and testosterone is therefore a potential alternative for treatment of GC induced sarcopaenia. However, longer and larger trials are needed, both to study the long-term effects on muscle mass and function as well as safety.

Glucose homeostasis was adversely affected during treatment with GH. Fasting glucose increased after GH, both when administrated alone and in combination with testosterone. Insulin resistance increased after co-administration of GH and testosterone. Two patients reached 2-h glucose levels during OGTT above 11.1 mmol/l, thereby fulfilling the criteria for diabetes mellitus. The relatively high GH dose administrated in the study is probably the main reason for the adverse effect on glycaemic control. Support for this conclusion comes from the fact that the majority of the patients reached supraphysiological levels of IGF-I after treatment with GH. GH is known to deteriorate insulin sensitivity after 6 weeks of treatment in GHD patients, which then returns towards baseline values after 6 months (199). The initial and temporary decrease in insulin sensitivity has been explained by the lipolitic effect of GH. In *Paper IV* this was reflected by an increase in fat oxidation and increased triglyceride concentrations in serum. In future studies, glucose metabolism should therefore be carefully monitored as patients with mild hypercortisolism are prone to have impaired glucose tolerance (80), which can deteriorate further with GH treatment.

**Concluding remarks**

One-hundred years have passed since Harvey Cushing presented his first patient with the syndrome that bears his name. During these 100 years enormous progress has occurred in the field of clinical endocrinology, although many questions remain to be answered. This thesis has shed light on some of the unanswered questions regarding the influence of GC exposure on outcome in patients with GC deficiency and CS. The main clinical implications of this thesis concern the importance of adequate GC replacement dosing and the increased knowledge on adverse outcome at long-term follow-up in patients with CS in remission; issues that are constantly relevant for the clinical endocrinologist and the patients with these disorders.

However, numerous topics still remain to be solved and the results of this thesis have contributed to creating further unanswered questions. How can we improve GC replacement in order to prevent undesirable consequences of over- and/or under-treatment? What explains the individual variance in outcome after treatment of CS? What is the optimal hormonal treatment for patients diagnosed with pituitary adenomas during childhood? Similarly, there is a need for further interventional studies on GC induced sarcopaenia, a growing problem in the elderly. Hopefully, these challenging topics will receive further attention in the near future and result in improved patient care in the end.

## GENERAL CONCLUSIONS

- GC replacement in hypopituitary women is independently associated with reduced BMD and a higher prevalence of osteopaenia, even when relatively low GC doses are used. GC replacement does not significantly affect cardiovascular risk factors at an average daily hydrocortisone dose of approximately 20 mg.

- Adult patients with hypopituitarism due to CO-CD have compromised final adult height as compared with adults treated for CO-NFPA, despite a more severe hypopituitarism in the latter group, indicating long-term consequences of GC exposure in childhood.

- Patients with CS in remission have attentional deficits and impairment in numerous domains of cognitive function at long-term follow-up that cannot be explained by co-existence of affective disorder or chronic fatigue. The cognitive and attentional deficits suggest a more global involvement of brain function than has previously been suggested.

- Short-term treatment with GH and testosterone increases skeletal muscle mass in men on chronic, low dose GC treatment. When GH and testosterone are administrated in combination, the increase in skeletal muscle mass was greater than after GH and testosterone alone. These findings suggest that combination treatment with GH and testosterone has the potential to reduce or prevent GC-induced sarcopaenia.

## SUMMARY IN SWEDISH – SAMMANFATTNING PÅ SVENSKA

Glukokortikoider är hormoner som bildas i binjurarna och har en stor inverkan på kroppens ämnesomsättning. Den viktigaste glukokortikoiden hos människa är kortisol. Överproduktion av kortisol kallas Cushings syndrom och kännetecknas av bukfetma, muskelförtvining, benskörhet, högt blodtryck, nedsatt sockertolerans samt minnes och koncentrationssvårigheter. Cushings syndrom kan orsakas av tumörer i hypofys eller binjurar, eller vara ett resultat av långvarig behandling med glukokortikoider (i vardagligt tal kallad kortisonbehandling).

Denna avhandling bygger på fyra studier där konsekvenser av långvarigt överskott av glukokortikoider undersöktes. I en stor studie på patienter med hypofyssvikt hade kvinnor med kortisonbehandling lägre bentäthet än kvinnor som inte behövde behandling med kortison. I en annan studie på vuxna patienter som behandlats för Cushings syndrom under barndomen såg man att patienterna var kortare än normala befolkningen och att förekomsten av högt blodtryck var hög. I en studie på patienter som tidigare framgångsrikt behandlats för Cushings syndrom var ett flertal kognitiva funktioner nedsatta vid långtidsuppföljning i jämförelse med friska individer. Slutligen visade det sig att behandling med tillväxthormon och testosteron ökade muskelmassan hos män med långvarig kortisonbehandling.

Sammanfattningsvis visar denna avhandling att långvarigt överskott av glukokortikoider har ett flertal negativa hälsorelaterade konsekvenser för patienter som står på kortisonbehandling samt patienter som tidigare har behandlats för Cushings syndrom. Vidare har man sett att behandling med tillväxthormon och testosteron hos män med långvarig kortisonbehandling är ett tänkbart behandlingsalternativ för att motverka kortisonorsakad nerbrytning av muskler.

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