Non-functioning pituitary adenomas and hypopituitarism

Studies on morbidity and mortality

Casper Hammarstrand

Department of Internal Medicine and Clinical Nutrition
Institute of Medicine
Sahlgrenska Academy
University of Gothenburg
Gothenburg, Sweden

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casper.hammarstrand@gu.se

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“The measure of greatness in a scientific idea is the extent to which it stimulates thought and opens up new lines of research”

- Paul Dirac

To my family

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Sahlgrenska Academy, University of Gothenburg
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ABSTRACT

Background and aims: Hypopituitarism is most commonly caused by benign pituitary tumours, often resulting in insidious symptoms that develop over a long time. Due to either a mass effect exerted by the tumour, or due to the surgical treatment, hormonal deficiencies may arise, requiring hormone replacement. Based on four studies, this dissertation aimed to investigate the influence of glucocorticoid and growth hormone replacement on mortality, morbidity and surrogate markers for cardiovascular disease.

Methods: In *paper I*, the influence of glucocorticoid replacement on mortality was studied in patients with NFPA with standardised mortality ratio, using the general population as reference, and hazard ratio calculated. In *paper II*, standardised incidence ratio (SIR) for malignant tumours was calculated for patients with NFPA using the general population as reference. In *paper III*, the influence of growth hormone replacement therapy on comorbidities was studied. Finally, in *paper IV*, changes in the concentration of LDL-cholesterol were compared between patients with growth hormone replacement and a random population sample of men and women.

Results: *Paper I* showed that glucocorticoid replacement doses of more than 20 mg per day were associated with excess mortality. In *paper II*, the overall incidence of malignant tumours was increased in NFPA patients. *Paper III* showed that the risk of type 2 diabetes mellitus and cancer was not increased in growth hormone replaced patients. Finally, in *paper IV*, LDL-cholesterol in growth hormone replaced patients was decreased above the secular trends seen in the general population.

Conclusions: Glucocorticoid replacement doses higher than 20 mg per day are associated with increased mortality. Patients with NFPA have an increased risk of developing cancer. However, growth hormone replacement seems to be safe concerning the risk of developing comorbidities, including cancer. Furthermore, growth hormone replacement can be considered beneficial for the lipid profile.

Keywords: Non-functioning pituitary adenoma, hypopituitarism, glucocorticoid replacement, growth hormone replacement, morbidity, mortality, cancer, LDL-cholesterol

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SAMMANFATTNING PÅ SVENSKA

Icke-hormonproducerande hypofysadenom (NFPA) är den vanligaste tumören i hypofysregionen näst efter prolaktinom och debuterar ofta med symptom relaterade till tumörens masseffekt på den omkringliggande vävnaden (1, 2). Synbortfall orsakat av tumörens volymsinverkan på synnerven och synnervskorsningen samt bortfall av hypofysens hormonproduktion, hypofysinsufficiens, är de två vanligaste symtomen vid hypofystumörer (3).

Sekundär binjurebarkssvikt på grund av hypofysinsufficiens innebär en ökad morbiditet och mortalitet (4, 5, 6, 7, 8). Huruvida det beror på tumören eller hormonbristen och dess ersättningsbehandling är inte fullständigt klarlagt. Tidigare studier har dock påvisat ett samband mellan icke-fysiologisk ersättningsbehandling med glukokortikoider och en ökad mortalitet både hos patienter med NFPA och hos patienter med akromegali (9, 10).

Risken, hos patienter med NFPA, för att utveckla elakartade tumörer är sparsamt studerat och föremål för debatt. En ökad incidens av hjärntumörer hos patienter med hypofysadenom har påvisats i tidigare studier, i synnerhet hos patienter som erhållit strålbehandling mot hypofysen (11, 12). Huruvida incidensen för andra cancerformer är ökad är oklart i nuläget. Det råder ingen konsensus i tidigare studier och det föreligger ofta betydande begränsningar i form av små patientmaterial, kort uppföljningstid, selektions-bias och/eller heterogena studiepopulationer rörande tumöretiologi och behandling (13, 14, 15, 16).

Patienter med hypofysinsufficiens och obehandlad brist på tillväxthormon har ökad andel kroppsfett med central fetma (17). Ogynnsam metabol profil med bland annat insulinresistens, dyslipidemi och ökad prevalens av hypertension, ateroskleros, hjärtkärl- samt cerebrovaskulär mortalitet har även påvisats hos dessa patienter (4, 5, 18). Ersättningsbehandling med tillväxthormon har visat gynnsamma effekter på de ovan nämnda metabola konsekvenserna av tillväxthormonbrist, men långtidseffekterna av ersättningsbehandling med tillväxthormon är än så länge ej väl utredda.

Denna avhandling bygger på fyra studier där konsekvenser av hormonell ersättningsbehandling med glukokortikoider och tillväxthormon samt cancerindincidensen hos patienter med NFPA undersöktes. I den första studien hade patienter med sekundär binjurebarkssvikt och daglig ersättningsdos med glukokortikoider >20 mg en ökad mortalitet jämfört med normalbefolkningen, medan ersättningsdoser ≤20 mg inte var associerad med en ökad

mortalitetsrisk. I den andra studien sågs en ökad cancerincidens hos patienter med NFPA. I den tredje studien var incidensen av diabetes mellitus typ 2 förhöjd hos patienter med NFPA utan tillväxthormonbehandling men inte hos patienter med ersättningsbehandling med tillväxthormon. Vidare var cancerincidensen hos patienter med tillväxhormonbehandling inte förhöjd jämfört med normalbefolkningen. Slutligen visade det sig att långvarig ersättningsbehandling med tillväxthormon var associerat med minskande LDL-kolesterolnivåerna i högre grad än vad som sågs i normalbefolkningen under motsvarande tidsperiod.

Sammanfattningsvis visar denna avhandling att patienter med NFPA och sekundär binjurebarkssvikt ej har en ökad mortalitet vid dygnsdoser av hydrokortison upp till och med 20 mg. Dessutom har avhandlingen visat att patienter med NFPA har en förhöjd risk att drabbas av cancer. Vidare har man sett att ersättningsbehandling med tillväxthormon förbättrar lipidprofilen och verkar ej medföra en ökad risk för att utveckla komorbiditeter, inklusive cancer.

LIST OF PAPERS

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

I. Hammarstrand C, Ragnarsson O, Hallén T, Andersson E, Skoglund T, Nilsson A G, Johannsson G, Olsson D S. **Higher glucocorticoid replacement is associated with excess mortality in patients with non-functioning pituitary adenoma.** *European Journal of Endocrinology*. 2017 Sep;177 (3):251-256.

II. Olsson D S, Hammarstrand C, Bryngelsson IL, Nilsson A G, Andersson E, Johannsson G, Ragnarsson O. **Incidence of Malignant tumours in Patients with Non-Functioning Pituitary Adenoma.**
Endocrine-Related Cancer. 2017 May;24 (5):227-235.

III. Hammarstrand C, Ragnarsson O, Bengtsson O, Bryngelsson IL, Johannsson G, Olsson D S. **Comorbidities in patients with non-functioning pituitary adenoma: influence of long-term growth hormone replacement**.
European Journal of Endocrinology. 2018 Oct 1;179(4):229-237.

IV. Hammarstrand C, Olsson D S, Koranyi J, Trimpou P, Landin-Wilhelmsen K, Johannsson G. **Growth hormone replacement therapy in adults with hypopituitarism: A longitudinal case-control study on lipid metabolism**.
Manuscript.

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OVERVIEW OF PAPERS

	Paper I GC replacement and mortality	Paper II NFPA and cancer	Paper III GH replacement and morbidity	Paper IV GH replacement and LDL-cholesterol
	Retrospective cohort study	Registry study	Retrospective cohort study	Longitudinal case-control study
Study period	1987–2014	1987–2011	1987–2014	1995–2008
Subjects (no.)	392	2795	426	342
Outcomes studied	Overall mortality depending on GC replacement dose	Cancer incidence	Cause specific morbidity in patients with and without GH replacement	Influence of long-term GH replacement on LDL-cholesterol
Registries included	Patient and Death Registries	Patient and Cancer Registries	Patient and Cancer Registries	
Results	HR (95% CI) Overall 1.88 (1.06–3.33)	SIR (95% CI) Overall 1.22 (1.11–1.33)	SIR (95% CI) Diabetes mellitus type 2 Without GH: 1.65 (1.06–2.46) With GH replacement: 0.99 (0.55–1.63)	Difference between groups. Adjusted means (95% CI) Men: -0.275 mmol/L (-0.479; -0.072) Women: -0.598 mmol/L (-0.789; -0.407)
Conclusion	GC replacement doses of more than 20 mg/day are associated with increased mortality	NFPA patients have an increased incidence of malignant tumors	Long term GH replacement can be considered safe concerning the risk of developing comorbidities including diabetes mellitus type 2 and cancer	Long-term GH replacement decreased the LDL-cholesterol concentration in patients with GHD above the secular trends seen in the general population

Abbreviations: GC, glucocorticoid; NFPA, non-functioning pituitary adenoma; GH, growth hormone; HR, hazard ratio; SIR, standardised incidence ratio

ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
FSH	Follicle-stimulating hormone
GC	Glucocorticoid
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone-releasing hormone
GHRT	Growth hormone replacement therapy
HC	Hydrocortisone
HCeq	Hydrocortisone equivalent
IGF-1	Insulin-like growth factor 1
LH	Luteinizing hormone
LDL-C	Low-density lipoprotein cholesterol
NFPA	Non-functioning pituitary adenoma
PA	Pituitary adenoma
PIT1	Pituitary-specific POU-class homeodomain transcription factor
SF1	Steroidogenic factor 1
T-PIT	T-box family member TBX19
TSH	Thyroid-stimulating hormone
VLDL	Very low-density lipoprotein

INTRODUCTION

History

The pituitary gland is a main endocrine regulator. The anatomical term stems from the notion, dating back to 400 BC, that the brain had a cooling function which was exerted by secretion of a bodily humor phlegm, *pituita* (19). This was suggested by Hippocrates of Kos "The Father of Medicine" (460-370 BC). Built upon this belief and the location of the gland, Galen (130-200 AD) hypothesised that the main role of the pituitary gland was essentially to secrete waste products, through phlegm, to the nose and throat (19, 20).

The significance of the pituitary gland as a key constituent of the endocrine system was not understood until the late 19th century (21). Observations of several clinical manifestations such as acromegaly were linked to abnormalities of the gland in the late 19th century by Pierre Marie (21).

As histopathological analyses developed, the understanding and knowledge of the pituitary functions grew on account of prominent scientists such as Harvey Cushing. He postulated the presence of the growth hormone in the pituitary (21). This laid the foundation for the hypothesis that excessive function of the pituitary gland was associated with various diseases, symptoms, and manifestations.

The pituitary gland consists of an anterior lobe (the adenohypophysis) and a posterior lobe (the neurohypophysis). The endocrine cells of the adenohypophysis produce several hormones, including thyroid-stimulating hormone, adrenocorticotropic hormone/ corticotropin, follicle-stimulating hormone, luteinizing hormone, prolactin and the growth hormone. The neurohypophysis releases neuropeptides that are synthesised in the hypothalamus i.e. antidiuretic hormone (also called vasopressin) and oxytocin (22, 23). The production and release of these hormones are controlled by feedback mechanisms, in which stimuli prompt their production, and once they reach certain levels, inhibiting signals are sent. In this way, the concentration of the pituitary hormones in the blood is maintained.

Pituitary adenomas

Pituitary adenomas (PA) are benign neoplasms arising from the pituitary gland and account for approximately 10-15% of all brain tumours (22). They are classified based on their size as

microadenomas, <10 mm, or macroadenomas, >10 mm. They are also classified based on their ability to produce and secrete excess hormones, as either clinically functioning or non-functioning (24). PAs are of different etiologies depending on which of the five major cell types of the adenohypophysis they arise from, hence classified as: prolactinomas, GH-producing adenomas, ACTH-producing adenomas, TSHomas and, if clinical symptoms indicate hormonal hypersecretion is absent, non-functioning pituitary adenomas (NFPAs). The latter, however, often originate from gonadotropic cells and produce gonadotropins or gonadotropic subunits, although not to a clinically significant degree (25). When hormonal hypersecretion of a PA is present, specific endocrine and metabolic consequences arise. Prolactinomas, causing hyperprolactinemia, are associated with clinical features such as amenorrhoea or oligomenorrhoea, erectile dysfunction, infertility, and galactorrhoea (24). Acromegaly is caused by hypersecretion of GH with clinical signs such as coarse facial features, enlarged feet and spade-shaped hands along with clinical symptoms such as arthralgia, and symptoms of carpal-tunnel syndrome (26). Moreover, hypertension, cardiomyopathy and impaired glucose tolerance are long-term consequences of acromegaly (27). ACTH-producing adenomas causing Cushing's disease are associated with features including weight gain, visceral obesity, hypertension, dyslipidaemia, impaired glucose tolerance atherosclerosis, fatigue and cognitive impairment (28). TSHomas are rare and the clinical manifestations of hypersecretion of TSH are similar to other thyroid diseases causing thyrotoxicosis (29).

The classification of pituitary adenomas was recently changed by adopting a pituitary adenohypophyseal cell lineage as the main lodestar in classifying adenomas (30, 31). Several transcription factors determining the differentiation of neuroendocrine cells have been identified such as: PIT1 (pituitary-specific POU-class homeodomain transcription factor), which leads to differentiation of somatotrophs, lactotrophs and thyrotrophs; T-PIT (T-box family member TBX19) transcription factor, leading differentiation of corticotrophs via the regulation of proopiomelanocortin lineage; and SF1 (steroidogenic factor 1), which leads to gonadotroph cell differentiation (30, 31). An adenoma displaying more than one pituitary hormone expression is defined as plurihormonal, and are either PIT1-positive or have variable lineage combinations. Furthermore, null-cell adenomas are pituitary tumours without a distinct cell lineage which are immunonegative for both pituitary hormones and pituitary transcription factors.

Most gonadotroph adenomas are clinically non-functioning, with immunohistochemistry staining positive for β-LH, β-FSH and α-subunits in a variable expression (32, 33, 34). Of the

NFPA subtypes, gonadotroph adenomas are the most common, followed by corticotroph, PIT1 lineage and null-cell adenomas (34).

Prolactinomas are the most common PA subtype that accounts for 40-60% of the clinically diagnosed cases (35). NFPAs are the second most common etiology of PAs, accounting for 14-51% (36, 37) with an annual incidence of 0.65-2.34 cases per 100 000 inhabitants (8, 38).

The studies in this dissertation were performed before the new classification system was introduced and used the previous histological classification system which was based on clinical features and immunohistochemical assessment.

Clinical presentation

The clinical manifestation of a NFPA relates to the tumour's mass effect on neighbouring structures such as the meninges, optical chiasm, or cavernous sinus causing symptoms such as headaches, visual impairments, visual field defects or ophtalmoplegias (38). The mean age at diagnosis ranges in previous studies between 50 and 60 years (37, 39, 40). Symptoms occur depending on the direction in which the tumour grows (3). Suprasellar extension with pressure on the optic chiasm is associated with visual impairments, most commonly bitemporal haemianopsia, and the pathogenesis of headaches, which is present in approximately 25% of macroadenoma patients (3), is suggested to be mediated by increased intrasellar pressure and stretching of the meninges (41). Furthermore, hypothalamic damage due to the tumour, surgery or radiotherapy can disrupt the hypothalamic control mechanism of appetite, thirst, temperature and sleep (26, 42).

The clinical presentation of NFPAs varies from asymptomatic patients to symptoms of severe headache and acute hypopituitarism secondary to pituitary apoplexy (43, 44). The latter is a life-threatening condition when untreated because of hypocortisolism following the loss of ACTH production (43). However, the onset is more often gradual, explained by the low growth propensity of microadenomas (45, 46), and the time to diagnosis is easily delayed due to subtle or lacking symptoms up to three years reported by Drange et al. (47). Larger tumours typically cause more symptoms, and drive patients to contact the healthcare. Thus when detected, NFPAs are often classified as macroadenomas (24), constituting approximately 50% of pituitary tumours in surgical series (48). In addition, with the increasing use of computed tomography

and magnetic resonance imaging (MRI) for indications other than pituitary disease, pituitary incidentalomas are diagnosed more frequently than before (44). Reported rates of micro- and macroincidentalomas in MRI studies are 10-38% (49, 50) and 0.16-0.3% (51, 52), respectively. In addition, a large study reviewing MRI scans of 2598 participants reported that NFPAs accounted for most of the incidentalomas, but was the second most common pituitary tumour after prolactinomas (53).

Hypopituitarism

Mass lesions in the hypothalamic-pituitary region may cause partial loss of the anterior pituitary function or total loss, panhypopituitarism (43, 54). With gradual tumour growth and subsequent increase in intrasellar pressure causing hypopituitarism, symptoms are often mild and developing over a long period of time (41, 43). Hormone deficiencies typically occur in a specific order with GH loss, followed by gonadotropins, TSH and ACTH (55). However, in cases of more acute onset of hypopituitarism such as hypophysitis or pituitary apoplexy, the order of hormonal loss may differ. Smaller tumours < 1 cm, microadenomas, are more common and rarely cause hypopituitarism compared to macroadenomas (≥ 1 cm) (43). This is understood by the mechanism in which the mass effect of the tumour compresses portal vessels or gives rise to increased intrasellar pressure which prevents the passage of hypothalamic hormones (41). The clinical presentation of hypopituitarism depends on which hormonal axis is affected and to what extent. Symptoms are often initially vague, and the onset in most cases gradual (43). Tiredness and weakness are general symptoms associated with hypopituitarism (55). More specifically, gonadotropin deficiency can present with menstrual irregularities or amenorrhoea in women and infertility, in men and women, or testosterone deficiency in men (43). ACTH deficient patients may experience weight loss, muscle weakness and low blood pressure (55). In addition, underexposure to cortisol may also cause adrenal crises (43). Patients with growth hormone deficiency often have low energy, central fat distribution and decreased lean body mass (43). Furthermore, symptoms of TSH-deficiency are weight gain, dry skin and loss of body hair as well as constipation (55). Hypopituitarism with affected GH or gonadal axis is most common with reported frequencies of 61-100%(54, 56, 57, 58, 59, 60) and 77% (3), respectively. The third is adrenal insufficiency 17-62% (54, 56, 58, 60, 61, 62, 63, 64) and lastly central hypothyroidism 8-81% (54, 57, 58, 59, 60, 61, 63, 64, 65).

Not only can NFPAs cause hypopituitarism, but the mass effect of the tumour may, in approximately 35% of the cases (3), give rise to elevated serum prolactin levels as dopamine

delivery from the hypothalamus is hindered when the tumour mass compresses the pituitary stalk, thus reducing the inhibition of prolactin production from lactotrophic cells (38).

The insulin tolerance test is the gold standard for diagnosing pituitary ACTH deficiency. However, a low morning cortisol concentration (< 80 nmol/L) is sufficient to diagnose complete ACTH deficiency (43). In patients with intermediate cortisol results (>80 and <300 nmol/L), a stimulation test is needed to clarify the cortisol reserve (43). In these cases and as an alternative to the insulin tolerance test, the less labour-intensive short Synacthen test can be used for detecting ACTH deficiency (66, 67). Although glucocorticoid replacement aims to achieve physiological cortisol exposure in ACTH deficient patients, the current replacement strategies fail to mimic the natural circadian rhythm of cortisol release and there is no reliable biomarker to use as replacement dose guidance (68, 69). Instead, physicians rely on data on endogen cortisol production in healthy individuals and clinical parameters in patients when evaluating the glucocorticoid dose; fatigue, nausea, and weight loss are under-treatment symptoms, whereas over-treatment symptoms such as hypertension, weight gain, and increased fasting glucose are harder to notice (68, 70).

Growth hormone deficiency can be diagnosed if the combination of a pituitary lesion, three other hormone deficiencies and a low serum IGF-1 concentration is present (71). In less apparent cases, dynamic pituitary function tests such as the insulin tolerance test or GHRH-arginine stimulation test are used for diagnosing GHD (43). Before commencement of GH replacement, there are important hormone interactions to consider. Untreated GHD may mask central hypothyroidism and secondary adrenal insufficiency that are revealed once GH replacement is initiated. Furthermore, because of high first-pass hepatic metabolism of oral oestrogen causing GH resistance with less serum IGF-I response, women on such treatment need higher doses of GH (72).

Treatment of NFPA

The treatment options for NFPAs include transsphenoidal surgery, radiotherapy (23), and active monitoring and surveillance. The growth propensity of microadenomas is low (45, 46) and in the absence of neurological symptoms, active monitoring is an often utilised option. Although active monitoring is used, hormonal replacement therapy is given if needed. In contrast, patients with macroadenomas > 15 mm have an increased risk of symptomatic tumour growth (45, 73,

74), and tumour regrowth is a negative prognostic factor associated with increased mortality (75).

Surgical treatment, primarily with a trans-sphenoidal approach, is indicated when visual field defects or neurological deficits caused by the tumour are present. In addition, radiological signs of impending visual loss or in some cases headache may warrant surgical intervention (44). A systematic review and meta-analysis of 43 studies of 6400 patients who underwent transsphenoidal surgery indicated a remission rate of between 42 and 97% (median of 77.9%) and higher remission in microadenoma (23).

In selected cases, when there is extrasellar tumour growth or the tumour is not amenable to trans-sphenoidal surgery, transcranial surgery is an alternative (44). However, residual tumour and recurrence rates are unfortunately not uncommon with reported recurrence rates during five and ten years of follow-up of 24.4% and 51.5%, respectively (76). Traditionally, treatment with adjuvant radiotherapy has been used more frequently but is a last therapeutic intervention because of considerable side effects such as hypopituitarism, visual field defects and increased risk of cerebrovascular events and secondary neoplasms (1, 3, 11, 44), despite potent efficacy regarding progression-free survival (77).

Gaps of knowledge

Hypopituitarism is associated with increased morbidity and mortality (4, 5, 6, 7), but whether it is due to the inherent properties of the underlying tumour, tumour treatment or the hormone deficiency and its treatment is not fully clarified. However, higher glucocorticoid replacement doses are associated with a worse cardiometabolic profile, indicating the adverse effects of excess exposure to glucocorticoids over time (18). In addition, previous studies have shown an association between higher daily glucocorticoid doses, ≥25 mg in patients with acromegaly and ≥30 mg in patients with NFPA, and increased mortality (9, 10). However, whether the mortality is increased or not using lower glucocorticoid replacement doses has not been demonstrated.

In patients with NFPA, the risk of developing malignant tumours is sparsely studied and subject to discussion. However, previous studies demonstrate an increased incidence of brain tumours in patients with pituitary adenoma, especially in patients receiving radiation therapy to the pituitary gland (11, 12). Whether the incidence of other cancers is increased is sparsely studied. and there are often significant limitations in small patient materials, short follow-up time,

selection bias, or heterogeneous study populations regarding tumour etiology and treatment (13, 14, 15, 16). However, increased mortality and morbidity in hypopituitary patients due to cancer have been reported, although not associated with GH replacement (78). Furthermore, a recent study in patients receiving GH replacement during childhood showed that the overall mortality was associated with the underlying condition rather than the cumulative dose of GH (79).

Studies analysing the impact of GH replacement on the risk of developing type 2 diabetes mellitus are conflicting (80, 81) and further studies, also including other comorbidities, are needed. Patients with hypopituitarism and untreated growth hormone deficiency have an increased proportion of body fat and central obesity (17). In addition, an unfavourable metabolic profile with insulin resistance, dyslipidaemia, and increased prevalence of hypotension, atherosclerosis, cardiovascular and cerebrovascular mortality is also present in these patients (4, 5, 18). GH impacts the lipid and lipoprotein metabolism through different mechanisms (82), one of which is by upregulating the expression of LDL-receptors in the liver, thus increasing the uptake of LDL-cholesterol from the circulation (83) and thereby reducing an important cardiovascular risk factor. Replacement treatment with GH has shown beneficial effects on the above-mentioned metabolic consequences of GH deficiency (84, 85), but the long-term effects are not clarified. Whether a potentially positive long-term effect of GH replacement on LDL cholesterol translates to a reduced risk of cardiovascular disease remains to be investigated.

Table 1: Overview of studies investigating glucocorticoid replacement.

	Zueger et al. 2012	**Sherlock et al. 2009**	**O'Reilly et al. 2016**	**Hammarstrand et al. 2017**
Study design	Retrospective analysis	Retrospective analysis	Retrospective analysis	Retrospective analysis
Study period	Pituitary function and GC replacement assessed 2011	1990-2006	1999-2014	1987-2014
Subjects (n)	106	501	519	392
Outcomes studied	Overall mortality based on hydrocortisone replacement doses	All-cause mortality after radiotherapy and hypopituitarism and replacement therapy	Clinical presentation, treatment strategies, pituitary function, vitality status and mortality.	Overall mortality depending on HC replacement dose
Conclusion	Daily HC replacement doses ≥30 mg are associated with increased overall mortality in NFPA patients with secondary adrenal insufficiency.	ACTH deficiency is associated with increased mortality in acromegaly. High hydrocortisone doses in ACTH-deficiency, ≥25mg/d are associated with increased mortality.	Daily HC replacement doses ≥30 mg in NFPA patients with secondary adrenal insufficiency are associated with increased mortality.	HC replacement doses of more than 20 mg/day in NFPA patients with secondary adrenal insufficiency are associated with increased mortality.

Abbreviations: GC, glucocorticoid; HC, hydrocortisone; NFPA, non-functioning pituitary adenoma

AIMS

- To investigate the impact of the daily glucocorticoid replacement dose on mortality in patients with hypopituitarism due to NFPA
- To investigate the incidence of malignant tumours in patients NFPA
- To investigate the impact of growth hormone replacement therapy on the incidence of type 2 diabetes mellitus, cerebral infarction, myocardial infarction, sepsis, fractures and malignant tumours in patients with growth hormone deficiency due to NFPA
- To compare changes in LDL cholesterol concentration in hypopituitary patients receiving growth hormone replacement with changes seen in a random population sample during the same time period

PATIENTS AND METHODS

A unique personal identification number is assigned to all permanent residents of Sweden which allows for identification in registers used by all healthcare providers in Sweden.

Paper I

This was a retrospective cohort study where patients with NFPA followed between 1997 and 2011 were included and the start of the study was January 1, 1987. The patients were derived from the uptake area of Sahlgrenska University Hospital, mainly the western region of Sweden. Medical charts were reviewed in order to collect data and confirm correct NFPA diagnosis. The tests for secondary adrenal insufficiency were performed according to local clinical practice using a Synacthten test, and if inconclusive, an insulin tolerance test was performed. NFPA patients diagnosed with secondary adrenal insufficiency and receiving glucocorticoid replacement were compared against NFPA patients with intact HPA-axis concerning the outcome, mortality. For patients receiving glucocorticoid replacement other than hydrocortisone, a hydrocortisone equivalent dose was calculated (86, 87). In order to evaluate the impact of glucocorticoid dosage on mortality, subgrouping depending on their daily and weight-based hydrocortisone equivalent dose was performed.

Paper II

This study was conducted as a national register study. Patients with an NFPA diagnosed at between 1997 and 2011 were identified through the Swedish National Patient Register and included in the study. The diagnosis was documented at internal medicine, endocrine, neurological or neurosurgical unit and patient time at risk began when the NFPA was detected, dating back to 1987. The incidence of malignant tumours was studied and data was collected from the Cancer Registry (88). Patients were followed concerning the outcome, malignant tumours until either death occurred or the study's end (December 31, 2014). Reference data on the incidence of malignant tumours in the general population was gathered in the same manner.

Paper III

This was an observational cohort study where patients with NFPA within the western region of Sweden were identified in the same manner as per paper 2, utilizing the National Patient

Register. Patients diagnosed with growth hormone deficiency by standards contributed by the Growth Hormone Research Society were offered treatment with growth hormone replacement. Further divided into two groups depending on whether diagnosed with GHD or not. The primary endpoint, morbidity, was studied using data from patient charts, the National Patient Register and the Swedish Cancer Register.

Paper IV

This was conducted as a longitudinal, case-control study with information gathered prospectively. Patients with hypopituitarism within the western region of Sweden and verified growth hormone deficiency, of whom all had received growth hormone replacement therapy, was identified through ”the Gothenburg Study.” Then, data concerning the primary outcome, changes in LDL cholesterol, from growth hormone substituted patients was compared with that of the cohort of subjects from the WHO MONICA 1995, Gothenburg. The latter has been studied twice with ten years apart and the majority of GH substituted patients were followed over the same time period.

Statistical analysis

Descriptive statistical analyses are shown as mean with standard deviation or median with minimum and maximum, depending on the data distribution. For comparison of continuous variables between groups, independent *t* test and Mann-Whitney *U* test was used as appropriate. For dichotomous variables, Chi-square was used for comparison between groups.

Papers I-III

Standardised incidence and mortality ratios were calculated by comparing the observed with the expected number of events between the patient cohort and the Swedish general population. Person-years at risk were calculated from inclusion in the study to either death or the end of the study and stratified according to gender, 5-year age groups, and 1-year calendar periods. When calculating the expected number of events for each stratum, the Swedish population for every calendar year and the 5-year age group were used as reference. The observed number of events was compared to that expected by standardised incidence or mortality ratios. Subgroup analyses of absolute and weight-adjusted hydrocortisone equivalent doses were conducted. In paper I, standardised mortality ratio for subgroups not overlapping was compared (89). In order to adjust

for potential confounders, COX/regression models were used to calculate hazard ratio. This method was also used for internal analyses between patients with with secondary adrenal insufficiency and patients with intact hypothalamic-pituitary-adrenal axis.

Paper IV

Continuous data were summarised using mean, standard deviation, median, minimum and maximum. Categorical data were summarised using numbers and percentages. In addition, for continuous effect variables, when comparing the two independent groups, mean, standard deviation, number and adjusted least square means with standard error of the mean (SEM) for the groups and for the difference between groups (including 95% confidence interval and p-value) were presented and adjusted for age, BMI, Lipid lowering medication and DM Type 2 using analysis of covariance (ANCOVA). Histograms were presented along with nonparametric kernel density estimates.

RESULTS

Paper I

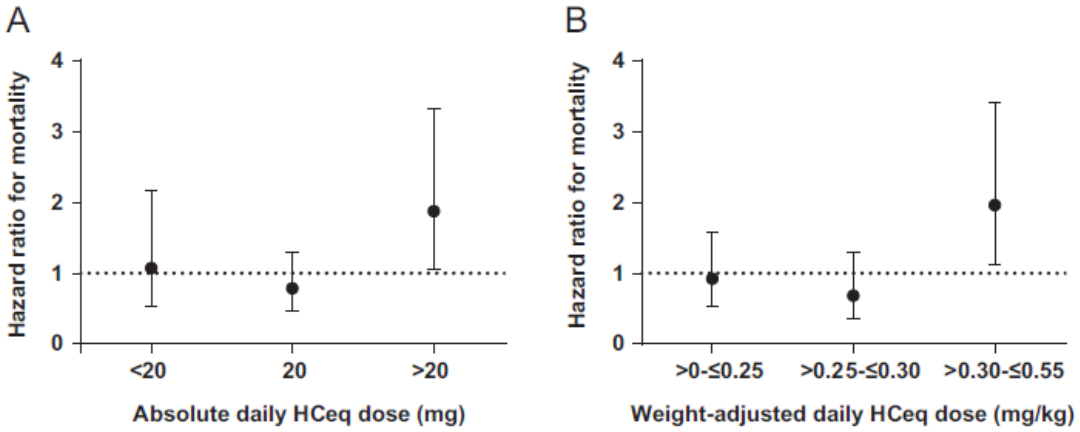
In all, 392 patients were included in the study – patient characteristics shown in table 1. During a mean follow-up time of 13 years, 106 deaths were registered. One hundred ninety-three (49%) patients were diagnosed with secondary adrenal insufficiency. Patients with and without secondary adrenal insufficiency had comparable SMR. When comparing SMR between groups, patients with a hydrocortisone dose of > 20 mg per day or a daily hydrocortisone equivalent dose of > 0.30-0.55 mg/kg had higher SMR than the patient reference group receiving daily hydrocortisone doses of 20 mg and >0.25-0.30 mg/kg respectively. Cox-regression adjusted for age at study start, gender and radiotherapy confirmed this finding (Figure 1).

Table 2: Characteristics of patients with NFPA included in the study. *Republished with permission of Bioscientifica Limited from European Journal of Endocrinology, Higher glucocorticoid replacement doses are associated with increased mortality in patients with pituitary adenoma, Hammarstrand et al. 2017. Copyright Clearance Center.*

	Total ($n=392$)
Gender, *n* (%)	
Men	252 (64)
Women	140 (36)
Age at diagnosis, years, mean ± S.D.	58.7 ± 14.6
Men, mean ± S.D.	58.9 ± 13.9
Women, mean ± S.D.	58.3 ± 15.9
Hypopituitarism, *n* (%)	313 (80)
Diabetes insipidus, *n* (%)	46 (12)
Hormonal replacement, *n* (%)	
- None	75 (19)
- Levothyroxine	272 (69)
- Glucocorticoids[a]	193 (49)
- Sex steroids	186 (47)
- Growth hormone	160 (41)
Mean follow-up time, years (range)	12.7 (0.1–28)
Patient-years at risk in the study	4961
Treatment with surgery, *n* (%)	287 (73)
Treatment with radiotherapy, *n* (%)	78 (20)

Patients treated with glucocorticoids due to non-endocrine chronic disorders were excluded ($n=13$).
[a]Includes replacement with hydrocortisone ($n=186$) and cortisone acetate ($n=7$).

Figure 1: Cox-regression adjusted for age at study start, gender, and radiotherapy. *Reproduced with permission of Bioscientifica Limited from European Journal of Endocrinology, Higher glucocorticoid replacement doses are associated with increased mortality in patients with pituitary adenoma, Hammarstrand et al. 2017. Copyright Clearance Center.*



Paper II

This study included 2795 NFPA-patients from Sweden, resulting in 26,664 patient-years at risk. The overall incidence of malignant tumours in the NFPA population was increased compared to that in the general population, SIR 1.22 (table 3). The overall increased incidence of malignant tumours was consistent when analysed without brain tumours, SIR 1.14. When analysing specific types of cancer, the incidence of malignant tumours of skin, malignant melanoma and neoplasms of the brain was increased, while the incidence of breast cancer was decreased in women.

Table 3: Standardised incidence ratio of all malignant tumours in 2795 patients with NFPA. *Republished with permission of Bioscientifica Limited from Endocrine-related cancer, Incidence of malignant tumours in patients with a non-functioning pituitary adenoma, Olsson et al. 2017. Copyright Clearance Center.*

Outcome	Expected No. of malignancies	Observed No. of malignancies	Standardised incidence ratio (95% CI)	*P* value
All	368	448	1.22 (1.11–1.33)	<0.0001
Men[a]	238	301	1.27 (1.13–1.42)	<0.0001
Women[a]	130	147	1.13 (0.95–1.33)	0.16
Patients with hypopituitarism	238	285	1.20 (1.06–1.34)	0.004
Patients without hypopituitarism	130	163	1.26 (1.07–1.46)	0.006
Patients treated with RT	11	17	1.56 (0.91–2.49)	0.11
Patients treated without RT	351	389	1.11 (1.00–1.22)	0.050
Analysed without malignancies diagnosed within three months after the NFPA diagnosis				
All	360	419	1.16 (1.05–1.28)	0.0027
Men[b]	233	284	1.22 (1.08–1.37)	0.0012
Women[b]	128	135	1.06 (0.89–1.25)	0.54
Analysed without brain tumours				
All	362	414	1.14 (1.03–1.26)	0.008
Men[c]	235	289	1.23 (1.09–1.38)	0.0007
Women[c]	128	125	0.98 (0.81–1.17)	0.85

[a]No significant difference was found between the overall incidence of malignancies for men and women (95% CI of the ratio of SIRs 0.92–1.38; *P*-value 0.27). [b]No significant difference was found between the overall incidence of malignancies diagnosed three months after the NFPA diagnosis for men and women (95% CI of the ratio of SIRs 0.94–1.42; *P*-value 0.18). [c]Men had a significantly higher incidence of malignancies (excluding brain tumours) compared to women (95% CI of the ratio of SIRs 1.01–1.57; *P*-value 0.038).
RT, radiotherapy.

Paper III

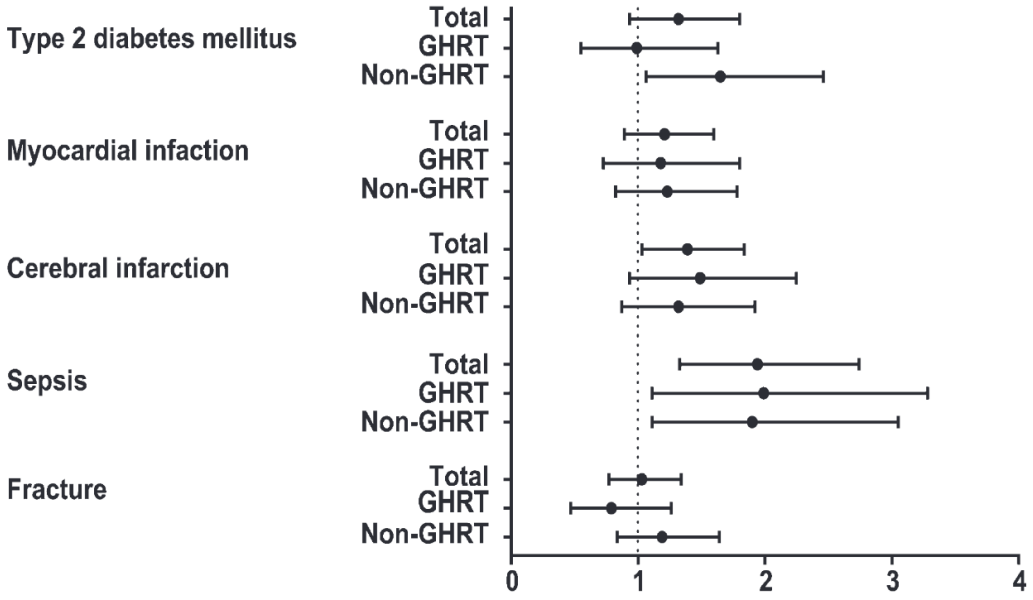
In total, 426 patients were included in the study of which 59% received growth hormone replacement (table 4). The incidence of diabetes mellitus type 2 was not increased for patients with growth hormone replacement compared to the general population, but was increased for patients without. The incidence of cerebral infarction and sepsis was increased compared to the general population, with SIR 1.39 and 1.94, respectively (figure 2). However, comorbidities such as cancer, myocardial infarction, and fractures were not increased in the cohort.

Table 4: Characteristics of 426 patients with NFPA enrolled in the study. *Republished with permission of Bioscientifica Limited from European Journal of Endocrinology, Comorbidities in patients with non-functioning pituitary adenoma: influence of long-term growth hormone replacement, Hammarstrand et al. 2017. Copyright Clearance Center.*

Characteristics	Patients with GHRT (*n*=207)	Patients without GHRT (*n*=219)	*P*
Gender, *n* (%)			0.02
Men	145 (70)	129 (59)	
Woman	62 (30)	90 (41)	
Mean (S.D.) age at start of study (years)	56.3 (11.5)	65.2 (15.0)	<0.001
Median (range) follow-up duration (years)	12.2 (0–24)	8.2 (0–27)	<0.001
Follow-up duration (patient-years)	2608	1991	
Median (range) GHRT duration (years)	11.7 (0–24)	–	
GHRT duration (patient-years)	2474	–	
Radiotherapy, *n* (%)	72 (35)	25 (11)	<0.001
Surgery, *n* (%)[a]	196 (95)	112 (51)	<0.001
Replacement therapy, *n* (%)			
Glucocorticoids	139 (67)	87 (40)	<0.001
L-Thyroxine	195 (94)	111 (51)	<0.001
Sex steroids	153 (74)	81 (37)	<0.001
Mean (S.D.) BMI (kg/m^2)[b]	28.5 (4.5)	26.5 (4.4)	<0.001
Treated for hypertension, *n*/total *n* (%)[c]	117/207 (57)	114/218 (52)	0.44

[a]Surgical route was transsphenoidal in 243 (79%), transcranial in 59 (19%) and unknown in 6 (2%); [b]missing data in 42 (9.9%) cases; [c]missing data in one (<1%) case in the group without GHRT.
GHRT, growth hormone replacement therapy.

Figure 2: Standardised incidence ratio (95% CI) of various comorbidities in NFPA patients. *Reproduced with permission of Bioscientifica Limited from European Journal of Endocrinology, Comorbidities in patients with non-functioning pituitary adenoma: influence of long-term growth hormone replacement, Hammarstrand et al. 2017. Copyright Clearance Center.*



Paper IV

In this longitudinal study, 342 patients were included and patients were followed over a median time period of ten years. The concentration of LDL cholesterol was significantly reduced to a greater extent in patients receiving growth hormone than in controls and was most evident in women who had a mean adjusted difference of -0.598 mmol/L (P<0.0001). In men, the adjusted mean difference in the concentration of LDL cholesterol was -0.275 mmol/L (P=0.0083). In women, we also observed a secular trend in the form of reduced total cholesterol in both patients and controls, more so in patients who had a mean adjusted difference of -0.408 mmol/L (P=0.0001).

DISCUSSION

This thesis explores important variables regarding morbidity and mortality in patients with pituitary adenomas, mainly NFPAs. Since patients are most often diagnosed in their fifties (61, 90, 91, 92, 93) and often receive life-long hormonal replacement treatment, it is essential to analyse the long-term effects of such treatment. To this end, we have conducted both cohort studies where patients were included in an unselective manner and followed prospectively (*Papers I*, *III*, and *IV*), and a national register-based study (paper II). This thesis has demonstrated:

1. Higher treatment doses of GC in patients with secondary adrenal insufficiency due to NFPA is associated with increased mortality

2. An overall increased incidence of malignant tumours in patients with NFPA

3. The incidence of cancer and diabetes mellitus type 2 in GH-replaced patients was not increased

4. GH deficient patients receiving long-term GH replacement had a greater LDL-C reduction compared to the background population

Glucocorticoid replacement and mortality

Paper I comprised 392 patients with NFPA, half of which had secondary adrenal insufficiency and received GC replacement. The study shows that higher daily glucocorticoid replacement doses are associated with increased mortality compared to patients with NFPA with intact hypothalamic-pituitary-adrenal axis and the general population. We analysed absolute hydrocortisone equivalent (HCeq) doses and weight-based HCeq doses with overall consistent results. The adverse effects of high and non-physiological GC replacement include higher BMI, total cholesterol, LDL cholesterol and triglycerides (18). Traditionally higher doses of glucocorticoid replacement (94, 95) reduce increasingly, based on the discovery that the endogenous cortisol production is significantly lower than the treatment doses for patients with secondary adrenal insufficiency (96, 97). Zueger *et al.*, O'Reilly *et al.*, and Sherlock *et al*. have reported an association between higher daily hydrocortisone (HC) dose (≥30 mg, ≥30 mg, and ≥25 mg, respectively) and increased mortality (HR 4.00, RR 3.79 and SMR 2.82, respectively) for patients with NFPA and acromegaly (9, 10, 98). However, limitations in previous studies

include a small study size (10), a short follow-up period (98), and an underlying condition associated with increased mortality (9, 99). Our results align with these studies and further emphasise that even lower maintenance doses would be advisable in that HCeq doses already over 20 mg or 30 mg/kg per day led to increased mortality.

When analysing the specific causes of death, the number of events for each category in our study was too small to reach potential statistical significance. However, predominantly found in previous studies are cardiovascular and respiratory causes of death (4, 5, 43). In patients with hypopituitarism, it is difficult to accurately study each hormonal deficit and its potential impact on different outcomes, including mortality, when also considering the accompanying replacement treatments. GH replacement is associated with a reduction in the availability of administered hydrocortisone by reducing the activity of the enzyme 11β-hydroxysteroid dehydrogenase, which facilitates the conversion of cortisone to cortisol (100, 101). Cushing's syndrome, which to a mild degree can be induced by excessive glucocorticoid replacement, and GHD are associated with excess cardiovascular mortality (4, 5, 102) Indeed, both conditions share clinical features including altered body composition with visceral obesity and decreased lean body mass, dyslipidaemia, hypertension, and insulin resistance (17, 103, 104, 105, 106, 107), a group of cardiovascular risk factors (108, 109).

Likely influencing the outcome is also the degree of adrenal insufficiency, in that, partially insufficient patients may be over-treated under normal circumstances, hence exposed to increased cardiovascular risk over time. On the other hand, patients with complete adrenal insufficiency do not respond adequately with increased cortisol production during acute stress or infections. As a result, they may, in these situations, be under-treated, thus running an increased risk of death (110). A discrepancy between increased cortisol need, as during illness, and cortisol availability can result in adrenal crisis, which is a life-threatening condition if not treated promptly. In addition to the hydrocortisone maintenance dose, patients with secondary adrenal insufficiency are therefore also equipped with rescue treatment to take during acute stress or illness (43). However, infections as a cause of death are still overrepresented in patients with secondary adrenal insufficiency with adrenal crisis likely contributing in half of the cases (110).

NFPA and cancer

In *Paper II*, a register-based study with national coverage, we showed an increased incidence of malignant neoplasms of the skin and brain tumours in patients with NFPA. In contrast, the incidence of breast cancer was decreased. Previous studies investigating the incidence of cancer in patients with NFPA are scarce and conflicting (13, 14, 15, 16, 111, 112, 113). The main focus regarding pituitary adenomas and cancer has been towards patients with acromegaly, where between 15 and 25% of deaths are related to cancer (27, 114). As a mitogen, the concern is that GH and IGF-1 may cause *de novo* neoplasia, in particularly colorectal cancer (114). Moreover, an increased risk of prostate, colorectal and breast cancer in healthy individuals with serum IGF-1 levels in the upper normal range vs. lower normal range have been demonstrated (115).

The incidence of neither colorectal cancer nor hormone-dependent cancers such as prostate or breast cancer was increased. On the contrary, women with NFPA had reduced incidence of breast cancer, especially hypopituitary women, although not statistically significant. With the current study design, we could not assess the potential effect of hypopituitarism and replacement therapy on the risk of developing cancer. Hence, the following reasoning is mere speculation. With this in mind, our results encourages speculation about the possibly protecting effect of low exposure to sex steroids in developing breast cancer. Historically, hypophysectomy, was used as a treatment in selected cases of breast cancer under the hypothesis that eliminated GH production had a beneficial effect on cancer (116). Our results are consistent with data reported from large surveillance databases (16). Furthermore, we found an increased incidence of malignant neoplasms of the skin, which may in part, be explained by increased surveillance. Interestingly, hypopituitary patients were overrepresented indicating a hormonal impact possibly explained by adrenocorticotropin deficiency and thereby melanocorticotropin deficiency. Accordingly, these patients have paler skin and are more susceptible to damage from UV radiation.

Of interest was the near 6-fold increased incidence of brain tumours, consistent with some (11, 12, 113, 117, 118), but not all studies (13, 119). Pituitary radiation therapy may have influenced this finding, but somewhat surprisingly, none of the patients who developed a second brain tumour had received radiation therapy. However, only five percent of the patients received pituitary radiation therapy and many earlier studies had larger populations with radiation therapy and more extended follow-up periods. The most common subtype of brain tumours in general

is meningioma (120). The 10-fold discrepancy between prevalence in populations reported (120) and imaging studies (52) indicates that incidental detection during surveillance may contribute to this finding. Our study shows that the overall incidence of malignant tumours in patients diagnosed with NFPA is increased, but not due to classically hormone-related cancer subtypes.

Growth hormone replacement and comorbidities

The main purpose of *Paper III*, comprising 426 patients, was to explore the incidence of comorbidities in NFPA patients with and without GHRT. Treated patients had received GHRT during a median period of 12 years. The incidence of cerebral infarction was increased in the cohort but not related to whether patients received GHRT or not. However, patients who had received pituitary radiation had twice the risk compared to the general population, although the number of events in the cohort was not sufficient to establish a potential difference between radiated and non-radiated patients. Results regarding the potential effect of radiation therapy on stroke are not consistent (4, 121, 122) and the subject remains to be fully clarified.

Previous studies have demonstrated increased mortality in hypopituitary patients (4, 5, 8, 123). Rosén *et al.* proposed untreated GHD as a contributing factor to these patients' adverse cardiovascular risk profile (4). More specifically, some of the negative consequences of GHD constitute central adiposity, dyslipidaemia, hypertension, reduced physical activity, increased intima-media thickness, and intimal plaques, as well as reduced arterial distensibility (104, 105, 124, 125, 126, 127). Although not assessed individually in our study, some of these risk factors may explain the increased incidence of cerebral infarction in the cohort. Interestingly, and somewhat surprisingly, the incidence of type 2 diabetes mellitus was increased for patients without GHRT by 65% compared to the general population. In contrast, patients with GHRT had a normal incidence. Safety questions have been raised concerning GHRT in relation to the reduction in insulin sensitivity that has been reported (128). A temporary decline has been noted up to a year after the commencement of GHRT (84). This is in line with an extensive study by Luger *et al.* reporting an increased incidence of type 2 diabetes mellitus presenting shortly after the commencement of GHRT (80). In contrast, others have shown no such association (81). Partly explaining the insulin resistance seen in adults with GHD is likely the increased abdominal fat which is positively affected by GHRT (84, 85). Upon subanalysis of GC replacement and severe infections, showing increased incidence of sepsis only in patients with

secondary adrenal insufficiency, a possible explanation may be inadequate GC replacement during infections and acute stress which is in line with a previous study (110).

As mentioned above, under *Paper II,* there is an ongoing discussion regarding GHRT and cancer development. The frequency of malignant tumours was not increased in the cohort, and in summary, this leads us to believe that long-term GHRT in patients with NFPA is safe concerning comorbidities.

Influence of growth hormone replacement on lipid profile

In *Paper IV*, a cohort-based case-control study, we investigated the changes in serum LDL-C in hypopituitary patients receiving long-term GHRT compared to changes seen in a random population sample. Patients with GHD and GHRT had a greater LDL-C reduction than changes seen in the control population with a median follow-up time of ten years. In addition, hypopituitary women had a more considerable lipid profile improvement than men.

Untreated GHD is associated with numerous cardiovascular risk factors, and atherosclerotic cardiovascular disease is the leading cause of death worldwide (17). Indeed, cardiovascular mortality is increased in hypopituitary patients with untreated GHD (4, 5). GH influences lipid metabolism via various mechanisms (82), including stimulation of lipolysis and increasing synthesis and secretion of VLDL (129). Furthermore, GH levels decrease after puberty with increasing age (130), whereas the inverse relationship applies to LDL-C (131). The hypothesis that GHD contributes to increased LDL-levels has been studied in both rat models and clinical studies and shows direct actions of GH on hepatic LDL receptor expression (83).

Several epidemiologic and genetic studies have established the importance of LDL-C in the pathogenesis of the cardiovascular disease (132, 133, 134). Silverman *et al.* displayed a 23% relative risk reduction for major cardiovascular events per 1 mmol/L in LDL-C reduction (135). Therefore, we believe that our results showing a reduction of 0.6 mmol/L is clinically significant in women with hypopituitarism who, compared to men, have worse cardiovascular mortality (8).

Strengths and limitations – methodological considerations

One of the significant strengths throughout *Paper I* and *III* are the unselected cohorts of patients with NFPA. Likewise, in *Paper II*, the inclusion of patients was solely based on NFPA diagnosis. Moreover, in cohort studies *I* and *III*, patients were derived from the same geographical area. Thus, likely reducing the effect of socio-economic confounding. It can be challenging to assess the effect of a single hormonal replacement treatment on the outcome when, in addition, accounting for numerous hormonal imbalances and their respective treatments. Therefore, tumours such as NFPA are an attractive model to study since hormonal hypersecretion is not present and thus not a confounding factor. Furthermore, all studies' follow-up periods and study sizes generally allow sufficient statistical power when analysing the primary outcome variables, including analyses of cancer subtypes in *Paper III*. In *Paper I*, patients were compared to the general population and against patients with the same pituitary disease with consistent results. One of the most interesting aspects of GH replacement is the beneficial effects on cardiovascular risk factors which have been demonstrated in controlled studies (136, 137). However, *Paper IV* is to our knowledge the first case-controlled study to investigate the secular trends of LDL-C with regard to GH replacement using a control group of randomly selected men and women followed over the same time period. In addition, blood analysis for patients and controls was performed in the same laboratory using the same methodology.

The disadvantages of studies with a retrospective design also apply to studies in this dissertation. For example, when records not designed for the study are used, the data quality may be inferior, and information on potential confounding factors may be lacking. In *Paper II*, information on the degree of hypopituitarism and hormonal replacement thereof would have supplied further understanding regarding the effect of hormonal status on the risk of cancer development. Likewise, the retrospective and non-randomised design of cohort studies *I* and *III* increases the risk of selection bias. In fact, patients with GH replacement were predominantly men, and patients without GH replacement were older and had less severe hypopituitarism, which may have influenced the decision of initiating treatment. However, when calculating SIR, age, gender, and calendar year are taken into account. Moreover, adherence to the replacement treatment is of interest when assessing the outcome. However, in *Paper I*, the cumulative exposure to glucocorticoids could not be assessed, nor was the adherence to GH replacement in *Paper III* objectively measured, although discussed during visits at specially dedicated

outpatient clinics. Furthermore, the results showing a positive effect of GH replacement are not likely exaggerated since these patients had a worse metabolic profile than the non-treated patients. Contrarily, the opposite can be argued.

A placebo-controlled, randomised study would be optimal in studying the secular trends of LDL-C concerning GH replacement. Unfortunately, we believe that such a study investigating the effects of GH replacement on lipid metabolism is unlikely to be performed due to ethical reasons. Hence, it is not easy to distinguish between the sole effect of GH on the lipid profile from the effect of other potential causal factors such as statin treatment. Many physiological factors influence the GH secretion such as age, gender, abdominal visceral fat, nutrition and exercise along with hormones such as steroid and thyroid hormones (101, 138, 139, 140, 141). The independent effect of each variable on GH secretion is difficult to determine because of complex interrelationships between the regulating factors of GH (141). In unreplaced GH deficiency, free T4 may be falsely high, thus potentially masking a partial central hypothyroidism (101). Replacement treatment with GH may not only reveal a central hypothyroidism but may also cause a relative adrenal insufficiency in patients with non-replaced partial adrenal insufficiency, by affecting the prereceptor metabolism of cortisol (101). Also, the lipid profile in patients with central hypothyroidism is affected by the adequacy of the thyroid hormone replacement. For example, insufficient doses of levothyroxine in patients with central hypothyroidism reduce the HDL-cholesterol concentration (142). In *Paper IV*, it can be speculated that the larger changes in the lipids seen in women than in men are to some degree explained by a difference in the adequacy of levothyroxine replacement since there was a larger difference between TSH and free T4 in women. Although not randomised, *Paper IV* can be seen as a controlled, follow-up study in a real-world clinical setting.

CONCLUSION

- GC replacement doses of more than 20 mg HC per day, in patients with secondary adrenal insufficiency due to an NFPA, are associated with excess mortality and patients with doses of 20 mg or less had a mortality risk similar to patients without GC replacement, and to the general population
- An increased overall risk of malignant tumours in patients with NFPA
- Long-term GH replacement in patients with NFPA could be considered safe with regard to the following comorbidities: diabetes mellitus type 2, cerebral infarction, sepsis and cancer
- Long-term GH replacement decreased the LDL-C concentration in patients with GHD above the secular trends seen in the general population

FUTURE PERSPECTIVES

This dissertation focused on hormonal replacement treatment in patients with NFPA, mainly concerning morbidity, mortality, and cardiovascular risk factors. Albeit many questions remain to be answered, this thesis has shed light on some of the unanswered questions, particularly concerning the effects of growth hormone and glucocorticoid replacement. The included studies are of different designs and are, in this matter, not comprehensive. Placebo-controlled, randomised trials would provide valuable evidence of causal relationships but can, on the other hand, be challenging to conduct due to factors such as the low prevalence of the disease, ethics, and funding. The primary clinical implications of this dissertation concern the importance of glucocorticoid dose on long-term outcomes and the beneficial effects of growth hormone replacement, along with safety questions surrounding the treatment. However, many topics warrant further investigation, such as the influence of the concentration-time profile for glucocorticoid replacement on outcomes and long-term studies of growth hormone replacement. Furthermore, in light of the favorable effects of GH replacement on LDL-C concentration, it would be interesting to investigate the effects on apolipoprotein B, which is a stronger predictor of cardiovascular disease (143). The observational studies of this dissertation can be regarded as forerunners of and encourage prospective studies.

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