

Thesis for the degree of Doctor of Philosophy (Medicine)

Aspects of diagnosis and treatment of hypopituitarism in adult life

by

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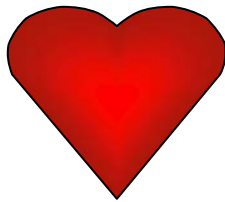
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The picture on the cover is from Fiesole, Tuscany, Italy
The poetry is from the book Three Fates by Nora Roberts, 2002

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In memory of my father



Livets gobeläng vävs med
rosenröda trådar av kärlek,
passionens djupröda nyanser,
förståelsen och förnöjsamhetens
lugna blå toner,
och humorns klart lysande silver

The tapestry of life is woven
with threads red as roses by love
the deep purple shades of passion
the compassion and contentedness'
calm blue tones
and the humour's clearly shining
silver

To my beloved

S S S

Abstract

Filipsson, H. 2009. Aspects of diagnosis and treatment of hypopituitarism in adult life, Department of Internal Medicine, Sahlgrenska Academy at University of Gothenburg, Sweden. ISBN 978-91-628-7694-4

Management of adult patients with hypopituitarism can improve with better characterisation of idiopathic pituitary insufficiency (IPI) and clearer diagnosis of central hypothyroidism (CH). Moreover, optimised treatment strategies for glucocorticoid (GC) replacement therapy and of long-term growth hormone (GH) in GH deficiency (GHD) are needed.

This thesis contains four studies addressing these issues. By evaluating patients with IPI, mutations generating hypopituitarism were identified in an unselected adult IPI population. A new allele constellation in a compound *PROP1* mutation was revealed in two siblings, with a phenotype of very late onset ACTH-insufficiency. Those cases were only detected in patients with documented childhood onset disease. A pilot study investigated the response of the thyroid gland after stimulation with 0.9 mg recombinant human thyreotropin (rhTSH) in patients with newly diagnosed CH and healthy controls. The untreated CH patients had lower free thyroxine response than controls. A database study containing 2424 hypopituitary patients, divided into ACTH-insufficient and ACTH-sufficient (AS) patients, demonstrated a clear GC dose-response relation with metabolic outcome. Patients with hydrocortisone equivalent doses of <20 mg/day had a similar metabolic profile as AS patients. In a large study on GHD patients on long-term GH treatment quality of life (QoL), body composition, and metabolic outcome were evaluated during 4-month-GH-discontinuation in a double blind, placebo controlled design. QoL deteriorated, body composition moved towards a GHD state and metabolic parameters were impaired during placebo treatment.

These studies infer that genetic hypopituitarism should be searched for in IPI cases, especially in childhood onset disease and where there is a family history. The diagnosis of CH can be improved by an rhTSH test. In many cases, doses of GC can be reduced in ACTH-insufficient patients in order to improve their metabolic outcome and continuous long-term GH replacement is needed to maintain beneficial effects on QoL, body composition, and metabolism.

Keywords: hypopituitarism, pituitary, diagnosis, treatment, GHD, central hypothyroidism, genetic, idiopathic, ACTH insufficiency, discontinuation

Abstract

Filipsson, H. 2009. Aspekter på diagnostic och behandling av hypofyssvikt i vuxenlivet, Institutionen för Internmedicin, Sahlgrenska Akademien, Göteborgs universitet, Sverige. ISBN 978-91-628-7694-4

Handhavandet av vuxna patienter med hypofyssvikt kan förbättras av en bättre karakterisering av idiopatisk hypofyssvikt (IH) och klarare diagnostik av central hypothyreos (CH). Dessutom, behövs optimerade behandlingsstrategier för glukokortikoidersättningsbehandling och av långtidsbehandling med tillväxthormon (GH) vid tillväxthormonbrist (GHD).

Denna avhandling består av fyra studier som berör dessa områden. Genom att värdera en oselektad vuxen population av patienter med IH, påvisades mutationer som gav hypofyssvikt. En ny allell-konstellation av en sammansatt *PROP1* mutation konstaterades hos två syskon med en fenotyp av sent debuterande ACTH-svikt. Dessa fall påvisades enbart hos patienter med dokumenterad debut av sjukdomen under barndomen. I en pilotstudie undersöktes svaret från sköldkörteln efter stimulering med 0,9 mg rekombinant humant thyreotropin (rhTSH) hos patienter med nyligen påvisad CH och friska kontroller. Dessa obehandlade CH patienter hade lägre fritt thyroxin-svar än kontroller. En databasstudie innehållande 2424 hypofyssviktiga patienter, vilka delades in i ACTH-sviktiga och patienter med bevarad ACTH funktion (AS), visade ett klart dos-respons samband med glukokortikoid (GC) doser och metabolt utfall. Patienter med hydrokortisonekvivalenta doser <20 mg/dag hade en jämförbar metabol profil som patienter med AS. Slutligen, i en stor studie av GHD patienter med lång-tids-GH-behandling värderades livskvalitet (QoL), kroppssammansättning och metabolt utfall under en 4 månaders- period utan GH-behandling i en dubbel-blind placebo-kontrollerad studie design. QoL försämrades, kroppssammansättningen förändrades mot ett GHD-liknande tillstånd och metabola parametrar förvärrades under placebo behandlingen.

Dessa studier innebär att förekomsten av genetisk hypofyssvikt borde undersökas vid fall av IH, särskilt vid debut av sjukdomen i barndomen och där familjär historik förekommer. Diagnostiken av CH kan förbättras med ett rhTSH test. Doser av GC kan, i många fall, minskas hos ACTH-sviktiga patienter i syfte att förbättra deras metabola status och kontinuerlig långtids-GH behandling behövs för att bebehålla de fördelaktiga effekterna på QoL, kroppssammansättning och metabolism.

Manuscripts included in the thesis

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

- I. Detection of genetic hypopituitarism in an adult population of idiopathic pituitary insufficiency patients with growth hormone deficiency
Filipsson H, Savaneau A, Barbosa E L J, Barlier A, Enjalber A, Glad C, Palming J, Johannsson G, Thierry B
Manuscript
- II. Exploring the use of recombinant human thyrotropin in the diagnosis of central hypothyroidism
Filipsson H, Nyström E, Johannsson G
European Journal of Endocrinology 2008 Aug;159(2):153-60
- III. The impact of glucocorticoid replacement regimens on metabolic outcome and comorbidity in hypopituitary patients
Filipsson H, Monson JP, Koltowska-Häggström M, Mattsson A, Johannsson G
Journal of Clinical Endocrinology and Metabolism 2006 Oct;91(10):3954-61
- IV. Discontinuation of long-term GH replacement therapy – a randomised, placebo controlled trial in adult GH deficiency
Filipsson H, Barbosa E L J, Nilsson AG, Norrman L, Ragnarsson O, Johannsson G
Manuscript

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IA Summary in English

This thesis focused on areas within diagnosis and treatment of pituitary insufficiency where improvements are needed. Approx 10% of all patients diagnosed with hypopituitarism has no clear aetiology, which is called idiopathic pituitary insufficiency (IPI). Within this group, a subgroup has been identified, in which hypopituitarism is explained by genetic causes. Usually, genetic hypopituitarism appears in childhood because of short stature, absence of puberty or occurrence of ACTH-insufficiency. An adult growth hormone deficient (GHD) population of IPI was investigated to determine genetic causes of the disease. Each patient's clinical characteristics decided, from previous experience, the genetic test performed. This observational study consisted of all IPI patients identified in the files of 373 hypopituitary patients. Of the 50 identified cases, it was possible to ask 39 patients to participate and 25 of those were selected for further genetic analyses, as they did not have isolated GHD or diabetes insipidus, which reduced the probability of a genetic cause, unless there were family cases or septico-optic-dysplasia. A compound heterozygous *PROP1* mutation, not previously reported, was detected in a sibling pair, whose hormonal insufficiencies had presented during childhood. A unique clinical feature was the very late onset of adrenal insufficiency. No mutations were detected in the sporadic cases.

Central hypothyroidism (CH) can be difficult to diagnose, as guidance from TSH is lacking. Therefore, the response of the thyroid gland to recombinant human thyrotropin (rhTSH) was evaluated in a pilot study with an open randomised controlled design. Hypopituitary patients and healthy controls were stimulated with two different doses of rhTSH intramuscularly, with one week in between. Patients with treated CH had a lower response in peripheral thyroid hormone levels to rhTSH than controls. Patients with untreated CH responded better, but lower than controls. Lastly, hypopituitary patients with preserved TSH secretion and controls had a similar response to rhTSH. However, all individuals had comparable increase in thyroglobulin after stimulation, which implied different mechanisms for thyroglobulin formation and thyroxine production in CH.

To evaluate whether type (hydrocortison (HC), cortisone acetate, prednisolone, dexamethasone) or dose levels (<20 mg HC equivalent dose (eq), 20-30 mg HCeq or >30 mg HCeq) of glucocorticoid (GC) replacement affected the metabolic outcome in hypopituitary patients, 2424 GHD patients in the KIMS database were studied. This open, observational, non-interventional study evaluated patients with ACTH insufficiency compared to ACTH sufficient (AS) patients before and after one year of GH treatment of IGF-I, lipids, glucose metabolism, anthropometry and morbidity. HC appeared marginally worse than to cortisone acetate. Patients with HCeq doses <20 mg/day were similar metabolically to AS patients, whereas HCeq doses >20 mg/day had inferior metabolic outcome. These metabolic disturbances sustained after GH replacement.

Whether the effects of GH sustain after GH discontinuation, as some studies suggest, or deteriorate were investigated in patients on long-term GH treatment. This randomised, double blind, placebo controlled study included 60 adult GHD patients, retrieved from a cohort of 180 eligible patients, who were treated with GH for >3 years (mean 10 years). After a 3-month-run-in period, patients were randomised to GH/placebo for two 4-month periods: and anthropometry, lipids, insulin sensitivity, muscle power, quality of life (QoL) and physical activity were evaluated at randomisation, cross-over and end of study. In addition, body composition (computer tomography, dual X-ray absorptiometry and bioelectric impedance) was measured and the change in fat mass, muscle mass and water were calculated. Patients deteriorated in QoL during placebo and a clear movement towards GHD state was observed during discontinuation, with an increase in waist circumference, cholesterol, LDL-cholesterol, CRP and body fat, and a decrease in extra cellular water and muscle volume. However, insulin sensitivity improved during placebo. This study highlighted that continuous GH treatment is needed for all GHD patients.

IB Summary in Swedish/ Sammanfattning på svenska

Hypofysen är en centimeterstor körtel belägen under hjärnan. Den styr flera av de hormonproducerande körtlarna: sköldkörteln i dess bildning av ämnesomsättningshormon, binjuren i dess bildning av kortisol, mjölkproduktionen via hormonet prolaktin och produktionen av könshormoner från testiklar och äggstockar. Dessutom gör hypofysen tillväxthormon (GH), styr vattenbalansen och förlossningsarbetet (Figur 1). När hypofysens hormonproduktion skadas uppstår en brist på hormoner som hypofyssvikt. Symptomen vid hypofyssvikt varierar beroende på vilket hormon som faller bort. Dessutom kan hypofyssviktiga patienter få synpåverkan eller huvudvärk pga förstoring av hypofysen. Detta ses ibland vid hypofystumör - en godartad knuta i hypofysen, vilket är den vanligaste orsaken till hypofyssvikt.

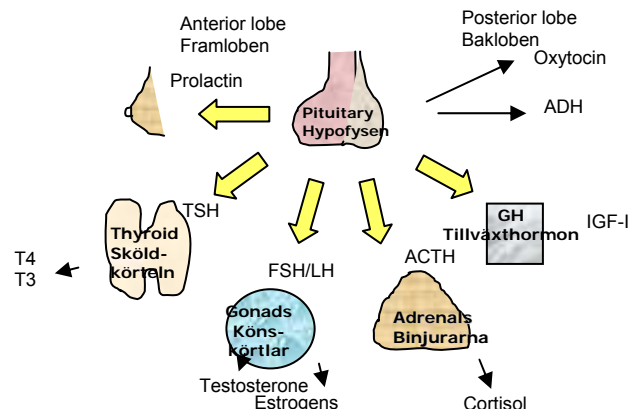


Figure 1 The hormonal system of the pituitary
Hormonellsystem från hypofysen

Hos ca 10% av hypofyssviktiga patienter kan inte någon bakomliggande orsak till den bristande hormonproduktionen identifieras, sk idiopatisk hypofyssvikt (IH). En del av dessa fall kan bero på en kraftig hjärnskakning, men på senare år har även genetiska orsaker, som kan påverka hormonproduktion, påvisats inom gruppen av oförklarad hypofyssvikt. Därför undersöktes 25 IH patienter, som sorterats fram från 373 hypofyssviktiga patienter, med lämpliga mutationsanalyser, beroende på röntgenfynd och kliniska symptom hos den enskilda patienten. Två fall av mutation påvisades hos ett syskonpar, en sk *PROP1* mutation. Sammansättning av mutationen hade tidigare inte rapporterats och i den kliniska bilden förekom en mycket senare debuterande kortisolbrist än vad som vanligen förekommer vid *PROP1* mutationer.

Diagnostiken av bortfallen produktion av sköldkörtelhormon (TSH) från hypofysen kan vara svår. I en pilotstudie kunde det bekräftas att patienter med TSH-brist har ett lägre svar från sköldkörteln av sköldkörtelhormon jämfört med friska kontroller. Detta test kan därför vara en hjälp i diagnostiken av TSH-brist.

Drygt 2000 patienter ur en stor internationell databas, KIMS, studerades före och efter ett års GH-behandling för att värdera effekten av olika sorter och doser av kortisonersättningsbehandlingar hos patienter med brist på på kortisol och GH. Patienter med kortisoldoser <20 mg/dygn uppvisade inte någon avvikelse i midjemått och blodfetter jämfört med patienter utan kortisolbrist. Patienter med högre dygnsdoser däremot, hade en sämre metabol profil. Detta innebär att vi, i många fall, skall behandla med lägre kortisoldoser än vad som idag ges vid hypofyssjukdom.

GH-effekterna kommer långsamt och har i en del studier visat sig kunna bestå även efter utsättande. Genom att sätta ut GH i 4 månader hos 58 patienter, som i genomsnitt använt tillväxthormon i 10 år, visades att livskvaliteten försämrades samtidigt som midjemåttet, kolesterol och fettmassan ökade jämfört med perioden då patienten hade GH. Detta är ett starkt argument för fortsatt GH-behandling till patienter med GH-brist.

II Abbreviations

A	adenine	IGF-I	insulin growth factor 1
AA	amino acid	IGF-II	insulin growth factor 2
Ab	antibodies	IGFBP	IGF binding proteins
ACTH	adrenocorticotrophic hormone	IGHD	isolated GHD
ADH	anti diuretic hormone	IPI	idiopathic pituitary insufficiency
AE	adverse event	IS	insulin sensitivity
ALST	appendicular LST	ITT	insulin tolerance test
AT	adipose tissue	LBM	lean body mass
BCM	body cell mass	LDL-C	low-density lipoprotein cholesterol
BF	body fat	LH	luteinizing hormone
BIA	bioelectric impedance analysis	LST	lean soft tissue
BMC	bone mineral content	L-T4	levo-thyroxine
BMD	bone mineral density	MRI	magnetic resonance
BMI	body mass index	MT	muscle tissue area
BP	blood pressure	N	Newton
bTSH	bovine TSH	NICE	National Institute for Clinical Excellence
C	cytosine	NFPA	non-functioning pituitary adenoma
CA	cortisone acetate	NHP	Nottingham Health Profile
CEM	Centre of Endocrinology and Metabolism	NTI	non-thyroidal illness
CH	central hypothyroidism	PCR	polymerase chain reaction
CO	childhood onset	P-glucose	fasting plasma glucose
CPHD	combined pituitary hormone deficiencies	PGWB	Psychological General Well-being
CRP	c-reactive protein	PRL	prolactin
CT	computer tomography	PROP-1	prophet of Pit-1
DBPC	double blind placebo-controlled	PSI	pituitary stalk interruption
DM	diabetes mellitus	QoL	quality of life
DNA	deoxyribonucleic acid	QoL-AGHDA	QoL-Assessment for GHD in Adults
DX	dexamethasone	rhTSH	recombinant human TSH
DXA	dual x-ray absorptiometry	RIA	radioimmunoassay
ECS	extra-cellular solids	rT3	reversed T3
ECW	extra cellular water	SAE	severe AE
11 β HSD	11- β -hydroxysteroid dehydrogenase	SDS	standard deviation score
EPP	ectopic posterior pituitary	SHBG	sexual hormone binding globulin
FFM	fat free mass	SITT	short insulin tolerance test
F-glucose	fasting glucose	SMM	skeletal muscle mass
4-C	four compartment model	SNP	single-nucleotide polymorphism
FSH	follicle stimulating hormone	SOD	septo-optic dysplasia
FT4	free T4	SST	short ACTH stimulation test
FT3	free T3	T	thymine
GalNac	N-acetylgalactosamine sulphate receptors	T4	thyroxine
GC	glucocorticoid	T3	triiodothyronine
GH	growth hormone	TBI	traumatic brain injury
GHD	GH deficiency	TBW	total body water
GHRH	growth hormone releasing hormone	Tg	thyroglobulin
GHRP-6	growth hormone related peptide 6	TRH	thyrotropin releasing hormone
GnRH	gonadotropine releasing hormone	TSH	thyroid stimulating hormone
HbA1c	glycosylated haemoglobin	TT4	total T4
HC	hydrocortisone	TT3	total T3
HCEq	HC equivalent	UK	United Kingdom
HDL-C	high-density lipoprotein cholesterol	VLDR	very low density lipoprotein
HPG	hypothalamus-pituitary gonadal	VNTR	variable number of tandem repeat
HRT	hormonal replacement therapy	WC	waist circumference
HU	Hounsfield units	W/H	waist hip ratio

III Introduction

A. Aetiology of hypopituitarism

1. General remarks

Hypopituitarism acquired in adult life is often a result of pituitary or peripituitary tumours and their treatment (1-3), most frequently represented by the non-functioning pituitary adenoma (NFPA) (4, 5). In a large study (6), the prevalence of hypopituitarism from pituitary tumours was 28/100 000 individuals and the non-tumour origin of hypopituitarism represented approximately 30% of cases. Several authors have listed the spectrum of non-tumour causes of hypopituitarism (3, 6, 7) (Table 1).

Table 1 Aetiology of non-tumour hypopituitarism

Irradiation	Tuberculosis
Surgery	Hemochromatosis (13)
	Histocytosis X
Pituitary apoplexia (8)	Intra-pituitary abscess
Shehaans syndrome (9, 10)	Traumatic brain injury
Empty sella (11, 12)	Subarachnoidal bleeding
Autoimmune hypophysitis	Genetic hypopituitarism
Granulomateus hypophysitis	Idiopathic hypopituitarism
Wegners granulomatosis	
Sarcoidosis	

However, in 10.2-11% of cases, no obvious cause hypopituitarism is detected. This group is defined as idiopathic pituitary insufficiency (IPI) (6, 14) and with the awareness of subarachnoidal hemorrhage (15), post-traumatic hypopituitarism (16, 17) and hypophysitis (18) as potential causes of hypopituitarism, this group has further reduced. Several genetic mutations have been discovered in humans (19) and explain some of the IPI cases. In an unselected adult growth hormone deficient (GHD) population, it is still unclear to what extent genetic hypopituitarism can be detected (Paper I).

2. Genetic hypopituitarism

The mature anterior pituitary consists of five hormone producing cell types, each identified by the hormone produced: somatotrophs (growth hormone (GH)), thyrotrophs (thyroid stimulating hormone (TSH)), corticotrophs (adrenocorticotrophic hormone (ACTH)), gonadotrophs (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), and lactotrophs (prolactin (PRL)). From the posterior lobe, two hormones are secreted: oxytocin and anti diuretic hormone (ADH) (20, 21). Hormonal production of the pituitary depends of a cascade of signalling molecules and transcription factors that guide the cells in the development of the pituitary (19, 21). Different factors are needed for organ commitment, cell proliferation, cell patterning and terminal differentiation (Figure 2).

In humans, alterations of genes encoding several of these factors have been identified as causing hypopituitarism (Table 2); both isolated GHD (IGHD) and combined pituitary hormone deficiencies (CPHD) (19, 21). The most common cause of CPHD

identified is a *PROP1* mutation (22) that is often observed in childhood, causing short stature due to GH- and TSH- deficiencies. Frequently, no spontaneous puberty occurs and an ACTH-deficiency may develop later in life (23, 24). The phenotype is highly variable (19, 22, 25). In *PROP1* mutations, magnetic resonance imaging (MRI) discloses either a hypoplastic anterior pituitary or an intrasellar mass (19, 23, 26). MRI may be useful guidance in other cases of genetic hypopituitarism in confirming septo-optic dysplasia (SOD), a triad of optic nerve hypoplasia, midline brain anomalies (which includes absence of septum pellucidum and/or corpus callosum) and hypopituitarism, in *HESX1* mutations, cerebellar abnormalities in *LHX4*, and ectopic posterior pituitary (EPP) in *HESX1*, *LHX4* and *SOX3* mutations (19). In addition, the appearance of the pituitary stalk (27) provides essential information, as a normal pituitary stalk and a eutopic posterior pituitary gland are always found in the cases of *POU1F1* or *PROP1* mutations. However, in the group of hypopituitary patients with SOD (28-30), EPP (27, 31, 32), stalk interruption (30), and extra-pituitary abnormalities (30) genetic causes are rare.

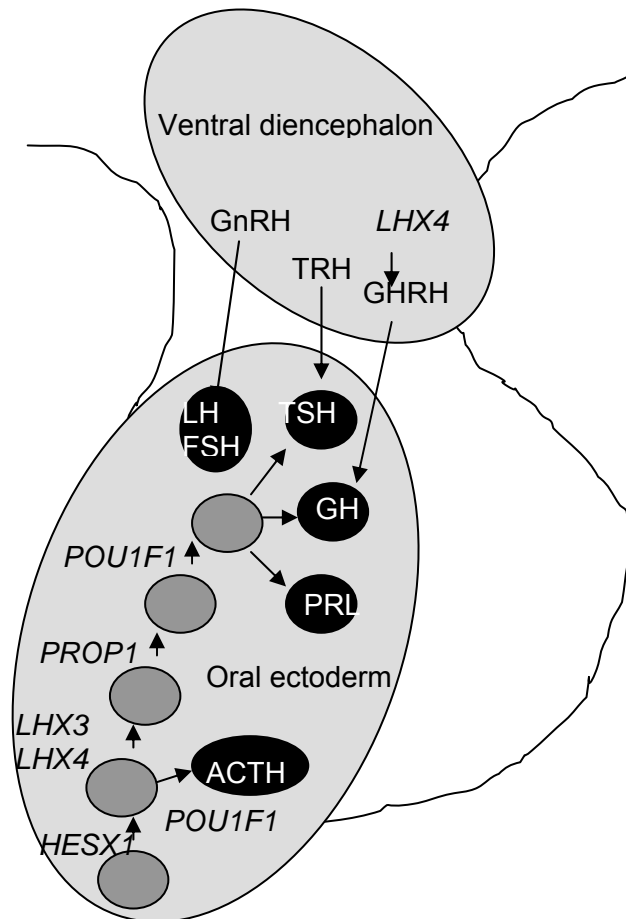


Figure 2 Different transcription factors and signalling molecules interplay in the development of the hormonal producing cells of the pituitary: thyroid stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH), follicle stimulating hormone (FSH), luteinizing hormone (LH), growth hormone (GH), prolactin (PRL). The picture is adopted from Dattani (19)

Genetic hypopituitarism is much more common in familial hypopituitarism than in sporadic cases (26, 28, 30, 32, 33). Childhood onset (CO) of disease is common, but in rare cases the phenotype may be subtle with normal final height (34, 35). In addition, most studies on the occurrence of genetic hypopituitarism originate from a paediatric population (22, 26, 28, 31) or from mixed populations of adults and children (23, 24, 29, 30, 32, 33). That some mutations may have an adult onset (AO) phenotype cannot be excluded. In addition, some adult IPI patients may have CO of hypopituitarism before the area of genetic testing. The aim of Paper I was, therefore, to investigate the existence of genetic mutations in patients with IPI collected from an adult GHD population.

Table 2 Phenotype and Genotype in genetic hypopituitarism. Adopted from Kelberman (21).

Genotype	Phenotype	Inheritance
<i>GH1</i>	IGHD, small or normal AP	R, D
<i>GHRHR</i>	IGHD, small AP	R
<i>HESX1</i>	GHD, APH, EPP	D
<i>POU1F1</i>	GH, TSH, PRL deficiencies, usually severe, small or normal AP	R, D
<i>PROP1</i>	GH, TSH, LH/FSH, PRL deficiencies, evolving ACTH insufficiency, small, normal or enlarged AP	R
<i>HESX1</i>	GH, TSH, LH/FSH, PRL, ACTH deficiencies, APH, EPP	R, D
Syndromes		
<i>HESX1</i>	SOD, APH, EPP, absent infundibulum, ACC	R, D
<i>LHX3</i>	GH, TSH, LH/FSH, PRL deficiencies, short neck, limited rotation, small, normal or enlarged AP, short cervical spine	R
<i>LHX4</i>	GH, TSH, ACTH deficiencies, small AP, EPP, cerebellar abnormalities	D
<i>SOX3</i>	IGHD and mental retardation, panhypopituitarism, APH, infundibular hypoplasia, EPP	X-linked
<i>GLI2</i>	Holoprosencephaly and multiple midline defects	D
<i>PITX2</i>	Riegers syndrome	D
IGHD=isolated growth hormone deficiency AP(H)= anterior pituitary (hypoplasia) R=recessive D=dominant		EPP=ectopic posterior pituitary PRL=prolactin SOD= septo-optic dysplasia ACC= agenesis of corpus callosum

B. Diagnosis of hypopituitarism

The number of studies evaluating proper diagnostic approaches for GH-, FSH/ LH-, TSH- and ACTH-deficiencies is not equally distributed; the majority concerns the diagnosis of GHD. Tests for evaluating ACTH production are also well described, however, there are few studies concerning the diagnosis of TSH-insufficiency with modern laboratory methods.

1. Somatotroph axis (Figure 3)

Where there is an intention to treat, patients should be tested for GHD if they belong to one of three groups: 1 patients with signs and symptoms of hypothalamic-pituitary disease; 2 patients that have received cranial irradiation; and, 3 patients with traumatic brain injury (TBI) or subarachnoid haemorrhage (36). The only exception to performing a stimulatory test for confirming the GHD diagnosis is when the patient has three or more pituitary hormone deficiencies and an insulin-like growth factor I (IGF-I) level below the normal reference range, as the chance of the patient being GHD is >97% (36).

The levels of serum IGF-I is primarily affected by GH and nutritional status, but also age, gender, circadian variation, genetic factors, sub-optimally treated chronic diseases and severe medical conditions influence IGF-I levels (37, 38). However, IGF-I is normal in 50-60% of adult patients with severe GHD (38, 39), and is possibly explained by withheld binding protein concentrations (40). Therefore, IGF-I is not a sensitive test for GHD in adults.

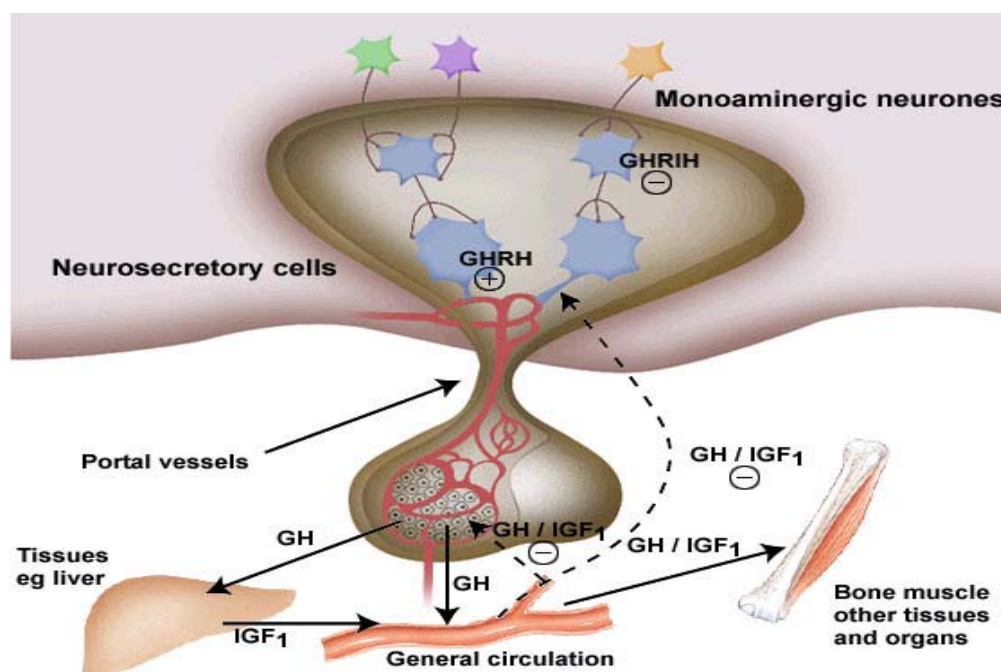


Figure 3 The GH/IGF-I system. The production of GH in the pituitary is regulated by the stimulatory hormone GHRH and the inhibiting hormone somatostatin and by a negative feedback from GH/IGF-I. GH stimulates the production of IGF-I, mainly from the liver, and these two hormones affect most tissues. Picture by courtesy to Pfizer, Inc, Stockholm, Sweden.

The stimulatory test of choice is the insulin tolerance test (ITT) (36, 41-43). However, modifications of the ITT have been suggested in respect to BMI and pre-stimulatory glucose levels (44). In addition, glucose-infusion after hypoglycaemia maintains counter-hormonal responses but reduces the risk of the hypoglycaemia (45). In ITT, hypoglycaemia produces a GH peak that is >5 µg/L in most healthy individuals and <3 µg/L in severe GHD. With ITT, severe GHD can be ruled out from the reduced GH secretion that accompanies normal ageing and obesity (42). However, an insufficient peak may also be a consequence of a recent burst of GH from the somatotrophs, making them refractory for the next stimuli, which infers a second test of GH secretion in uncertain cases. In addition, cut-off values depend on the GH assay used, and therefore, the cut-off may need to be adjusted accordingly (42).

If the ITT is contraindicated, GH stimulation with growth hormone releasing hormone (GHRH) in combination with arginine or the GH secretagogues GH-related peptide 6 (GHRP-6) have proved as reliable as the ITT. However, the cut-off levels for GHD in these tests need to be adjusted for BMI (36, 42, 43). Among other classical provocative tests, the glucagon test is established as diagnostically reliable, whereas the diagnostic reliability of arginine alone has been questioned. The clonidine test is not useful in adults (42). In irradiated patients, damage to the pituitary/hypothalamus system develops progressively, beginning with a hypothalamic impairment. The reliability of ITT is better the first 5 years after irradiation; in cases of negative test results from the combination test with GHRH, ITT should be considered. Thereafter, ITT, GHRH-Arginine and GHRH-GHRP-6 tests are reliable and concordant (42).

2. Gonadotroph axis

The characteristic hormonal pattern in hypogonadotrophic hypogonadism is low peripheral hormone (testosterone or oestrogen) in combination with low normal or normal FSH and LH levels (46, 47). PRL secretion requires evaluation (46, 47) because of its inhibitory effect on gonadotropine releasing hormone (GnRH) secretion; prolactinomas are the most common pituitary tumour, representing 40% of cases (48) and cause a substantial number of hypogonadism cases. In addition, stalk interruption in NFPA slightly increases PRL with secondary effects on GnRH production (49). Therefore, clinical evaluation of the hypothalamus-pituitary gonadal (HPG) axis should include analyses of gonadotrophs, oestrogen or testosterone and PRL (47) although knowledge on the efficacy of current diagnostic tests is limited. Partial androgen deficiency is under discussion especially within the context of the ageing male.

3. Thyrotroph axis

Central hypothyroidism (CH) is a rare cause of hypothyroidism with a prevalence in the general population of 1:80 000-1:120 000 individuals (50). In CH, the bioactivity of TSH (51) is reduced because of inadequate hypothalamic stimulation that causes the pituitary to secrete abnormally glycosylated TSH. TSH in this form has a longer half-life than normal TSH (52), which explains the normal and sometimes slightly elevated levels of TSH seen in CH (53). Hence, TSH production is adjusted for temporal needs.

Thyrotropin releasing hormone (TRH) has a major effect on the posttranslational maturation of the oligosaccharides chain of TSH (54) that plays an important role for TSH's biological properties (51, 52), as the degree of sialylation and sulfonation on the chains determines the clearance of TSH from the circulation. Clearance of TSH by the liver is dependent on the sulfonated residues, as the uptake is regulated by the N-acetylgalactosamine sulphate receptors (GalNac). Pituitary derived TSH, which is predominately sulfonated, and bovine TSH (bTSH), which is solely sulfonated, are cleared predominately by the liver. Recombinant human TSH (rhTSH), which is solely sialylated is excreted by the kidneys and hereby escapes the regulating properties of GalNac (55). The clearance rate is 2-fold lower for rhTSH than pituitary TSH, which results in a 10-times increase in plasma concentration of rhTSH after three hours; however, biological activity appears lower with rhTSH than pituitary TSH (56).

Through its receptor, TSH regulates intracellular metabolism of the thyrocyte (Figure 4) (57-59) in the production of triiodothyronine (T3) and thyroxine (T4). In CH, low T3 and T4 levels are detected; however, in mild hypothyroidism thyroid hormone

levels may be within the lower normal range (60-63). In addition, diagnosing partial CH may be blurred by the 25% intra-individual variation of free T4 (FT4) (60), which also suggests that CH cases are found when thyroid hormone levels are in the lower parts of the reference range. Therefore, a decrease of FT4 >20% in a patient with pituitary disease is indicative of CH (64).

There are, however, some concerns in the diagnosis of CH. Patients with non-thyroidal illness (NTI) may have values that overlap with those of CH. Therefore, analysis should be repeated and evaluated in the light of the clinical situation. A clue to distinguishing these two conditions is the evaluation of T3 (50, 65). Moreover, even though the mechanism is unclear, patients with adrenal insufficiency may present with an elevated TSH and a slightly lower FT4, mimicking CH and mild primary sub-clinical hypothyroidism (66).

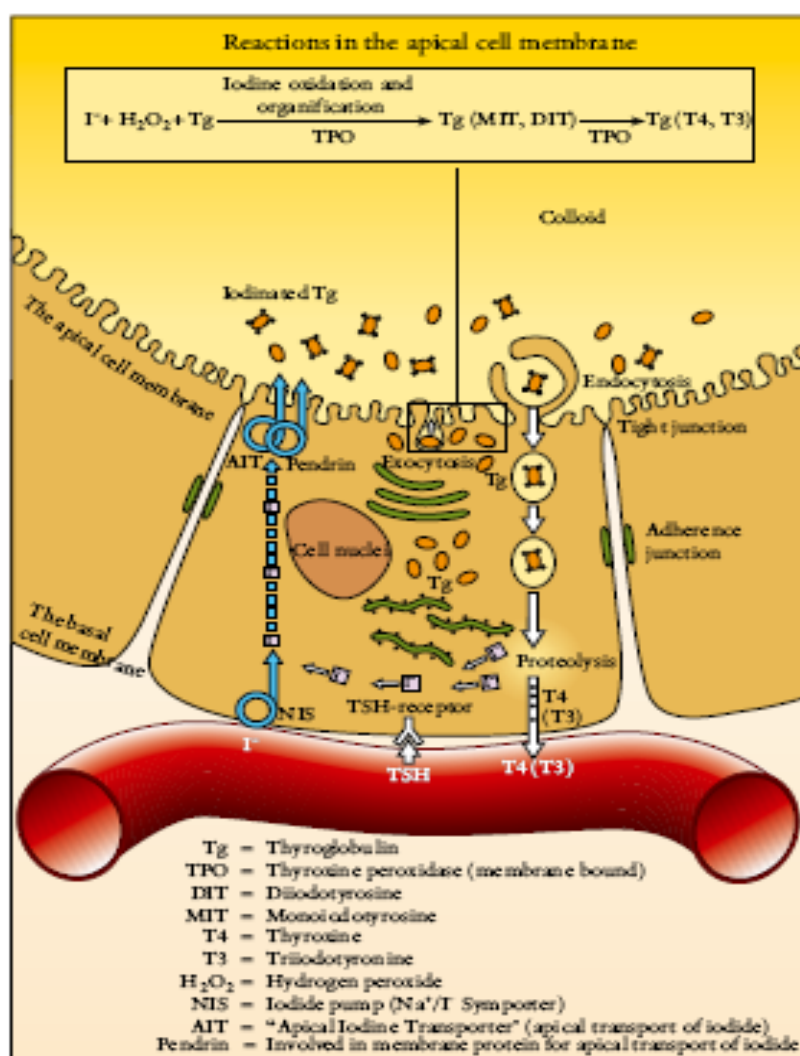


Figure 4 When TSH stimulates its receptor, all production steps in the thyroid hormone synthesis in the follicular cell increases. There is an influx of iodine via the sodium-iodine symporter and a transport of iodine to the apical membrane where it is coupled to thyroglobulin. Simultaneously, iodinated thyroglobulin is mobilised by endocytosis from the colloid and thyroid hormones are released from thyroglobulin into the blood circulation. The picture is from the book "Tyroidea sjukdomar (thyroid diseases) by Berg G et al Media Center TVB AB and Nycomed AB, 2007. Reprinted with the permission of Nycomed, AB, Stockholm, Sweden.

Because of the uncertainty of using basal thyroid hormone levels in the evaluation of CH, other tests have been developed. Patients with CH have a blunted nocturnal surge (67, 68) in TSH circadian secretion (69-71). However, this may be found in NTI (72), in postoperative patients (73, 74), during starvation (75), and in severe primary hypothyroidism (76). The TRH stimulation test is used in the diagnosis of CH (77, 78), but its value has been questioned (79, 80). In addition, in some early studies,

bTSH stimulation was considered for the diagnosis of CH (81, 82), but it was later established that an inactive gland in CH can be stimulated to resume thyroid hormone synthesis after numerous bTSH injections (81). The use of bTSH was terminated due to commonly occurring allergic reactions (83) and the appearance of neutralising and haemagglutinating antibodies (83, 84). The measurement of FT4 is currently the most sensitive test for CH (50, 64, 65) and an additional test to clarify diagnosis is warranted.

The primary aim of Paper II was to investigate whether the stimulation of the thyroid gland with rhTSH could distinguish between patients with CH and those who were TSH-sufficient.

4. Corticotroph axis

The normal cortisol physiology (85-90) is the basis of the diagnosis of adrenal insufficiency. A morning serum cortisol <100 nmol/L (91) has a specificity of 100% but only a sensitivity of 50% (80). A morning serum cortisol concentration >400 nmol/L (92) or 500 nmol/L (80, 93, 94) is considered normal. However, a provocative test is often needed, particular for patients with morning cortisol levels in-between 100-500 nmol/L.

ITT (95) is considered the gold standard for assessing the adequacy of the corticotroph axis. An intact axis is indicated by a peak cortisol of >500 nmol/L, however, cut-offs depend on the method used. However, ITT is labour-intensive and contraindicated in certain patients (3, 80, 94).

The results of ITT and the short synacthen test (SST) are correlated (96, 97), as ACTH-insufficiency leads to reduced ACTH receptor expression in the adrenal gland (98). Most often, a cortisol peak >550 nmol/L is considered a normal response (80). However, results of the SST are unreliable within 2 weeks after pituitary surgery or other acute pituitary insult (91, 99). As administration of 250 µg ACTH in the SST represents a massive supraphysiological challenge, a low-dose ACTH test is proposed as a more sensitive test and suggested (100), as a replacement for the high dose SST and ITT for initial evaluation of the corticotroph axis in patients with pituitary disease. However, clinicians choice of test and how they are used vary widely (101).

C Treatment of hypopituitarism

1. Somatotroph axis

The first placebo-controlled trials of GH treatment in GHD adults were reported in 1989 (102, 103) after recombinant GH became available (1). These and other studies defined the clinical syndrome of adult GHD, which is characterized by visceral adiposity, decreased lean body mass (LBM), reduced muscle strength and exercise capacity, elevated low density lipoprotein cholesterol (LDL-C) and c-reactive protein (CRP), reduced bone mineral density (BMD), dry skin and impaired psychological well-being (1, 104, 105) (Figure 5).

Hypopituitary patients have excess cardiovascular and cerebrovascular mortality (5, 106-108), which has been designated to untreated GHD or treatment modalities of the pituitary disease, such as cranial irradiation (5). However, a large study from the United Kingdom (UK) determined no indications of increased mortality in untreated GHD patients (5), but the number of patients assessed for GHD was low. Mortality data from long-term GH replacement therapy from controlled trials are yet unavailable, but a positive outcome of GH replacement is indicated by a study (109) where

Cushings' disease	GHD
Moon face	Dyslipidemia
Buffalo hump	Increased BMI
Visceral adiposity	Decreased lean body mass
Bruises	Visceral adiposity
Skin atrophy	Insulin resistance
Insulin resistance	Osteoporosis
Osteoporosis	Increased vascular mortality
Increased vascular mortality	

BMI=body mass index

Figure 5 The similarity of patients with Cushings' disease (overproduction of cortisol) and growth hormone deficiency (GHD). Similar symptoms and signs are marked in grey.

the morbidity in myocardial infarction, cerebrovascular disease and malignancies were increased in untreated GHD patients, but are similar or lower than to the normal population in GH treated GHD patients. Moreover, mortality in cardiovascular and cerebrovascular diseases of patients in the KIMS (Pfizer international database) is correlated to IGF-I standard deviation score (SDS) levels and is normal when IGF-I SDS is in the upper half of the normal reference range after treatment (110). The question about mortality reduction by GH will eventually be answered.

The efficacy of GH treatment has been evaluated in long-term studies (111-114) with a transient reduction in body fat during the first 3-years and progressive improvement of total cholesterol and LDL-C. After an initial deterioration, HbA1c decreased progressively (111). In addition, muscle mass and LBM increase and muscle strength improve, at least during the first 5-years of treatment (112, 114). Improvement in quality of life (QoL) occurs, predominantly, during the first year of GH treatment, but a successive improvement is observed (113, 115). After 8 years of GH treatment, the Swedish GHD population attained the same QoL-AGHDA score as the normal population (113).

In the late 1990s, dosing based on body surface area and body weight was abandoned in favour of individualised dosing (116, 117) guided by clinical and biochemical responses. The recommended starting dose of GH is 0.2 mg/day in young men, 0.3 mg/day for young women and 0.1 mg/day for older patients, reflecting the larger need in women and the reduction of GH production by age. It is administered subcutaneously in the evening to mimic the higher GH secretion during the night (36).

The efficacy of GH treatment is monitored by measurements of body composition and serum IGF-I levels, which should be maintained below the upper limit of normal reference range. In addition, total cholesterol, LDL-C, fasting glucose and diastolic blood pressure (BP) need yearly assessment (36). This monitoring reflects the effects designated to GH: reduced body fat (1, 118-120), increased muscle mass (1, 118, 121), LBM (1, 118, 119), extra cellular water (ECW) volume (1, 104, 120) and BMD (1, 120). GH reduces total cholesterol, LDL-C (1, 122, 123) and CRP (124, 125) in GHD patients, and, after an initial worsening of glucose metabolism, it improves insulin sensitivity (1, 126), possibly because of the reduction in visceral fat. GH replacement is reported to increase physical performance (118, 121, 127) and improve QoL (1, 113-115, 128-133). However, data on QoL are inconsistent, with three

randomised, double-blind studies unable to detect any change in QoL (118, 134, 135).

2 Gonadotroph axis

Testosterone replacement in men aims to restore serum testosterone and androgen male characteristics (80).

Younger women are given hormonal replacement therapy (HRT) to restore menstruation patterns, especially as an increased cardiovascular mortality is demonstrated in untreated hypogonadism (5); transdermal preparations are recommended (37, 80, 136). The results of large epidemiological studies have influenced the guidelines on HRT for women of peri- and post-menopausal ages (80). HRT is only advised between 50-59 years for symptomatic relief and is not recommended for older ages because of negative cost-benefit ratio.

Androgen therapy may be indicated in females if symptoms of androgen deficiency occur in combination with low androgen levels (80). Treatment with dihydroepiandrosterone is not generally recommended, but can be used for individual patients, mainly in clinical trials: large, randomised placebo-controlled trials are still missing. Studies in which hypopituitary women use transdermal testosterone are reported, but long-term safety data is still lacking (80, 137).

3. Thyrotroph axis

The vast majority of CH patients are treated with levothyroxine (L-T4) (50) and combination therapies with T3 and T4 have not proven superior (138, 139). In TSH insufficiency, TSH for judging an appropriate thyroxine replacement level is lacking, making thyroxine replacement more arbitrary, with risks of subclinical hypo- and hyper-thyroidism (140-146). In children with documented mild CH and short stature, increasing serum FT4 from the lower third to near the upper third during 6 months of thyroxine therapy, significantly increases growth velocity (147). Thus, minor thyroid dysfunction may have detrimental effects on patient outcome.

Recommendations for adequate thyroxine replacement in adult patients with CH are based on a few reports (64, 65, 139, 148, 149) that direct dosing by weight, TSH- and FT4-levels. In 1999, Ferretti et al (65) describe a mean dose of thyroxine of 1.5 ± 0.3 µg/kg body weight that is modified according to age, targeting normal free T3 (FT3) without signs of over-replacement. Five years later, Alexopoulou et al (64) report treatment with a mean dose of 1.6 ± 0.5 µg/kg body weight/day results in suppressed TSH in 75% of patients. This is in accordance with another study evaluating body weight-guided dose with empirical titration (139). Moreover, Shimon et al (149) observed in 2002 a suppression of TSH below 0.1 mU/L that predicted euthyroidism in 92% of cases, rather than 34% when TSH was >1 mU/L. Finally, Carrozza et al (148) recommend FT4 to be mid-normal or in the upper part of the reference range. FT4 is predominately a marker of hypothyroidism, and FT3 is more sensitive for detecting hyperthyroidism (65).

4. Corticotroph axis

The daily cortisol production rate is substantially lower than previously reported 5.7 mg/m²/day or approximately 9.9 mg/day (150). However, cortisol production level is estimated to be between 9 and 11 mg/m²/day (151). Approximately 5–10% of the circulating cortisol is free, which mediates the glucocorticoid (GC) effect in peripheral tissues (152-154). Cortisol has a similar affinity for both the mineralcorticoid receptor and the GC receptor, but the activity of 11-β-hydroxysteroid dehydrogenase

(11 β HSD) type 2 protects the mineralcorticoid receptor from over-stimulation by converting active cortisol to inactive cortisone, allowing aldosterone to interact with its own receptor (155). The type 2 isoform inactivates cortisol in the kidney; whereas, 11 β HSD type 1 principally performs the reverse action of converting cortisone to cortisol in the liver and visceral adipose tissue (156) (Figure 6a). When expression of these 11- β isoenzymes in peripheral tissues is altered, corticosteroid action is modified. A sexual dimorphism with lower 11- β -HSD type 1 activity in women than in men has been identified (157, 158).

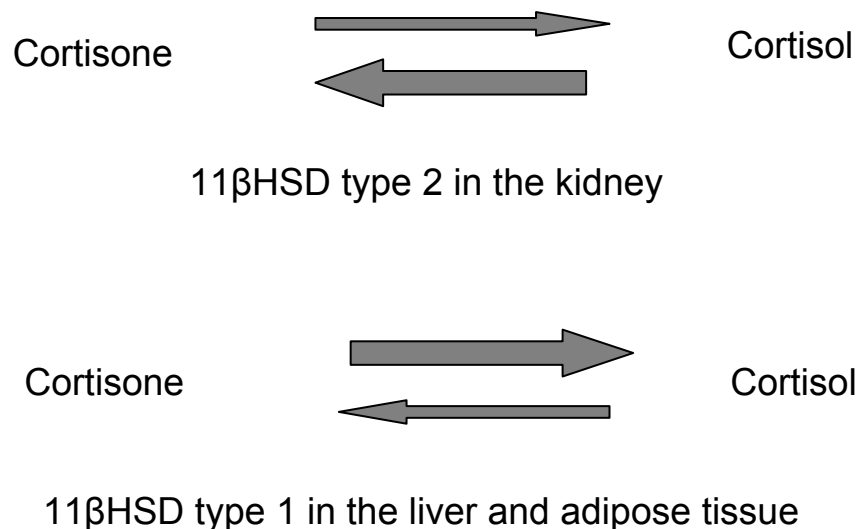


Figure 6a 11 β HSD exists in two forms. Type 2, which is mainly found in the kidney, converts the active hormone cortisol to the inactive prohormone cortisone thereby protecting the mineralcorticoid receptor from over stimulation. Type 1 performs the reverse reaction in the liver and in adipose tissue activating cortisone to cortisol.

The aims of GC replacement therapy are to mimic the circadian serum steroid profile, to respond to the increased need for cortisol during physical and physiological stimulation and to achieve normal well-being, normal metabolism and favourable long-term outcome (3), avoiding under- (159) and over-replacement (160, 161). Hydrocortisone (HC) is the name of synthetic cortisol, and cortisone acetate (CA) is a synthetic analogue that is metabolised in the liver by 11 β HSD type 1 to the active HC form. Both HC and CA have anti-inflammatory and mineralcorticoid effects and are short-acting (8 to 12 h), especially HC, which has a serum half-life of 1.7 h (162). Prednisolone has an intermediate duration of action and a greater anti-inflammatory effect than mineralcorticoid activity (159). Dexamethasone (DX) has mainly anti-inflammatory activity with no mineralcorticoid effect, and is longer-acting, with a half-life of approximately 36 to 72 h (152). HC is probably the most commonly used GC for replacement therapy. However, some European centres also use CA, mainly for practical reasons. Prednisolone and DX have been used for replacement therapy in selected patients (3). The equivalent doses for these steroids are 20 mg HC = 25 mg CA = 5 mg prednisolone = 0.65 mg DX (163, 164) and are based on the anti-inflammatory properties of GC, which cannot, by guarantee, be transferred to replacement equivalences.

Practically, administration of HC and CA multiple dosing is needed. When HC is administered twice daily, two thirds of the total daily dose is typically administered in

the morning and the remainder in the afternoon at 1600 to imitate circadian cortisol production (165). Low cortisol levels in the afternoon may be partly prevented by administering HC on a thrice-daily regimen (166, 167). Thrice daily administration of a daily dose of 25 mg CA, achieves more physiological cortisol levels than twice daily regimens (168).

Historically, 30 mg/day of HC was given to patients with adrenal insufficiency (169), but through the use of serum cortisol day curves and 24-h urinary free cortisol measurements to determine total daily HC or CA dose (167, 170), the mean daily dose was reduced to 20 mg HC (170) or 25 mg CA (168).

The effect of GC on bone and cardiovascular risk factors has been assessed (154). Zelissen et al. (171) determined in 1994 an inverse correlation between lumbar BMD and increasing dose of HC/kg body weight in men with Addison disease, but not in women. Five years later, Wichers et al. (172) conducted a randomised double-blind study in patients with ACTH-insufficiency, who were treated for three periods of 2 weeks with 15, 20 or 30 mg/day of HC. Osteocalcin levels (a marker for osteoblast activity) fell as HC doses increased but resorption markers remained unchanged.

In 1995, al-Shoumer et al. (173) reported that hypopituitary patients on GC replacement were more insulin resistant in the mornings when HC was administered than with mornings when no HC was administered. In contrast, the same year Dunne et al. (174) determined no significant difference in fasting glucose or glycosylated haemoglobin (HbA_{1c}) levels after a reduction in HC dose from 30 mg to 15 mg over a 3-month period. This is in accordance with a report from McConnell et al. in 2002 (175), who studied 15 ACTH-insufficient patients in a randomised cross-over study on either intravenous HC (to mimic the physiological cortisol production) or 15 mg + 5 mg HC administered orally. Moreover, subjective health status in cortisol insufficiency becomes impaired with increasing GC doses (176). In an open non-controlled study of 11 panhypopituitary patients with untreated GHD, treated with 20-30 mg HC/day, the patients were instructed to reduce the HC dose to 10-15 mg/day. After 6-12 months reductions in body mass index (BMI), weight, total and abdominal body fat measured by dual X-ray absorpometry (DXA), total cholesterol, triglycerides and QoL-AGHDA score were observed (177). Hence, there are indications that higher GC doses used in replacement therapy may result in or augment features traditionally connected to GHD (Figure 5) and that lower doses are advocated.

A large randomised double-blind study of different HC doses is still lacking. However, in Paper III, the metabolic outcome of different GC doses was evaluated in a large cohort of hypopituitary patients before and after GH treatment.

5. Interactions between hormonal systems

In GHD men, GH replacement reduces total testosterone because of reduced sexual hormone binding globulin (SHBG) levels: free testosterone remains constant. Hence, GHD does not mask central hypogonadism (178), but careful monitoring is required as diagnosis of hypogonadism is mainly based on total testosterone levels. Furthermore, testosterone enhances the metabolic effects of GH in hypopituitary men, which could explain some of the sexual dimorphism in response to GH (179).

However, in GHD women, oral oestrogen replacement lowers IGF-I levels, which has a clear clinical implication as the GH dose needed to achieve target serum IGF-I levels increases. Therefore, transdermal oestrogens, avoiding the first passage effect of the liver, are recommended (37, 80, 136). In addition, transdermal oestrogens reduce induction of hepatic protein production, which may be beneficial by reducing procoagulatory factors and acute phase proteins that lower the vascular risk: SHBG

levels are also reduced, which increases free testosterone (80). This has clinical implementations in women receiving oral oestrogens with low androgen levels and who could benefit from transdermal administration to lower SHBG and increase free testosterone in order to reduce symptoms of androgen deficiency (80).

In addition, both transdermal and oral oestrogens increase thyroxine binding globulin and patients need higher L-T4 replacement doses when these therapies are combined (64). GH replacement increases conversion of T4 to T3 and decreases that of T4 to reversed T3 (rT3) (180-182). Therefore, careful monitoring of thyroid function is mandatory during GH treatment (181), as it may induce hyperthyroidism and the L-T4 dose need be reduced. In addition, GH therapy may unmask undiagnosed CH (63, 182).

The thyroid hormonal system in hypopituitary patients is influenced by GC replacement (64, 183). Adrenal insufficiency may increase TSH, mimicking subclinical hypothyroidism, which underlines the need for proper diagnostic assessment and the replacement of GCs before thyroxine replacement (66): euthyroidism may trigger an adrenal crisis by accelerating the metabolism of cortisol.

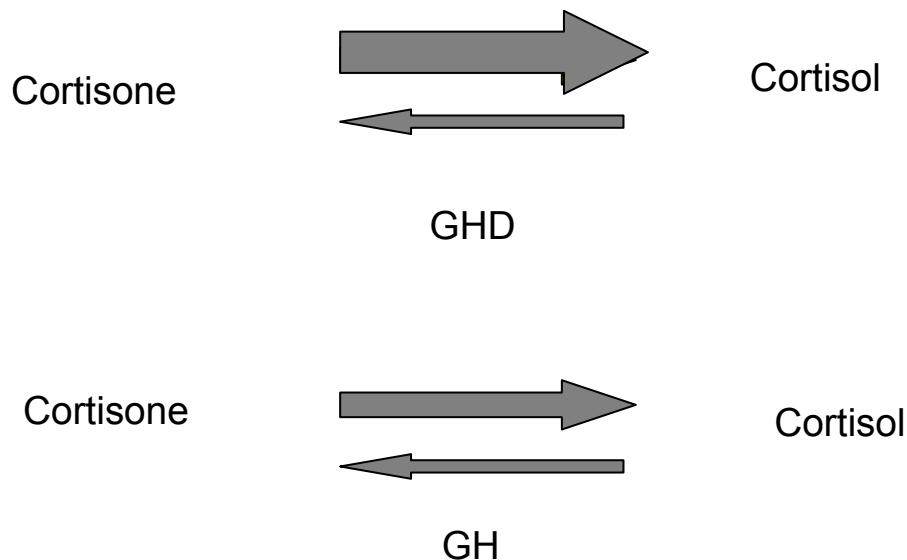


Figure 6b The 11β HSD type 1 enzyme activity is enhanced in growth hormone deficiency (GHD), thus exposing the tissues to more cortisol, whereas GH replacement inhibits the type 1 shuttle

In GHD, 11β HSD type 1 activity is increased (184, 185), suggesting augmented tissue exposure to GCs (Figure 6b). This could explain some of the metabolic features associated with hypopituitarism and severe GHD (Figure 5). GH therapy restores 11β HSD type 1 activity (184-186) (Figure 6b), which implies that GH therapy in GHD adults alters the serum cortisol profile, with a reduced cortisol concentration in blood after oral administration of HC. This effect may be important in patients with partial or total ACTH deficiency with sub optimal cortisol replacement, resulting in a risk of clinical overt cortisol deficiency after GH therapy commences (186). Moreover, in untreated GHD patients, HC - but not CA in equivalent doses - can result in supra-physiological cortisol-tissue exposure, which is attenuated by GH replacement. In addition, patients treated with CA are more vulnerable to the inhibitory effect of GH on 11β HSD type 1, with a reduction in serum cortisol levels (187).

D Discontinuation of GH replacement

1. General remarks

Pituitary replacement therapies are usually continued throughout life as pituitary function is permanently damaged. However, there are mechanisms without cell destruction, such as impaired blood flow (188) or increased intra-sellar pressure (189) that give a chance of recovering pituitary function if the beneficial conditions are restored. Recovery of hormonal production may be considered after pituitary surgery (190), after medical treatment of PRL-producing tumours (191, 192), after TBI (16), and after autoimmune hypophysitis (18), and replacement therapy may be discontinued. In addition, GH treatment is discontinued in adolescents to evaluate persistent GHD.

2. Discontinuation of GH

Since 2007, it is recommended that patients with CO GHD should be re-evaluated for continuous GH therapy after completed growth of height. GH testing is not needed in patients with genetic hypopituitarism, in cases with >3 hormonal deficiencies or in non-GHD paediatric indications, Turners syndrome and small for gestational age, as these non-GHD patients have no proven benefit of continuous GH treatment (36).

The effects of GH discontinuation in adolescent patients have been evaluated by several research groups (193-196). A 2-year-discontinuation in CO GHD adolescents produces an accumulation of important cardiovascular risk factors: higher total cholesterol, higher LDL-C, lower high-density lipoprotein cholesterol (HDL-C) and increased total body fat and abdominal fat (193). A cohort of adolescent patients with CO GHD has been retested (194), and the true GHD patients were randomised to GH or placebo and were compared with those without GHD. After 2 years of discontinuation, lipids, glucose metabolism, body composition, BMD, echocardiogram, exercise test, and QoL measures were comparable with the group that had received continuous GH treatment. The effects on QoL in young adults with CO-GHD was evaluated one year after withdrawal and one year after re-institution of GH treatment (196). One-year-discontinuation of GH treatment led to a decrease in QoL within 6 months, which was counteracted within 6 months after restart of GH treatment. Finally, in adolescents, the psychological general well-being (PGWB) score indicates greater impairment in GHD patients than in the GH-sufficient control group at baseline (195) and the authors conclude that discontinuation of GH in late adolescent life does not risk immediate deterioration of perceived QoL. To summarize, some studies indicate that GH discontinuation in GHD adolescents is safe, whereas other studies present contrary results.

GH treatment is not indicated in all countries to the entire group of GHD adults because of its high costs. In Sweden, the expense of GH treatment is within the top 10 of the most expensive medical products sold *via* the pharmacy (Figure 7a-b) (197). The high expenditure for GH has forced some countries to restrict prescription to the patients having the lowest QoL, which is considered the most cost effective group (198) as in this group QoL improves the most (132). Since 2003 in the UK, the National Institute for Clinical Excellence (NICE) has demanded a QoL-AGHDA score ≥ 11 before GH treatment and an improvement of at least 7 points after 9 months of treatment (198). The rationales for selection of QoL as a sole criterion for prescription is unclear, in fact, changes in body composition is not correlated to perceived QoL (199).

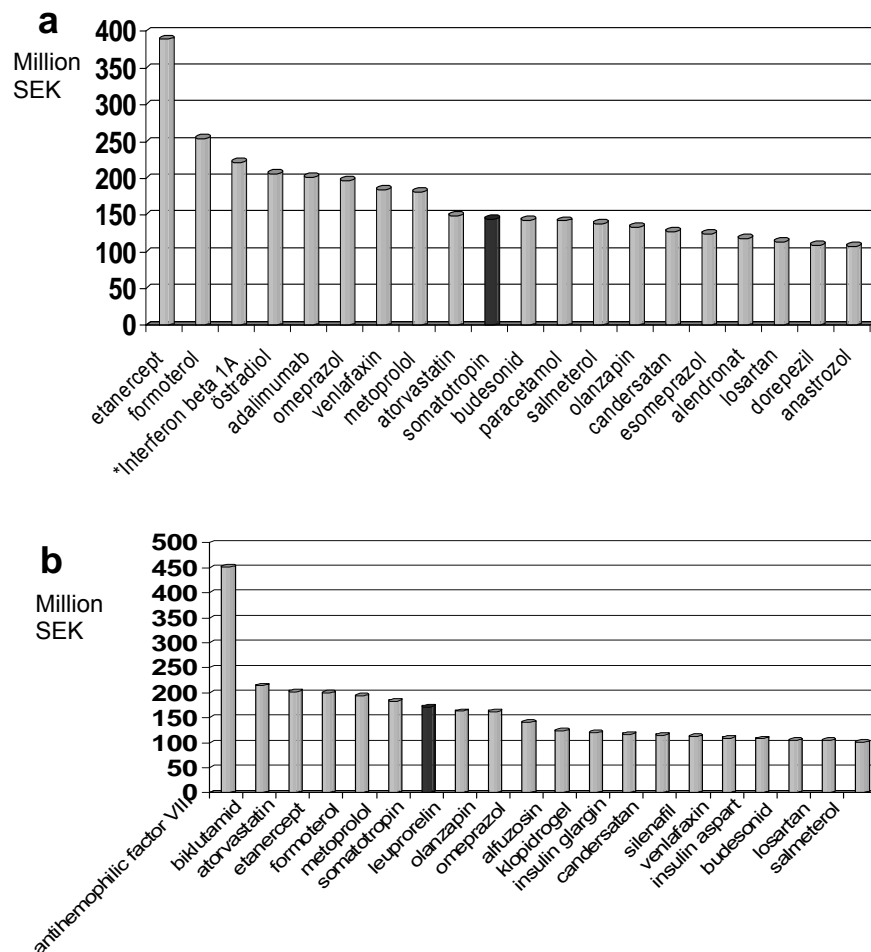


Figure 7a-b The 20 most expensive medical product sold by the pharmacies in Sweden; **a** women **b** men In darker shading the cost for GH (197).

Can GH be discontinued in adults temporarily with sustained effects? In GHD adults, a few discontinuation studies have been performed since 2000. After 18 months without GH, an increase in body fat and a decrease in LBM are observed in 40 men with adult-onset GHD. However, BMD continued to increase during GH discontinuation (200). Moreover, a study of 3-month-GH-discontinuation in adults implied worsening in QoL, but only in the questionnaire SF-36 and not the Nottingham Health Profile (NHP) score (201). In addition, in a 3-month double blind, placebo-controlled (DBPC) trial discontinuation of GH, 12 out of 21 adults with severe GHD discontinuation of GH caused detrimental psychological effects (113). From semi-structured interviews, the key psychological symptoms of GH discontinuation were: reduced energy, increased daytime drowsiness, crying episodes, depression, irritability and physical symptoms were: increased pain in joints and muscle, weight gain and changes in skin, hair and nails. However, only three QoL sub-scores gained statistical significance and GH treatment duration prior to discontinuation was only on average 6 months, which may influence the results, as QoL continues to improve years after GH treatment commences (113).

Discontinuation also provides information on the effects of long-term continuous GH replacement, when all the beneficial effects are likely to be obtained. A GH semester may lead to preserved QoL, maintained metabolic outcome, and reduced expense for the nation, or, the patient's situation may deteriorate, which would strengthen arguments for continuous GH treatment in adult life (Paper IV). These are important as-

pects, as by the end of 2009 in Sweden, the current GH prescription mode will be audited and this may lead to its re-appraisal.

IV Aims

The aim of this thesis was to focus on the parts of hypopituitarism within aetiology, diagnosis and treatment that are still clinical dilemmas. The aim of each paper was:

- In a group of patients with idiopathic hypopituitarism identify cases with genetic hypopituitarism and evaluate the efficacy of genetic screening in an unselected group of adult hypopituitary patients (Paper I).
- To examine if the diagnosis of CH can be improved with using additional testing by TSH stimulation and to evaluate the biology of an unstimulated thyroid gland (Paper II).
- To investigate the impact of different GC replacement regimes and dose exposure on metabolic outcome (Paper III).
- To investigate long-term GH replacement therapy on QoL and metabolic outcome in adult hypopituitary patients (Paper IV).

V Study design

All studies were approved by the Ethical Committee of the University of Gothenburg and patients signed informed consent after receiving oral and written information about the study. Papers II and IV were authorized by the Medical Product Agency in Uppsala, Sweden

A Paper I

Paper I is an observational study collecting blood samples from the cases of IPI at the Centre of Endocrinology and Metabolism (CEM) since it started in 1990. Therefore, it is not regarded as purely cross-sectional. After being identified as IPI, defined as no identifiable cause to their hypopituitarism, patients were invited to participate in the study either by letter or in connection to their ordinary visit to CEM.

B Paper II

This was a prospective, randomised single-blinded trial with two doses of rhTSH. All subjects underwent a routine clinical investigation, including an electrocardiographic registration. In the CH group, L-T4 substitution was replaced with 20 µg triiodothyronine (Liothyronin®, Nycomed AB, Sweden) administered thrice daily five weeks before study-start because of its shorter half-life. Triiodothyronine substitution was discontinued one week before rhTSH injection and L-T4 substitution was re-instituted after study-completion.

At 9 am, all participants received an intramuscular gluteal injection of 0.1 and 0.9 mg rhTSH (Thyrogen®, Genzyme, Boston, USA) given in random order with one-week in-between. Before each injection, safety routine blood samples were taken and thyroid hormone, thyroglobulin (Tg), and Tg antibodies (ab) specimens were collected at 45 min before, immediately before and 2-, 3½-, 7-, 24-, 48- and 72-hours after each injection. IGF-I and insulin levels were measured before the first rhTSH injection and any side effects were recorded concurrently at visits.

C Paper III

The KIMS database is an open, observational, non-interventional pharmaco-epidemiological survey of adult GHD replacement with Gentropine® (subcutaneous recom-

binant human GH) treatment that was initiated in January 1994 (14). Currently, nearly 13 000 patients are registered from 31 countries. The two largest contributors are the UK and Germany, followed by USA and Sweden and represent approximately 60% of patients enrolled in KIMS. The database is monitored closely by KIMS to ascertain proper registration.

At baseline, before GH treatment, medical history, age of onset of pituitary disorder, GHD diagnosis and number of additional pituitary hormonal deficiencies are documented and QoL is assessed. Anthropometric measurements and BP are recorded and blood samples are collected for IGF-I, lipids, fasting glucose (F-glucose, and HbA1c. These parameters are reported into KIMS on a yearly basis during GH treatment. In addition, adverse events (AEs), defined as any untoward medical occurrences regardless of their causal relation to GH, are reported (202).

D Paper IV

This was a randomised, placebo-controlled, double-blinded study with a crossover design. After inclusion, other replacement therapies were optimised during a 3-month run-in period before randomisation. Stratification was by age (≤ 45 / >45 years) and sex. Patients were treated for two periods of 4 months: one period with GH and one period with placebo.

At inclusion, thyroid hormone levels, testosterone (men), and IGF-I were assessed. At visit 2 (baseline), visit 3 (cross-over), and visit 4 (end of study), weight, length, BP, IGF-I, SHBG and highly sensitive CRP were collected. Moreover, specimens of total cholesterol, HDL-C, LDL-C, triglycerides, P-glucose, HbA1c, and serum insulin were stored and a short insulin tolerance test (SITT) was performed. Patients completed three QoL questionnaires and body composition was determined by computer tomography (CT), DXA, and bioelectric impedance. In addition, muscle function and physical activity were assessed.

E Considerations on study designs

In Paper II, a longer interval than 1 week between injections would be more appropriate, as thyroid hormone levels had not completely returned to baseline levels before the next injection. However, this was a pilot study and a longer interval would have increased the risk of profound hypothyroidism in both groups of CH patients.

As Paper III is built from the information about patients' concomitant medications, insufficient reporting to the registry may undermine the validity. There are continuing efforts to facilitate and improve the reporting, especially of concomitant medications. In an ongoing comparison between the reporting of severe AE (SAE) and AE in KIMS and another large Swedish registry, the rikshIA, the accuracy of AE reporting was gauged by cross-referencing individual cardiovascular events in KIMS and rikshIA (all cardiovascular events on a cardiac intensive care unit). There is a concordance of an average 29% match on cardiovascular events and 86% for myocardial infarctions. The low average accuracy can be explained by many AE are collected outside intensive care units, which will not be reported into the rikshIA, also underlined by the high accuracy for myocardial infarctions (203).

In Paper IV, a study design with a fixed number of patients is accompanied with risks, even though the power was calculated from available QoL data. In case of negative results, the number of participants may be too small. In addition, a double-blinded design with fixed length of study periods cannot exclude a positive effect being detected if the study period was longer. If patients were randomised to either placebo or GH and were followed until interim analyses detected a difference this issue

would have been overcome. However, considering the large placebo effect in QoL, the study design of Paper IV was the best possible as the patients were their own controls. The length of the discontinuation period was a balance between an acceptable period of risk for the patients and the probable time space for developing aberrations, if any.

Moreover, an improvement to Paper IV would be an additional 4-month period separating the two periods of GH/placebo, where patients would be on open GH. This would have served as a washout period, making the two periods equal and minimizing the carry-over effects. Now, the patients receiving GH in first period may not be completely comparable with those receiving it in the second.

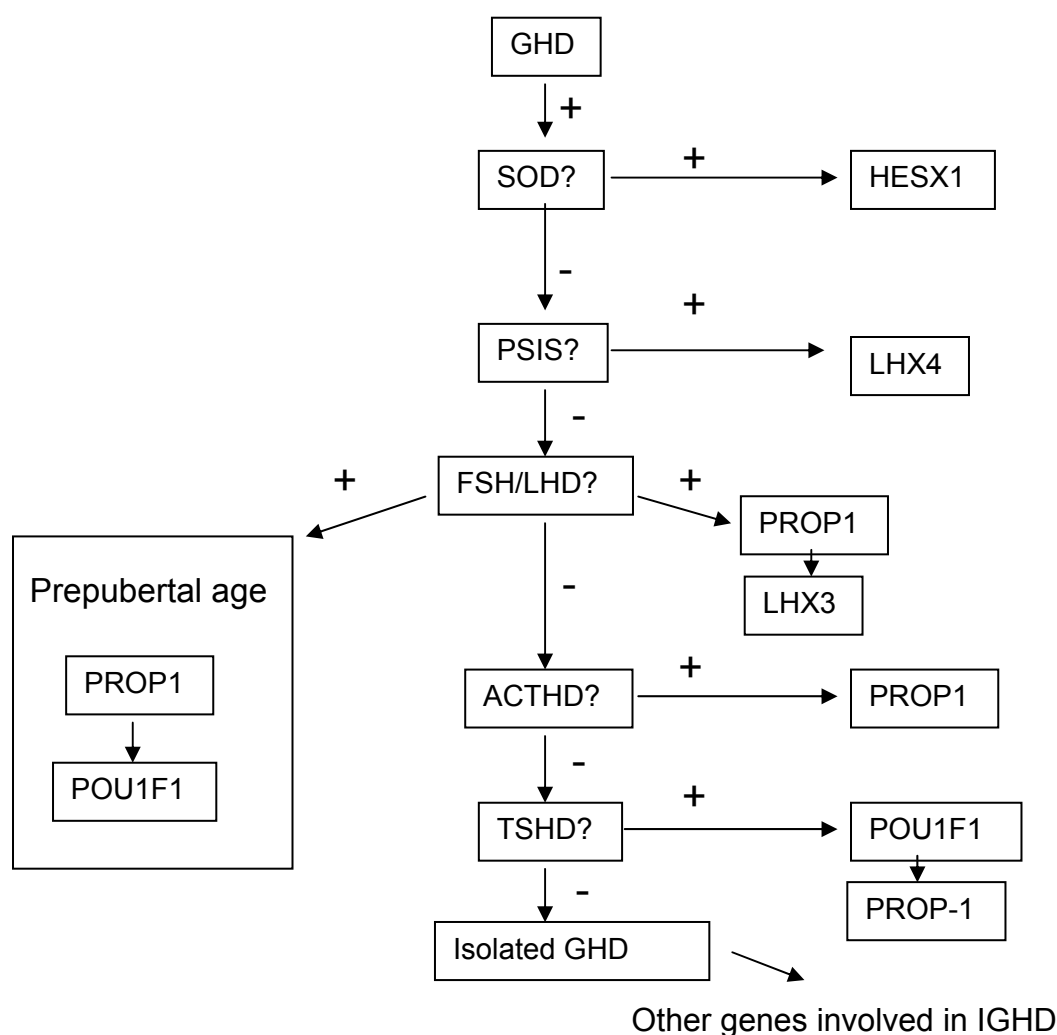


Figure 8 Algorithm of genetic mutation testing according to phenotype. Adopted from (30).

GHD=growth hormone deficiency
 SOD= septico-optic dysplasia
 PSIS=pituitary stalk interruption syndrome
 D=deficiency

VI Patients

A. Patients of the Endocrine Clinic at Sahlgrenska University Hospital, Göteborg, Sweden.

In Papers I, II and IV, patients were recruited from the CEM, an outpatient unit that recruits patients from the western part of Sweden, an area of >1.5 million inhabitants, representing 17% of the population in Sweden, for detection and treatment of patients with GHD. In Paper I, 373 files were searched for patients with IPI. Fifty patients were detected. After exclusion of four deceased patients, three lost on follow-up, two that was unwilling to participate, and two that were unable to understand informed consent, 39 patients with IPI were included in the study. Twenty-five patients were chosen for genetic testing according to the algorithm in Figure 8.

In Paper II, 18 patients with well-defined pituitary disease and pituitary insufficiency were recruited from the Endocrine clinic, whereof six were from CEM. These patients comprised of three groups: the CH group (n=6) was treated with L-T4 for CH; the newCH group (n=6) was newly diagnosed CH (established pituitary disease, an FT4 below normal range and additional pituitary insufficiencies) not yet replaced; and the nonCH group (n=6) had hypopituitarism but unaffected TSH secretion, which was reflected by normal pre-study FT4 levels. Six healthy controls were also included. The groups were matched for age, sex, and BMI (Table 3). The exclusion criteria were: current thyroid disease, presence of thyroperoxidase ab, cardiac disease, and treatment with antiepileptic, antipsychotic, or anticoagulation drugs.

Table 3 Demography of the study population, presented as mean and range, of patients with central hypothyroidism (CH), patients with newly diagnosed CH (newCH), patients with pituitary insufficiency but intact secretion of TSH (nonCH) and controls.

Subjects	NewCH (n=6)	CH (n=6)	Non-CH (n=6)	Controls (n=6)
Age (years) mean (range)	55.7(35-68)	56.2 (48-63)	55.7 (40-61)	51.8 (37-62)
Sex (female:male)	2:4	1:5	1:5	1:5
BMI (kg/m ²) mean (range)	26.9 (23.3-32.0)	26.6 (21.7-30.8)	26.0 (20.1-30.0)	25.4 (21.8-27.8)
Levothyroxine (µg)	0	125 (100-150)	0	0
Pituitary insufficiencies (number)				
GH	6 (unreplaced)	6 (replaced)	1 (replaced)	0
FSH/LH	6 ^a	5 ^b	3 ^c	0 ^c
TSH	6	6	0	0
ACTH	4	4	1	0
ADH	0	0	1	0
Pituitary diagnosis (n)				
Pituitary adenoma	5	3	3	
Histiocytosis		1	1	
Acromegaly, Surgery, Cured			1	
Hypopituitarism, idiopathic		1	1	
Mb Cushing, Surgery		1		
Pituitary Apoplexia, Surgery	1			

^a 2 males with unreplaced hypogonadism, 2 postmenopausal-aged women without oestrogens.

^b One postmenopausal woman on oestrogens. ^c One postmenopausal woman without oestrogens.
n=number.

In Paper IV, 60 patients were recruited from 180 eligible patients according to the inclusion criteria of well-defined pituitary-hypothalamic disease and a well-established GHD in adult patients, aged 25-75, with at least 3 years of continuous GH treatment and non-occurrence of exclusion criteria (active malignancy, diabetes mellitus (DM) with proliferative retinopathy, heart failure, respiratory insufficiency, pregnancy and lactation). To avoid an initial selection bias, all 180 were invited to participate of which 2/3 declined because of unwillingness, the travelling involved, lack of time, or reluctance to take the risk. All eligible patients were encouraged to reduce the risk of recruitment bias of healthier patients.

B. KIMS database

By 30 January 2004, the KIMS database (14) included 8836 GH deficient patients from 28 countries. The study in Paper III was conducted on European patients enrolled in the database. The exclusion criteria included: inaccurate data (n=1224); non-European patients (n=1391); <1 year of follow up (n=1063); non-naïve patients (n=1974); ACTH/GH producing adenomas (n=225); delayed GH treatment (n=117); patients commencing GC replacement after baseline (n=79); or, GH peak >3.0 µg/L in response to a stimulation test (n=339). Therefore, 2424 patients with severe GHD were available for study.

In Paper III, the patients included were divided into four groups according to their GC replacement regimen: 48.2% of patients received HC treatment (HC group, n=1168), 20.1% CA (CA group, n=487) and 2.1% Prednisolone (n=46) or DX (n=6) (PD group) and 30.0% of the patients were considered ACTH-sufficient (AS group, n=717). To be able to estimate the impact of GC dose and compare GC groups, a HC equivalent (HCeq) dose was calculated for all patients receiving GC (163, 204-206). GC patients were divided in three groups, with low (<20 mg/day, n=328), medium (20-29 mg/day, n=677) and high HCeq doses (≥30mg/day, n=664).

C. Considerations on patient selection

Patients being cared for at CEM are in most cases suitable for GH therapy, therefore excluding patients with active malignancy and patients at high age. In addition, a small number of patients have their follow-up elsewhere, which would mainly affect the eligible patient number for Paper I.

Choosing patients according to phenotype criteria in Paper I increased the risk of misclassifications and thereby a lower detection frequency. However, testing for all known mutations in every IPI patient is expensive and time consuming and the phenotypes of genetic hypopituitarism are well characterised. It is important to gain knowledge and algorithms when to test, as the genetic field is still expanding and new mutations may appear that may shed light on the IPI group. In Paper I, the results have the precautions of selection criteria and testing according to known mutations.

Furthermore, in Paper I, the selection for genotyping was dependant on the phenotype, which might not be entirely clear due to difficulties in diagnosing some hormone axes, such as CH. However, more pituitary hormone deficiencies may have developed in adulthood than during childhood, making the phenotypic picture clearer.

Moreover, as family cases point to genetic cause, ascertainment bias has to be considered. The number of sibling born is usually too low to allow the pattern of inheritance to become clear. Of two siblings, two cases may be affected, but chance can equally result in one healthy and one with the disease: family history would in this case be negative. Moreover, parents may consider not having more children, if the

first child is affected, and this would affect the presentation for the clinician. In addition, the disease may have different penetrance and also variable expressions, even within the same family, which also complicates phenotypes and the choice of genetic testing (207).

In Paper II, the control population was selected from colleagues and nurses and not according to the normal procedures through newspaper advertisement or randomly sent invitations to the normal population. However, as this was a biochemical study, which was assumed unimpressible by the patients, the choice of control group was acceptable.

In addition, in Paper II the newCH group was recruited with high probability of CH. However, in some cases, inclusion criteria were obtained within a couple of weeks postoperatively, which may have affected the thyroid gland's ability to respond to rhTSH. In mice, deprivation of TSH affects the thyroid gland within 24 hours and continues progressively during the following weeks (208) – which implies that the response was likely to be affected in these patients.

In Paper III, there were some caveats in the KIMS database; including selection biases, which excluded patients with contraindications to GH; treatment decision made by the physician, which further excluded some patients; the prescription of GH differs between countries, further selecting patients into KIMS; and patients with a short survival may not be considered for GH treatment. Furthermore, regular visits may lead to improved treatment of other pituitary hormone insufficiencies in the KIMS patients. Underreporting AE may be a risk in a registry.

In Paper IV, 2/3 of the eligible patients declined participation, which might be a recruitment bias. The 120 non-participating patients may be the ones who had the worst QoL before receiving GH and who would gain the most in QoL through GH treatment (132), but were reluctant to risk a deterioration in QoL. However, all patients were well informed about the study and the intention to gain evidence for continuous treatment will have motivated some. The study may have been strengthened by QoL assessment of ongoing GH treatment in the non-participating patients and further characterisation of that population to evaluate any major differences from the participating population. Unfortunately pre-treatment QoL scores were not available for all patients.

However, the patients selected had a baseline QoL-AGHDA score of median 2 (mean 3.38) that can be compared with the normative Swedish population score of 3.9 (age 50) (113). The patients recruited in Paper IV were 10 years older than the normal reference population and had been treated for 10 years - 2 years longer than the patients, who were reported to have normalised QoL-AGHDA score after 8 years (113). Age is considered to influence QoL AGHDA score positively by some (209), but not by others (210). The population studied in this cohort was close to the QoL AGHDA score reported for the normal population.

VII Methods

A. Genetic Methods

The process of transcription and translation is illustrated in Figure 9. The deoxyribonucleic acid (DNA) in the human genome is not static. A mutation is defined as a genetic variance that is detected in <1% of the population. Common mutations are entitled polymorphisms. The frequency of *de novo* mutations is low, thus, most mutations are inherited. Mutations can be point mutations, where a nucleotide is exchanged, deleted, or inserted, or changes within a chromosome. A point mutation may not necessarily change the amino acid (AA), if the exchanged nucleotide constructs a codon that codes for the same AA. However, a defect protein may result if the reading frame changes for one or several AAs or a stop codon is induced. Polymorphisms are often single-nucleotide polymorphisms (SNPs) or variable number of tandem repeat (VNTR) polymorphisms. Ninety percent of the variability of the human genome occurs as SNPs.

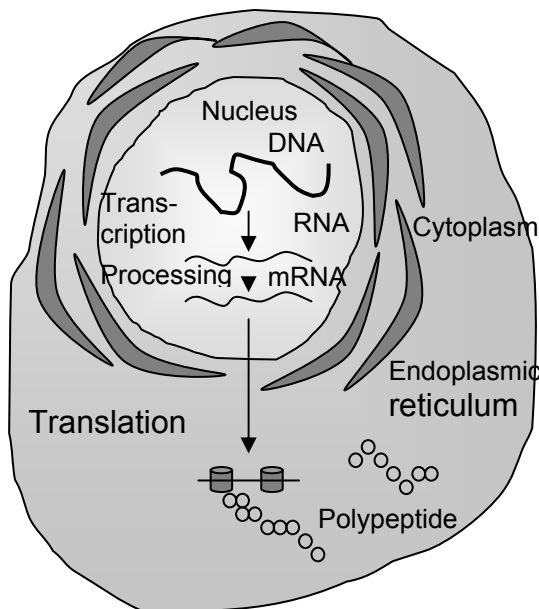


Figure 9 The process of transcription

RNA is transcribed from the double strain helix of DNA that is first single strained. Message RNA (mRNA) is produced by cutting of intrones – pieces of inactive genetic material from the RNA strain. MRNA is transported out from the nucleus to the ribosome where the polypeptide is translated.

1. Genotyping

In Paper I, genomic DNA was isolated from whole blood with FlexiGene DNA kit (Qiagen, Hilden, Germany). After identifying a sequence of the DNA of interest for a mutation, oligonucleotide primers are designed to flank the target sequence. Polymerase chain reaction (PCR) consists of cycles where the target DNA is multiplied - amplified. PCR consists of three successive reactions: first DNA is made single-strained by denaturation in heat; secondly, primers anneal to identify the target sequence; and, third a heat-stable polymerase synthesises new complementary strains to the single strain DNA beginning at the primers. After 30 cycles, the medium will contain amplified sequences of primers and target sequence, which will much easier be detected.

In Paper I, all coding exons of *HESX1*, *LHX4*, *PROP1*, *POU1F1* and *GH1* genes were amplified from genomic DNA (30) by exon flanking. The promoter, the enhancer, and the β isoform of the *POU1F1* gene encoding a 48-aminoacid fragment of 5' end of exon 2 (30) were also analysed. The same primers were used for sequencing.

B. Biochemical methods

1. IGF-I

In Paper III, two IGF-I methods were used (211, 212): Nichols radioimmunoassay (RIA) and Nichols Advantage system. An initial problem with the first RIAs was that IGF binding proteins (IGFBP) rapidly bonded to IGF-I, which could interfere with the subsequent ab steps. Precautions were taken and in the next generation RIAs, including the Nichols RIA, an acid-ethanol precipitation was used, which allowed the IGF-I to dissociate from IGFBPs, and large protein complexes were precipitated. This improved the purity of IGF-I, but there were still some methods containing IGFBPs. In the latest generations of IGF-I, including Nichols advantage, IGFBPs are first separated by acidification and a high concentration of IGF-II is added that binds to IGFBPs, which saturates binding sites, and complexes can be precipitated. However, some of the IGF-II may remain unbound and these methods require highly specific anti-IGF-I ab with very low cross-reactivity for IGF-II (40).

In Paper II the Nichols Advantage method was used, and in Paper IV, the IGF-I method according to Immulite, an immunoenzymo-chemolumino-metric method also with IGF-II, was used as Nichols methods was no longer available.

IGF-I is often expressed as IGF-I SDS and the reference range is mean \pm 2 SDS (211), as IGF-I is age and gender specific.

2. Thyroid hormone analyses

Immunoassays can be separated into non-competitive and competitive methods (213) (Figures 10a and b). In Paper II, TSH was determined with an immuno-chemoluminometric method (Architect, Abbot, USA), which is a non-competitive method. Non-competitive methods have good ability for detecting very low concentrations with a good sensitivity; however, this method deserves large molecules such as TSH. One disadvantage is the risk for high dose Hook effect, which produces false normal values.

In contrast to TSH, T4 and T3 are small molecules and a non-competitive immunoassay is not possible. Instead, competitive immuno-chemic methods are used. Competitive methods are sufficient for both small and large molecules; however, there is poorer detection capability at lower antigen concentrations (213). In Paper II, FT4, TT4, FT3, and TT3 were determined with the immuno-chemoluminometric methods (Architect, Abbot, USA).

The majority of the hormone produced from the thyroid gland is T4, which is converted by deiodinases to the active hormone T3 in peripheral tissues (214). T4 may also be converted to an inactive form, rT3, by deiodinase 3; rT3 differ in location of the third iodine from T3 (Figure 11). RT3 was determined by a radioimmunometric assay, which uses the competitive technique.

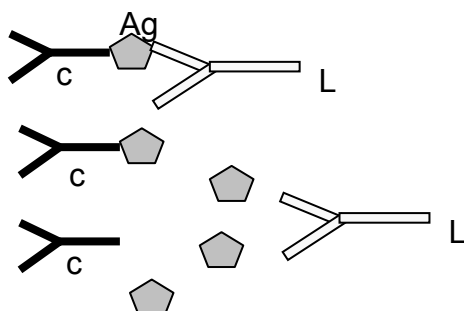


Figure 10a The principle of non-competitive immunoassays: The antigen (Ag) is identified by the capturing antibodies (C). Thereafter, a labelled antibody (L) is added that attaches to the antigen, which gives a detection signal proportionate to the antigen level.

Figure 10b The principle of competitive immunoassays: The labelled ag (L) competes with the antigene (ag) in the sample at the binding sites of the capturing antibody (C). The more ag in the sample, the less labelled ag is bound to the antibody, which results in an inversely proportional detection signal of the level of ag in the sample.

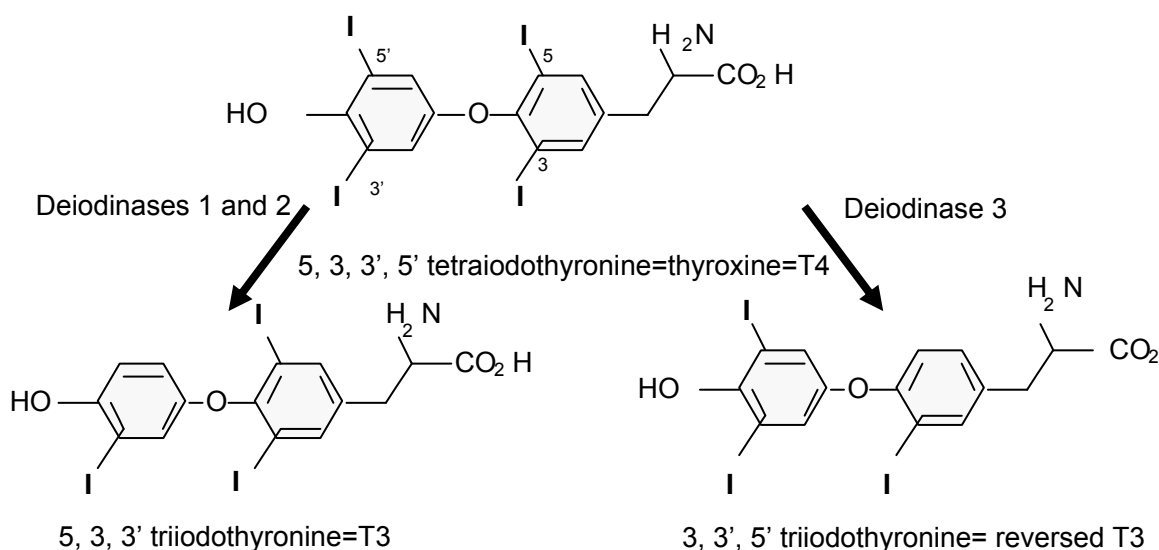
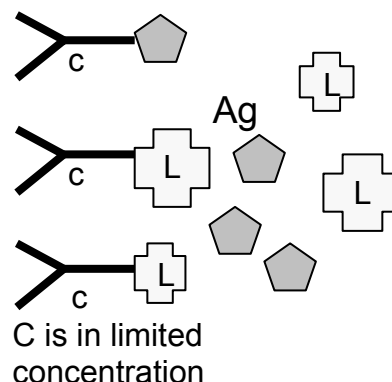


Figure 11 The deiodination of T4. T4, containing 4 iodine atoms (I), can be converted either by deiodinases 1 or 2 to the active hormone T3 or to reverse T3 (which is inactive) by deiodinase 3 depending on which iodine atom is removed.

Tg is a complicated molecule to measure because of its heterogeneity, and Tg analyses should always be accompanied with a test of Tg ab or Tg recovery, as cross-reactive ab are common (215). After TSH activates its receptor, iodinated Tg is endocytosed into the follicle cell and proteolytic degradation liberates bound T3 and T4 to be secreted into the blood (215) (Figure 4). Leakage of Tg to extra cellular fluid occurs normally to a low degree; however, the leakage increases when the cell mass is enhanced, the TSH receptor is stimulated or the integrity of the cells is damaged (215). In Paper II, Tg was analysed by a non-competitive immunofluorimetric method (Delphia) and Tg antibodies by Lumitest, which is a competitive method.

As a prerequisite for inclusion, thyroperoxidase ab was performed in all subjects of Paper II, with a competitive method (BRAHMS) that uses an immunoluminescence technique.

3. CRP

In Paper IV, CRP was determined by an immunoturbidimetric method (Modular, Roche). An anti-CRP ab on a latex coat reacts with the CRP within the sample and the antigen/ab complex is measured.

CRP has become a prognostic marker in coronar heart disease (216) and since 1999, highly sensitive CRP analyses have been developed, as a response to the demands for good detection and precision at lower levels.

4. Lipids

In Paper III, total cholesterol (217), triglycerides (218) and HDL-C (219) was measured centrally in one single laboratory. In Paper IV, a photometric method (Modular, Roche) was used for the analyses of lipids. LDL-C was calculated by Friedewald's formula (220) that is developed in normal subjects from the mass ratio 5:1 of triglycerides to that of very low density lipoprotein cholesterol (VLDL). In addition, when chylomicrons are undetectable, most of the triglycerides in plasma are contained in VLDL particles. LDL-C can be calculated from the formula:

$$\text{LDL-C} = \text{Total cholesterol} - \text{HDL-C} - (\text{triglycerides}/5).$$

5. Glucose metabolism

Glucose levels were measured in the fasting state of patients in Papers III and IV, and in Paper IV also during repeated SITT. In Paper III, glucose was measured locally by each participating centre, and in Paper IV, a photometric method was used for P-glucose. HbA1c was evaluated by chromatographic methods in Papers III and IV.

SITT was performed in fasting patients in the morning. Insulin was injected intravenously at 0.05 E/kg and P-glucose samples were collected up to 15 min after insulin injection. After the last P-glucose specimen, 10 ml of 30% glucose was administered and the patient had a light breakfast. In SITT, the glucose fall per minute was evaluated as a measure of insulin sensitivity (IS).

C. Anthropometric methods

In Papers II, III and IV, height was measured to an accuracy of half a centimetre and weight (kg) to one decimal place. BMI was calculated from the formula: weight/height squared (kg/m^2). Waist circumference (WC) was measured midway between the iliac crest and the lowest level of the thorax in the supine position in Paper III and in the standing position in Paper IV. Hip was measured as the maximal circumference in both Papers. BP was performed in supine position in Paper III, as in the majority of cases in Paper IV.

D. QoL questionnaires

1. Nottingham Health Profile

The NHP questionnaire assesses general well being and QoL and contains 38 yes/no questions divided into six different dimensions: pain (eight issues), energy level (three issues), sleep (five issues), emotional reactions (nine issues), social isolation (five issues) and physical mobility (eight issues) (221, 222). Subscore of the different dimensions was calculated as a weighted mean of related issues and was expressed between 0 and 100. The total score was the mean of the six subscales and a high score indicated poor QoL.

2. Psychological General Well-being

PGWB is a self-assessment questionnaire constructed to measure the individual's affective or emotional state (223). The 22 items are subdivided into six dimensions;

anxiety, depressed mood, positive well-being, self control, general health and vitality. A high total score indicates good emotional state.

3. Quality of Life-Assessment for GHD in Adults

QoL-Assessment for GHD in Adults (QoL-AGHDA) (224) was developed from in-depth interviews with adult GHD patients attending Christies Hospital in Manchester, UK, and is based on the concept that QoL is the degree to which human needs are satisfied. QoL-AGHDA is specially designated to assess the impact of GHD and GH treatment in adult patients and consists of 25 yes/no statements that describe main psychological conditions in adult GHD patients. A high QoL-AGHDA score indicates poor QoL (113, 225).

E. Body composition

These are the methods used in Paper IV.

1. Bioelectric impedance analysis

The main concept of the bioelectric impedance analysis (BIA) is that tissues rich in water and electrolytes are less resistant to an electrical current than lipid-rich adipose tissue (226). BIA measures were performed with poly-frequency BodyScout system. Impedance data was collected by 50 logarithmically spaced frequencies from 5 to 500 kHz. Extracellular and intracellular resistance were calculated and from these data, together with gender, height and weight, total body water (TBW), extracellular- and intracellular-water and fat free mass (FFM) and body fat (BF) were derived.

2. Dual X-ray Absorptiometry

When an X-ray is placed on one side of the body, the attenuation (intensity) of the beam on the opposite side is related to the thickness, the density, and the chemical composition of the tissues passed. Attenuation values for bone mineral content (BMC), lean soft tissue (LST) and BF are obtained, reflecting the different densities and chemical compositions (227). DXA was performed by a Lunar Prodigy scanner. FFM was defined as the sum of LST and BMC, and appendicular LST (ALST) as the sum of LST in arms and legs. Total body skeletal muscle mass (SMM) was calculated as $SMM = (1.19 \times ALST) - 1.65$ (228).

3. Computer Tomography

CT attains information of the tissue densities at each pixel. The anatomical location of the pixel can be identified as adipose tissue (AT), muscle, skin, viscera, or bone tissue (227). Formulas to calculate volumes for compartments are based on the area from a slice, the density of the tissue/organ and the weight and height, age and gender of the patient. This have minimised the number of slices needed and the radiation dose can be limited.

The CT examination (High Speed Advantage System and High Speed CT/i System (GE Medical Systems, Milwaukee, Wisconsin, USA) was performed and three slice positions were determined: the thigh image, abdominal image and liver image.

Examination data were transferred to a system for image analysis and the outer edges of the compartments were visually traced and determined, as previously described (229). In the thigh image, muscle tissue area (MT) and subcutaneous AT area were measured. In the abdominal image, subcutaneous AT area and intra-abdominal AT areas were determined, and in the liver image, the level of attenuation

was computed in Hounsfield units (HU). The cut-off value for diagnosis of fatty liver was liver attenuation ≤ 30 HU.

4. Compartment Models

In Paper IV, the four-compartment (4-C) model was used. The 4-C model is an extension of the the basic two-compartment model, where the body is divided into BF and FFM, and the three-compartment model, where FFM is further divided into water content and the remaining parts, mostly minerals and proteins (227) (Figure 12). However, for patients with depleted protein mass or low BMC, 4-C is more correct. In this model, the FFM mass was divided into three physiological compartments: body cell mass (BCM), ECW, and extra-cellular solids (ECS). BCM was calculated by subtracting ECW (BIA) from LST (DXA). The ECS compartment can be defined on the basis of total body calcium or BMC with DXA (227). In Paper IV, the 4-C model was built from the algorithm:

$$\text{Total body weight (kg)} = \text{BCM (kg)} + \text{ECW (kg)} + \text{BF (DXA) (kg)} + \text{BMC (DXA) (kg)}$$

CT and MR may also provide information on masses, if multiple slices are performed to reconstruct volumes. However, these should not be used in the models, because in many diseases the apparent volume can be normal and body composition abnormal (227).

A further expansion of the 4-C is the multi-compartment systems (anatomical, molecular, cellular and functional) (227). The five-compartment model is built from the molecular multi-compartment system and FFM is divided into total body proteins and total body water. Total body mineral is estimated from BMC plus soft mineral tissue. In addition, total body glycogen is calculated (230) (Figure 12).

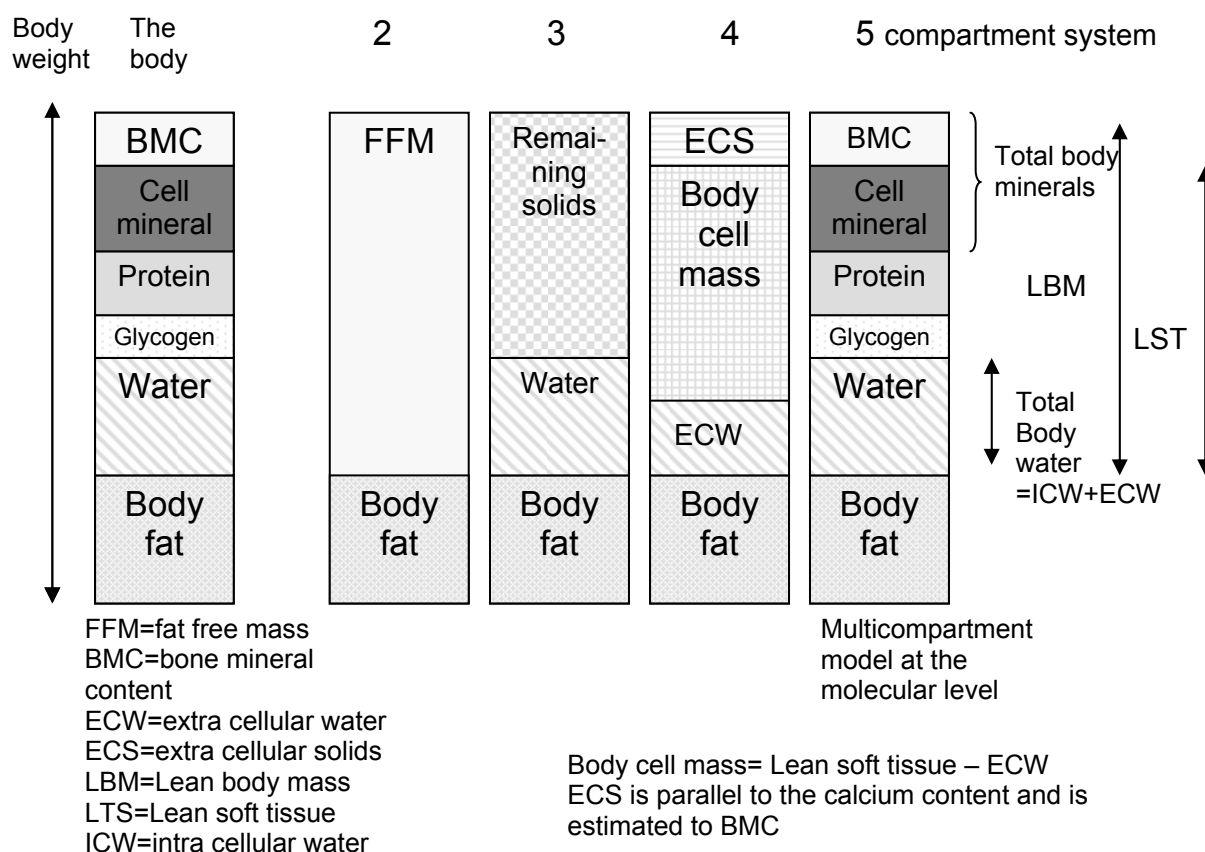


Figure 12 Schematic drawing of content in different compartment models.

F. Functional methods

1. Muscle strength

Right and left hand-grip strength were determined *via* electronic grip force equipment (Gripit®), which estimates the maximum momentary force and the mean force during 10 s in Newtons (N) with a variability of 4.4-9.1% (231).

2. Baecke questionnaire

Physical activity was evaluated through a questionnaire developed by Baecke et al in 1982 (232), who assessed indices of habitual physical activity at work, sport, and during leisure time. A high score indicates high physical activity.

G. Statistical methods

No statistics was performed in Paper I, as this was a descriptive study of the occurrence of mutations known to affect pituitary function.

In Paper II, the levels of thyroid hormones before and after rhTSH administration were analysed with paired t-test, and comparisons of hormone levels between groups were by unpaired t-test.

The statistical analyses in Paper III were covariance analyses for unbalanced designs. Treatment group comparisons and dose-response (linear compartment trend) tests were adjusted, in general, for age and sex. Additionally, analyses of BMI were adjusted for country. Adjustment for multiple comparisons was according to the Bonferroni method. The analyses of occurrence of AEs were with Fischer's exact test. Prevalence at baseline was compared with Chi-square tests.

Calculations on quantitative data in Paper IV were by paired t-test, as the number of participants was almost 60 this made the crucial demands of normal distribution and normal variability less important. In one variable, the number of specimens was <30 because of technical problems with the analyses, thus, the non-parametric Wilcoxon test was used. For qualitative variables, the QoL and Baeckes questionnaires, non-parametric test were used, as qualitative data are always categorical. In the SITT, IS was derived from the linear slope of P-glucose (233) between 4-15 minutes and was expressed as mmol/L glucose fall per minute and the correlation coefficient was determined with Pearsons correlation test.

H Considerations on Methods

1. Considerations on genetic methods

PCR is limited in that the amplification product is small, 0-5 kb, and the amount of material that can be cloned by one PCR is restricted. To repeat the same PCR is time-consuming and expensive. However, the major advantages with PCR are that it is quick, easy to perform and is very sensitive and robust. It is often possible to amplify DNA from badly degraded cells or those embedded in a medium (207); however, the latter was not possible in the deceased father of siblings in Paper I.

Testing a gene for a specific mutation or SNP is easier than sequencing the gene for any mutation. Therefore, it takes effort to identify a mutation not previously described, as might be considered in the sibling pair in Paper I with dominant inheritance, but no currently known mutation.

2. Considerations on biochemical methods

In Paper II, the expression of IGF-I in SDS enabled the comparisons of serum IGF-I levels between groups, and longitudinally. However, this did not overcome the prob-

lems with different IGF-I methods, and might have influenced the IGF-I results from different study periods and between different subgroups within the KIMS database.

In Paper II and IV, the same IGF-I method was used throughout studies, therefore, the absolute values were expressed. In Paper II, there were small differences in gender and age distribution of the groups, but this was unlikely to have influenced the results.

Insulin resistance is a major risk factor for the development of diabetes mellitus (234) and the euglycemic clamp technique is the gold standard for the investigation of IS (235). However, it requires sophisticated equipment and expertise, and is time consuming and expensive. ITT (235) may assess IS, but it is complicated by the counter-regulatory hormone responses and the risk of severe hypoglycaemia. The SITT used in Paper IV, is a validated instrument for assessing IS without the same risk of hypoglycaemia (233). SITT have a good correlation with the euglycemic clamp for evaluating IS (233) and is performed for only 15 minutes, before the counter-regulatory hormones are released.

3. Considerations on anthropometry

In KIMS, there is no guarantee that anthropometric measurements are performed in the same way by all physicians including patients, even though detailed instructions exist, and scales for body weight may be calibrated differently. The lack of total control over the performance of a study is a disadvantage with large observational database surveys. However, the advantage is the size of the surveys, which is unlikely to be archived in traditional multi-centre studies. These kinds of surveys have a lower degree of evidence than larger DBPC trials.

4. Considerations on QoL scores

Which QoL score is the best for assessing QoL in GHD and during GH treatment? Several questionnaires are used, but few have been validated in GHD patients. Even commonly used the NHP and PGWB scores are general QoL scores and not designed specifically for GHD (201, 221-223). The NHP sub-domains emotional reactions and energy (1, 133) and four sub-domains in the PGWB score (236) may be more sensitive for GHD. However, in discontinuation studies for 3-months (201, 237) or one-year (195), the NHP score was unable to detect any difference in QoL. QoL-AGHDA score is a disease specific questionnaire for GHD (113, 224, 225) and is a reliable tool for assessing QoL changes during GH treatment (113).

5. Considerations on body composition

BIA is considered to work well for patients with stable electrolyte and water balance (238). However, assessment of minor changes in FFM or BF is limited and longitudinal observations have less precision (238). In GHD, BIA may underestimate absolute amount and changes in BF during GH treatment (230), as BIA reflects the electrical conductivity in the extremities (226) and in GH treatment of GHD it is the central fat deposits that are primarily affected (14, 104). Moreover, a stable hydration level is important for the accuracy of the BIA method and GH treatment affects fluid distribution, hence BIA is an indirect measure of FFM and BF.

Concerns have been raised whether DXA may be affected by fluid changes in LST. There are small, but predictive errors in DXA soft tissue calculation that can arise after fluid distribution changes (239). The main weaknesses of this method is that the rays' travel distance through the body is unknown and the tissue is considered homogeneous; thus the result is less accurate in areas containing several tis-

sues, such as the abdomen (230). DXA may underestimate BF in patients with a large waist circumference (240).

There is no gold standard for measuring body composition. In endocrine diseases, such as GHD, there is considerable variation in the fluid component that may affect the reliability of measurements such as BIA and DXA. Therefore, CT would be the best option in this situation, especially as there is a large difference between the pixels of the attenuation for adipose and muscle tissues; however, CT evaluates an area that may also be affected if the water content of the patient is affected.

In Paper IV, a 4-C model was used that provided information on the protein and water content of the LST compartment: this information was not attained if with only DXA.

6. Considerations on functional methods

In GHD, exercise performance improves after GH treatment (127); however, it is unproven if physical activity changes in the measures performed. Functional evaluations of physical performance are not easily attained, as the questionnaires may not capture all aspects of altering physical function and handgrip measures evaluate the relative small muscles in the forearm. Handgrip has the advantage in that it is used in large trials and associated with functional outcome in patients (231, 241). The prerequisites for improved physical activity are muscle fibres, well-being, heart function and pulmonary capacity, which are all affected by GH treatment (1).

7. Considerations on statistics

The t-test is a robust test, meaning that the probability of rejecting the null hypothesis when it holds, is not seriously affected. T-tests are primarily sensitive to outliers, which tend to decrease efficacy and make it more difficult to detect real differences between groups. T-tests also demand a normal variance and a normal distribution. In Paper II, the analyses were performed with a t-test, even though the number of patients was limited. Therefore, the use of a non-parametric test might have been more appropriate. In addition, FT4 levels detected for many patients in the CH were <5.2 pmol/L, a range where the measurements are imprecise, which would argue for a non-parametric test.

A prerequisite for these analyses is that samples are independent. In covariance analyses (ANCOVA), the analysis is performed in respect to some other covariate (influencing factor). In Paper III the ANCOVA analyses were followed by post-hoc analyses. As the number of analyses increase, the risk for a type 1 error, i.e. the null hypothesis is wrongly rejected, is amplified. The Bonferroni method corrects for that risk. This means that instead of a level of significance of 5%, the significance level is divided by number of tests performed in an analysis. This guarantees that the simultaneous risk of a type 1 error is not higher than 5%. However, with Bonferroni corrections, achieving significance is more difficult.

In Paper III, the prevalence of diseases at baseline was evaluated with the Chi-square test, as these are categorical data. The null hypothesis implies independence of the variables, so knowledge of one disease does not give any information of the other disease. As the number of AE was low, the assumptions of the Chi-square test (the expected number shall not be <1 and the expected cell count should be at least 5 for at least 80% of the cells) were not fulfilled in Paper III, and the Fisher exact test (with very low power) was used.

VIII Main Results and Discussion

A Genetic causes of hypopituitarism in adults (Paper I)

1. Detection of a new allele combination in a heterozygous *PROP1* mutation

Among the 39 patients, two pairs of siblings were studied. In pair nr 18 and 19, the phenotypes were CO CPHD (GH+LH/FSH+TSH+ACTH) and a compound heterozygous mutation in the *PROP1* gene was detected in both siblings - codons 117 and 120: exon 3 p Phe 117 Ile (c 349 thymine (T)>adenine (A)) and p Arg 120 Cys (c 358 cytosine (C)>T). Additional analyses were performed on a healthy sister, who harboured the heterozygous mutation in exon 3 p Phe 117 Ile.

In family cases, *PROP1* mutations are detected in approximately 50% of cases and present as CPHD. Usually, patients have evolving deficiencies of GH, TSH, PRL and gonadotrophins, but the phenotype is highly variable with no correlation to genotype (21). The *PROP1* mutation results in a missense mutation of an AA change at codon 120 of arginine to cysteine, which leads to a protein with 12% retained function (242). Furthermore, the Phe 117 Ile mutation results in a T to A transversion at nucleotide 349, which produces a substitution of phenylalanine to isoleucine at codon 117, with a 5% protein binding capacity compared to normal (242). These mutations have been previously described (20, 242). Nevertheless, if Arg 120 Cys was described in a homozygous state (25, 33, 34, 242-244) and Phe 117 Ile in a homozygous (245) and in another form of compound heterozygous state (242) with other *PROP1* mutations, this allele combination has never been described before.

2. Phenotype of *PROP1* mutation -remarkable late debut of ACTH-insufficiency

The *PROP1* gene is essential for the differentiation of somatotrophs, thyrotrophs and gonadotrophs. The *PROP1* mutation has a variable phenotype (21, 26), also observed among the two cases in this study. Due to a currently unexplained mechanism, many *PROP1*-mutated patients develop ACTH-deficiency, most commonly during the second or third decade of life (23). The debut of ACTH-deficiency in the brother was remarkably late at age 53. However, there is a report of a sister from four siblings with the Arg 120 Cys mutation that developed ACTH-insufficiency in the fourth decade (243). In fact, 2.5 years before to diagnosis of ACTH-insufficiency, the patient was judged ACTH-sufficient, despite SST with a suboptimal increase in serum cortisol. A partial ACTH-insufficiency may have developed even though no symptoms had occurred. However, the debut is late.

3. A sibling pair with a dominant mode of heritage without proven mutations

Sibling-pair nr 1 and 3 consisted of one sister with childhood onset GHD in combination with adult onset TSH deficiency and uncertain development of ACTH insufficiency during pregnancy. The sister had a hypoplastic pituitary. The brother had CO IGHD. Both had an ectopic posterior pituitary. Analyses for known mutations in *PROP1*, *HESX1*, *LHX4* and *GH1* mutations gave negative results. The sister has two small children and the oldest daughter had verified IGHD.

The phenotypes of this sibling pair directed suspicion to the IGHD phenotype type II that has a dominant inheritance (Figure 13). The phenotype may be highly variable and even include other pituitary hormone deficiencies in the case of *GH1* splice site mutations (20, 246). However, a history of GH antibodies in the sister pointed at IGHD type 1a, but (20, 246) this problem was temporarily. MR findings in genetic IGHD are often normal and an EPP indicates at perinatal damage (27), only rarely is EPP detected in *GH1* mutations (27).

Inheritance	Type	Phenotype	Gene	Nature of mutations
Autosomal recessive	IA	Severe short stature, anti-GH antibodies on treatment	GH-1	Deletions, amino acid substitutions
	IB	Less severe short stature, No anti-GH antibodies	GH-1 GHRH	Splice site mutations, amino acid substitutions
Autosomal dominant	II	Less severe short stature, no antibodies	GH-1	Splice site mutations
X-linked recessive	III	Short stature (GHD) with agammaglobulinemia	?	?

Figure 13 The classification of mutations of isolated growth hormone deficiency (IGHD) (19).

4. No detected mutation in the sporadic cases

Even though mutations are few in large series of sporadic cases, no mutation was detected in the remaining non-familial cases (n=21). Patients selected for genotyping may have been misclassified, therefore, generating a low detection frequency. In addition, the mutation detection procedure might be inadequate, as direct screening of the coding exons of each gene did not allow, for example, ruling out potentially causal intronic variations. Furthermore, in an adult population, the number of other aetiologies increases with time, which lowers the frequency of genetic causes compared to that in children. An adult population may also suffer from the consequences of inappropriate replacement therapy, such as cortisol insufficiency (247) and unreplaced GHD (106), which is reported with increased mortality and leave fewer patients left for study. This may also be the case if genetic hypopituitarism influences life span, which is still an open question (248, 249). The frequency of genetic hypopituitarism in the Scandinavian countries is unknown until now, leaving the possibility for a lower frequency than in other countries.

5. Patients to be tested for genetic hypopituitarism

Genetic testing is expensive, time consuming and the phenotypes in childhood are well characterised. Therefore, selection criteria have emerged. Familial inheritance (27, 30), normally located posterior pituitary (32) and hormonal deficiency of pituitary origin (27), especially GHD in association with other anterior pituitary defects (30), indicate the most common genetic cause, the *PROP1* mutation. Conversely, sporadic cases (26), isolated GHD - especially in an adult population (246), presence of diabetes insipidus (21), hormonal deficiency of presumed hypothalamic origin (27), breech delivery (27), pituitary stalk interruption (PSI) (27) and EPP (27, 32, 250, 251) indicate causes other than currently identified genetic determinants, or as yet unknown genetic causes.

In this study, adult onset of hypopituitarism was a circumstance that decreased the likelihood of a genetic cause. Patients whose hypopituitarism developed during adulthood were included: this is a strength considering possible detection of unknown mild phenotypes with late-onset that would not have been caught during childhood.

In most cases, hypopituitarism associated with development gene mutations starts during childhood (23, 252) and only in rare cases do CPHD mutations present with a mild phenotype, with a normal final height (34). The *PROP1* mutated cases in this study were revealed at the age of 17, however, symptoms had emerged earlier. Therefore, the selection criterion of debut in childhood for genetic testing is supported.

B RhTSH testing in central hypothyroidism (Paper II)

1. Newly diagnosed CH patients have a poorer response to rhTSH than controls

Patients with newly diagnosed CH and no previous L-T4 treatment exhibited a less pronounced increase in thyroid hormone levels after administration of 0.9 mg rhTSH than controls (Figure 14a).

The rhTSH test may identify patients with CH, if $rT3 < 0.56$ nmol/L or $FT4 < 20$ pmol/L 48-72 hours after 0.9 mg of rhTSH (exact figures are assay specific). The peak FT4 overlapped, but the lowest FT4 level in the controls after 0.9 mg rhTSH was in one subject unable to leave specimens on two out of three occasions when FT4 used to peak. If this patient was excluded from the overlap analysis, no overlap existed between the newCH and the control group after 0.9 mg rhTSH. The discriminative value might be better than that observed in this study. Thus, a TSH-stimulation test may be useful in the diagnosis of CH.

Even at baseline, the controls and the newCH groups were separated in FT4, although some overlap existed. This is probably best explained by “regression to the mean” as the low thyroid hormone levels were used for selection into the study. NewCH patients with untreated GHD might have higher FT4 levels than they would have on GH replacement, because of a decreased peripheral deiodination of T4 to T3. Therefore, the CH of the newCH patients might be more severe than illustrated from FT4 levels.

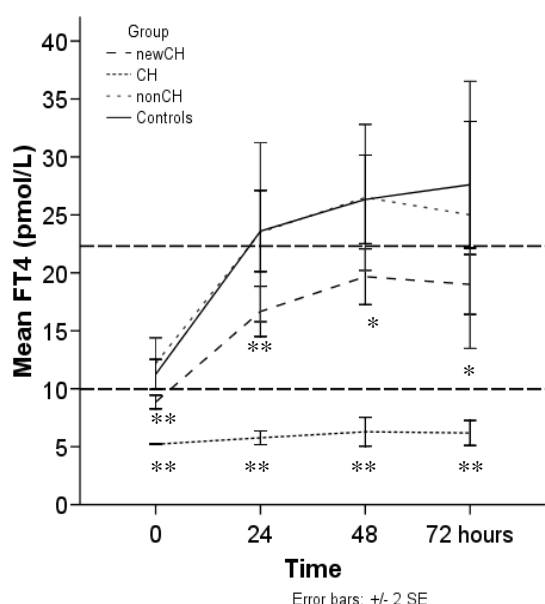


Figure 14a The figure shows the high-dose-rhTSH response (0.9 mg i.m) in six patients with previous treated central hypothyroidism (CH), six newly diagnosed CH (newCH), six patients with hypopituitarism without CH (nonCH) and six healthy controls in FT4. * $p < 0.05$, ** $p < 0.01$ newCH- or CH-group vs controls. Values are mean. Error bars = SEM. The horizontal broken lines represent normal range.

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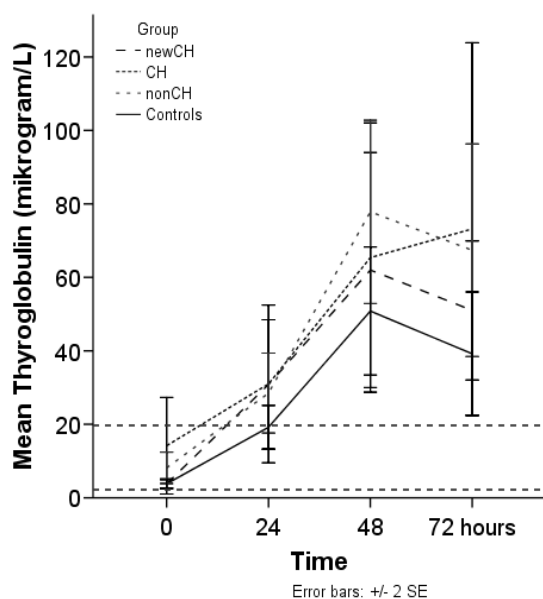


Figure 14b The high-dose-rhTSH response (0.9 mg i.m) in six patients with previously treated central hypothyroidism (CH), six newly diagnosed CH (newCH), six patients with hypopituitarism without CH (nonCH) and six healthy controls in thyroglobulin (without an extreme outlier in the CH group). Values are mean. Error bars=SEM

The horizontal broken lines represent normal range.

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Patients with and without TSH-deficiency displayed a similar increase in serum thyroglobulin levels in response to rhTSH (Figure 14b). This indicated the diminished response in thyroid hormones was not related to the unresponsiveness of the TSH receptor of thyroid follicular cells. One possibility for the less marked thyroid hormone response to rhTSH was decreased TSH receptor density in CH; however, this is contraindicated by the similar Tg increase among the groups. As endocytosis and, consequently, the removal of Tg from the lumen is diminished, the net result is a gradual accumulation of poorly iodinated Tg (253). This low-iodinated Tg has a low hormone content (254); hence, a smaller amount of hormones are released from the thyroid after TSH stimulation. This is probably the main reason for the blunted thyroid hormone response to rhTSH found in the two patient groups with CH. Individuals within the CH group probably had a less iodinated Tg than newCH individuals, most of whom apparently had partial stimulation of the thyroid with low – but detectable levels of thyroid hormones. Therefore, the sensitivity of this suggested diagnostic tool may be lower in mild cases and recent onset of central hypothyroidism.

2. The dormant thyroid gland in TSH-insufficiency can be awakened by TSH

Before the availability of rhTSH, an increase I^{131} thyroid uptake was observed in patients with CH after multiple repeated bTSH injections (81), demonstrating that a dormant gland can be activated. However, patients with severe CH (CH group) produced higher thyroid hormone levels after the second rhTSH injection, regardless of dose. This is most likely due to the activation of the thyroidal cellular system by TSH increasing the iodine content of the thyroid gland and thereby making it more responsive to the next stimuli (81).

3. Thyroglobulin increases similarly in all groups after rhTSH stimulation

The normal basal and stimulated Tg levels in CH (Figure 14b) contradict a reduction in the endocytosis of Tg. As the amount of Tg increases in the absence of TSH, the high concentration in the lumen may allow a large amount of Tg to be taken into the cell by endocytosis, in spite of the reduction in the volume of the endocytotic compartment. Moreover, although the major regulator of Tg synthesis is TSH (58, 59), insulin and IGF-I are able to stimulate Tg synthesis in the absence of TSH (255, 256). Insulin levels did not differ between groups and normal serum IGF-I levels were

found in all groups except newCH, where IGF-I levels were low, reflecting untreated GHD. Therefore, a reduction of basal Tg levels would be suspected in the newCH group. In addition to accumulation of Tg in the lumen, some other factor, yet unknown, may contribute to the normal Tg production in TSH-insufficiency.

C Metabolic outcome of GC replacement in hypopituitarism (Paper III)

1. CA has metabolic advantages over HC

The differences between types of GC and AS patients were few, HC-treated patients had increased HbA1c and prednisolone/dexamethasone-treated patients had increased waist-hip ratio, suggesting they may be less favourable choices for GC replacement than CA. Group size and country of origin (as mean BMI in general population differs between countries) could be confounding factors, although origin was statistically corrected for.

There are studies suggesting important differences between CA and HC. In untreated GHD patients, HC, but not CA, appears to produce a supra-physiological cortisol tissue exposure, which is attenuated by GH replacement. However, patients treated with CA are more vulnerable to the inhibitory effect of GH on 11 β HSD type 1, with a reduction of serum cortisol levels (187) and possible reduced tissue cortisol exposure (257). A drawback to calculating HCeq doses is that the estimates of bio-equivalent GC doses are based on the anti-inflammatory effect of GCs and not their metabolic effect. There are some indirect data suggesting that HC may have higher potency than CA with the traditional estimate (187). However, in the absence of an alternative method for normalising GC potencies, the published result has been maintained. DX and prednisolone are more potent GCs with longer plasma half-life and duration of action and they only need administering once daily. However, their high potency may lead to over-exposure to GC and thus contribute to the less favourable metabolic profile, as indicated in this study (258).

The results from Paper III, together with results from other studies, inferred that CA has benefits compared to HC, and should be advocated in the replacement strategy of ACTH-insufficient patients. In addition, dose adjustments of HC are often needed.

2. A dose-response between with HCeq dose and metabolic outcome

There was a clear GC dose response relationship with WC, serum triglyceride, total and LDL-C levels, and BMI (Figure 15a-d). Of interest was that patients receiving HCeq doses of less than 20 mg/day had a similar metabolic profile to those with an intact HPA-axis. This is, therefore, the first study to demonstrate that a mean GC replacement dose \geq 20 mg HCeq augments the metabolic perturbations associated with hypopituitarism and untreated GHD.

The association between GC and obesity, abdominal fat distribution and glucose intolerance is a recognised concept, illustrated by the situation in Cushing's syndrome. In GHD and obesity, there is an increased local 11 β HSD type 1 activity in adipose tissues (257, 259) resulting in increased local cortisol exposure (259). However, in this study, the relationship between these factors and dose of GC was not stronger in this large cohort of subjects, indicating that other endocrine insufficiencies may have strong modulating effects and attenuate some of the differences between the GC deficient and the sufficient patients. It is also possible that increased tissue exposure to GC, as a result of increased 11 β HSD type 1 activity occurring in both ACTH

deficient and replete GHD subjects, may exert a major influence over and above that resulting from exogenous GC dose. This is supported in a small study from 1995 by Dunne et al, who lowered the HC dose from 30 to 15 mg in patients, without detecting any metabolic differences (174).

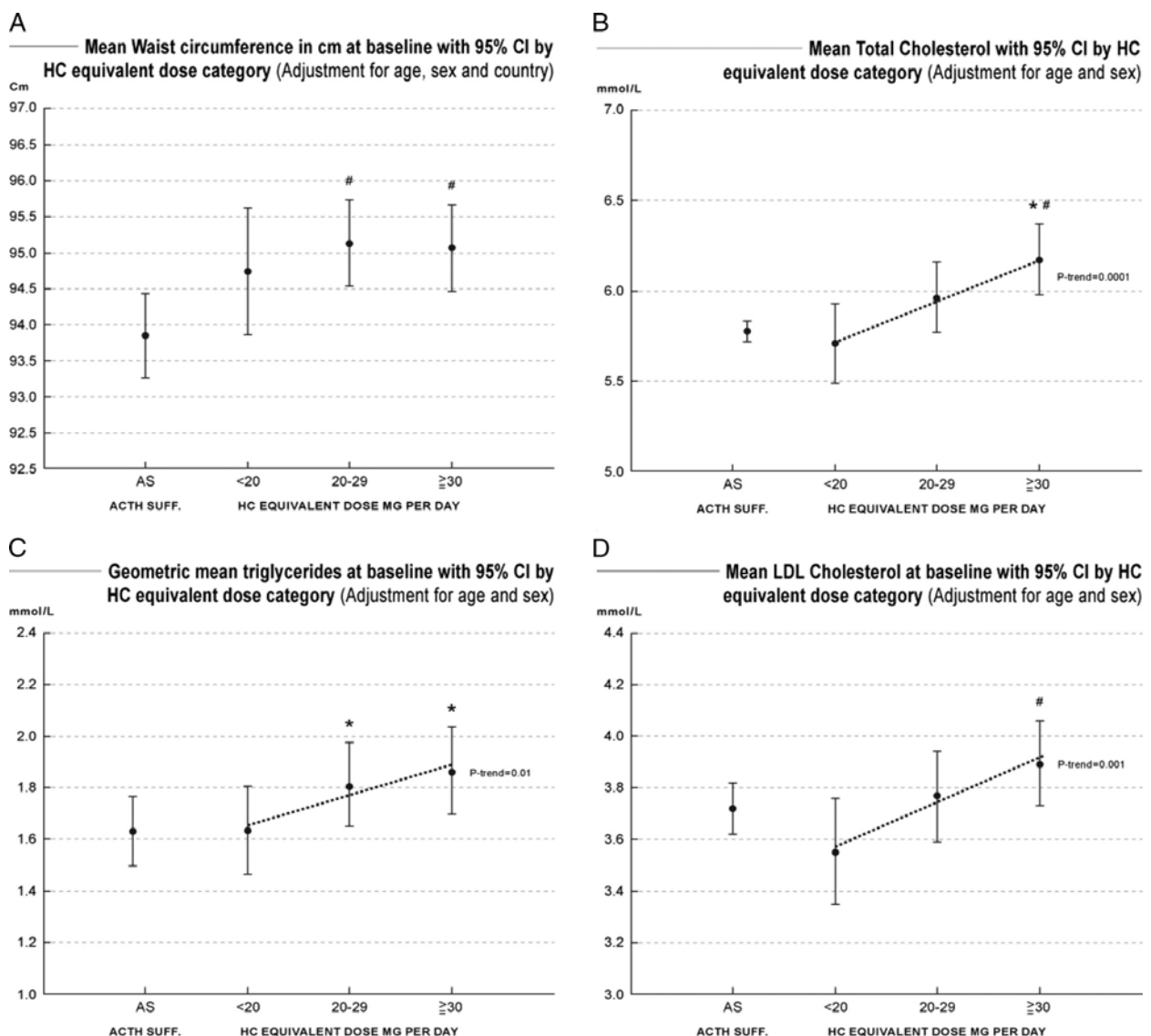
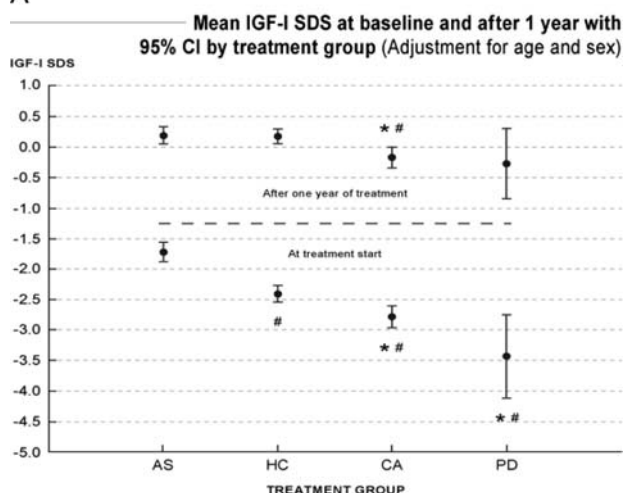


Figure 15a-d Hydrocortisone (HC) equivalent dose categories in ACTH deficient and ACTH sufficient patients with GHD before GH replacement. The broken line represents a dose response analysis within the glucocorticoid treated groups. **a** Waist, $*=p<0.001$ vs AS **b** Total cholesterol, $*=p<0.0001$ vs AS, $\#=p<0.0001$ vs <20 mg/day **c** Triglycerides^a, $*=p<0.001$ vs AS **d** LDL cholesterol, $\#=p<0.05$ vs <20 mg/day. ^aLogarithmic transformed triglycerides. Published in Journal of Clinical Endocrinology and Metabolism 2006 Oct;91(10):3954-61 Reprinted with the permission of Copyright ©2006 The Endocrine Society

3. Levels of IGF-I SDS were related to sort and dose of GC

Serum IGF-I SDS was lower in GC treated than in ACTH sufficient patients and the dose of GH was larger, probably reflecting the more pronounced hypopituitarism in this patient group. In addition, IGF-I SDS was higher in HC treated patients than in patients on CA and PD (Figure 16a) and an increasing dose of GC was associated with increased serum IGF-I, both at baseline and after one-year of GH treatment (Figure 16b).

A



B

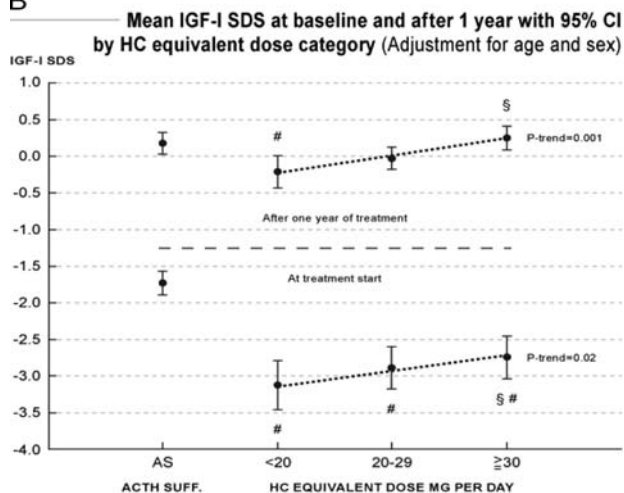


Figure 16a-b IGF-I SDS levels in 2424 GHD patients before and after one-year of GH, categorized according to **a** type and **b** hydrocortisone (HC) equivalent dose levels of glucocorticoid replacement in comparison with ACTH sufficient (AS) patients. CA = Cortisone Acetate, PD = Prednisolone/ Dexamethasone. SDS = standard deviation score.

a $\ast = p < 0.001$ vs HC adjusted for glucocorticoid dose, $\# = p < 0.001$ vs AS all four groups compared.

b $\# = p < 0.0001$ vs AS, $\S = p < 0.0001$ vs < 20 mg/day. The broken line represents a dose response analysis within the glucocorticoid treated groups.

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There are some data suggesting GC augments serum IGF-I generation (260) only in the presence of GH (261), and a direct hepatic effect of GC on IGF-I secretion after GH administration has been suggested (262).

In addition, total IGF-I levels may be reduced in obesity, predominantly with visceral localization (37). However, in this study increasing HCEq was associated with larger waist circumference, as well as higher IGF-I levels, which contradicted the previous statement. These possible mechanisms may support a direct GC effect on IGF-I levels and the above findings in the study.

4. Afterwards

The study of Paper III may also be put in the context of studies published afterwards. The pilot study of Danilowicz et al. in 2008 (177), where panhypopituitary patients without GH replacement were changed from 20-30 mg HC/day to 10-15 mg/day, underlines the message of Paper III that high GC doses often have adverse consequences. Danilowicz et al. evaluated metabolic parameters and QoL-AGHDA score and observed a significant weight reduction and lower total cholesterol and LDL-C concentrations; however, these results are difficult to interpret considering the uncontrolled study design. LBM, BMC and glucose homeostasis were not affected and QoL-AGHDA score improved after HC dose reduction. QoL-AGHDA score was not evaluated in Paper III but is currently evaluated. This is in accordance with the report of QoL in adrenal insufficient patients, where increased GC dose was associ-

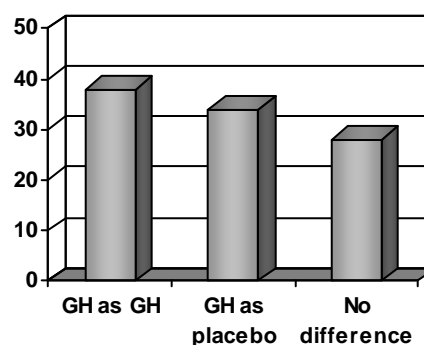
ated with impaired QoL (176). This study included both primary and secondary cortisol insufficient patients; however, it suffered from the selection bias from the 32% of patients receiving GH who achieved QoL that was worse than for unreplaced GHD patients.

D Discontinuation of GH in adult hypopituitarism (Paper IV)

1. The placebo effect was considerable

This study confirmed that after GH discontinuation for 4 months, patients presented many of the GHD features. However, a considerable placebo effect occurred in the patients' perception of their health (Figure 17) and as much as 34% of patients identified the GH period as the period when they received placebo.

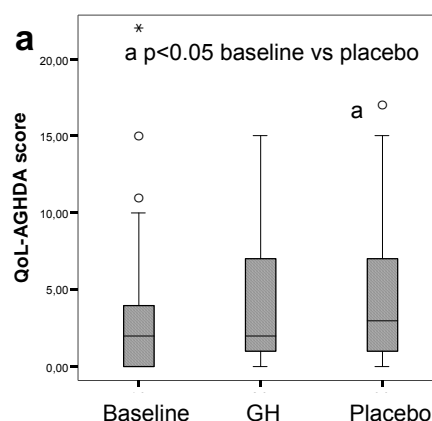
Figure 17 Patients own belief of treatment type. Of the 58 patients 38% correctly identified the GH period, whereas 62% of patients were unable to recognize the GH period (34% identified the placebo period as GH, 28% did not respond as they felt no difference), p NS=0.746 in the responders

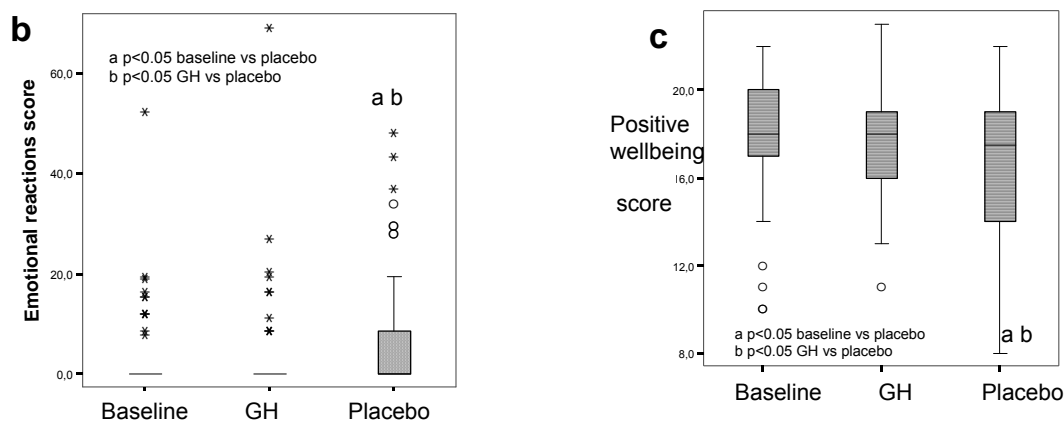


2. QoL deteriorated during placebo

In this study, some QoL variables were affected during non-GH treatment compared to the period patients received GH therapy: QoL-AGHDA score was impaired (higher) than at baseline (Figure 18a) during placebo and sub-variables emotional reactions and psychological well-being scores of the NHP and PGWB measures deteriorated during placebo compared to the GH period (Figure 18b-c).

Figure 18a-c QoL assessed by **a** Quality of Life-Assessment for Growth Hormone Deficiency in Adults (QoL-AGHDA), low score indicates good QoL; **b** Subscore of Emotional Reaction in the Nottingham Health Profile (NHP), low score indicates good QoL; and **c** Subscore of positive well-being in the Psychological General well being (PGWB) scores, high score indicates good QoL, in 58 hypopituitary patients before (baseline), during GH, and after 4-month-GH-discontinuation (placebo).





However, in therapy discontinuation studies, there is a risk of recruitment selection where patients with the worst initial QoL, and who improve the most with GH treatment (132), are reluctant to risk deterioration. The inclusion of these patients would emphasise these results further, as these patients are prone to more deterioration of QoL. Therefore, even though a lesser QoL effect was observed in this study, the results highlighted that QoL was impaired during discontinuation.

Moreover, in Sweden, GH treatment is universal to all adult GHD patients. This will affect QoL results, as the QoL effects are mixed by the influence of individuals not prone to QoL impairment after GH discontinuation. A similar study in the UK would have larger effects on the QoL data, as the GHD population is selected for treatment according to the NICE criteria (198) because of poor QoL. However, conducting the study in Sweden is an asset, as the recruitment selection is minimised and the results are valid for all GHD patients, as the metabolic benefit from GH does not correlate to QoL changes (199).

3. Discontinuation changed body composition towards a GHD state

The increased subcutaneous and visceral fat mass, decreased muscle area and ECW corresponded to the shifting into a GHD state and was consistent with a previous study on adult men (200), who discontinued GH after 18 months of treatment. Moreover, in the re-evaluation studies of adolescents, similar effects in body fat mass are observed as in this study (193), but data are not consistent (194).

Cortisol exposure in the adipose tissue increases when GH therapy is removed and is based on an increased cortisone to cortisol conversion from the enzyme 11 β HSD type 1 that is normally inhibited by GH (184, 185), which is probably the rationale behind the enhanced adipose tissue in this study, combined with a decline in the GH induced lipolysis (263).

In order to obtain anabolic effects and increased muscle function, a GH treatment period of more than 6 months is often needed (264), which is in agreement with the observation in this study. Questions over whether muscle function depend on GH have been raised, as longer-term GH studies are often open (264). Several studies have demonstrated short-term increase in thigh muscle size after GH (264), but whether this is a protein anabolic effect of GH or whether it reflects increased water content is open for debate. In discontinuation studies, LBM decrease (193, 200). In this study, ECW decreased in parallel with decreased BCM and muscle area. As the attenuation in muscle reflects the density, a maintained attenuation, as in this study,

indicates that the reduction in ECW is not the predominant cause of the decreased muscle area, a reduction in BCM may be a contributing factor. Conversely, as AT increased despite a reduction in ECW, it probably reflects a true increase in AT.

4. Impairment of metabolic parameters during GH discontinuation

This study ascertained increased serum total cholesterol concentration (+0.2 mmol/L), increased LDL-C (+0.25 mmol/L), increased CRP and increased WC (+ 1.5 cm), 4 months after discontinuing GH, as compared to during GH.

The effects in lipids, CRP and waist circumference were uncontroversial, as this is expected during GH discontinuation; however, this highlighted the metabolic consequences of discontinuation GH during a period as short as 4 months, as these are important cardiovascular risk factors and untreated GHD is connected with an increased mortality (5, 106, 107). For every 1 cm increase in WC, the cardiovascular risk increases by 2% (265) and for every 1 mmol reduction in LDL-C, the risk of major vascular events decreases by 20% (266). Therefore, in order to maintain the long-term metabolic effects of GH, continuous replacement with GH is needed.

5. An improvement of insulin sensitivity during GH discontinuation

In this study, HbA1c decreased and the insulin sensitivity improved during placebo, implying decreased insulin resistance.

GHD in adults is a state of insulin resistance (267) and patients on long-term GH treatment improved insulin sensitivity compared to the previous GHD state (111). When GH is discontinued abruptly; GH appears to have direct and immediate insulin antagonistic effects that overrule the effects of increased visceral fat mass on insulin resistance and result in enhanced insulin sensitivity during placebo treatment. Although significant, the increase in body fat may not have retrained to a full GHD state during four months of GH discontinuation.

IX Main findings

From this thesis, the main findings can be summarised as:

- Idiopathic hypopituitarism is a heterogenic state in which cases of genetic origin may be found, especially in familial cases. In addition, adult-onset of disease seldom points towards genetic causes, as the phenotypes mostly become symptomatic in childhood (Paper I).
- A compound heterozygous *PROP1* mutation with an allele variant association not previously reported was detected with a phenotype of very late onset ACTH-insufficiency in the brother (Paper I).
- Patients with newly diagnosed CH and no previous L-T4 treatment exhibited a less pronounced increase of thyroid hormone levels than controls after administration of rhTSH. Therefore, an rhTSH test may become useful as an adjuvant test in the diagnosis of CH (Paper II).
- Patients with and without TSH-deficiency displayed a similar increase in serum thyroglobulin levels in response to rhTSH, suggesting a responsive TSH receptor with similar sensitivity and functioning endocytosis. The reason to this is unknown (Paper II).
- Cortisone acetate may have some metabolic advantages over to HC during GC replacement therapy (Paper III).
- Patients receiving HCeq doses of <20 mg daily did not differ in their metabolic profile from patients with an intact hypothalamus-pituitary-adrenal axis. Moreover, with increasing HCeq dose, higher BMI, WC, total cholesterol, LDL-C and triglycerides were observed. These data suggested that many patients with ACTH-insufficiency were treated with too high glucocorticoid doses (Paper III).
- A 4-month period of GH discontinuation in patients on long-term GH replacement resulted in deteriorated QoL, increased subcutaneous and visceral AT, increased waist circumference, deteriorated lipid profile, increased CRP and improved insulin sensitivity compared to during GH treatment. This study highlighted the importance of continuous GH treatment in adult GHD patients in order to maintain beneficial long-term effects of GH replacement (Paper IV).

X Future Aspects

To conduct studies that bring knowledge to a meaning through direct consequences in improved patient care must be the purpose of all clinical research. Detecting a genetic mutation has implementations for the patient. Through an early postnatal diagnosis, the patient will gain optimal treatment for the pituitary deficiencies. Puberty outcome can be predicted and the development of additional insufficiencies determined. Avoiding over-treatment of pituitary axes that will be unaffected by the pituitary genetic disease is important. Moreover, families may be screened for an early diagnosis. As genetic considerations may be more common in the future, the detection procedure will be more generally available. A study of all IPI patients in Sweden with CO of disease would be warranted and produce a complete mapping of the frequency of mutations. In addition, trying to sequence new mutations in patients with familial concentration would be advocated. Conducting such a study in Sweden is attainable, as the Swedish health care system contains all hypopituitary cases with CO origin.

The concern about CH is more common than the number of CH cases detected. In addition, the sensitivity of existing diagnostic tools is inadequate, leading to both over- and under-treatment with L-T4. Before any conclusions can be drawn concerning

the discriminative value of the TSH test, the trials should be extended to patients with mild CH or with indefinite diagnosis. Unfortunately, there is no commercial interest in CH and the rhTSH needed is expensive, rendering the prospect of future studies uncertain. In addition, rhTSH test may be performed with a dose of 0.3 mg, which is well tolerated, and has proven useful in treatment before radioiodine of non-toxic goitre. Moreover, the model of TSH stimulation in CH presents good conditions for studying the effect on extrathyroidal TSH receptors.

Paper III has attracted much attention, and is cited by others: it confirms the indications from smaller studies that GC doses are too high. This paper has already had effects on the care of many patients. However, KIMS is an open surveillance study, connected with known limitations. To resolve the issue whether some GC doses within the normal replacement range are more beneficial than others are, a double-blind study of two different replacement categories must be conducted.

The Disco study (Paper IV) may have future implications as it argues for continuous GH treatment, especially in Sweden, as an audit will be performed during 2009 to evaluate the indications for GH treatment in adults. However, as the consequences of GH replacement are judged differently in different countries, it may have implications for other countries attitudes towards adult GHD treatment. This study may therefore be a future landmark. In addition, the major issue in GH treatment is its effect on the increased mortality in hypopituitary patients. A future possibility for acquiring more information is to compare the Swedish population, where GH treatment is administered to all adult GHD patients not having exclusion criteria, with a hypopituitary population, with similar socio-economic, health and mortality patterns, but where adult GHD treatment is not prescribed to everyone so mortality and morbidity can be evaluated.

XI Concluding remarks

This thesis contributes to the increasing knowledge of diagnosis and treatment of adults with pituitary insufficiency. The more we learn the more humble we become when faced with the complexity of the human body. The interplay between the pituitary and the peripheral responding organs is intricate, yet, with rough tools, we manage to replace hormones in these patients bringing them back to an improved QoL. Knowing this, and to have contributed in some areas to make things better, is a fortune and pushes life to its edges. Research brings a greater depth to knowledge for the individual clinician and a different understanding is gained. This thesis comprised several journeys. Firstly, it has been a journey through genetic mutations, central hypothyroidism, metabolic effects of cortisol and the mostly marks of GH. Secondly, it has been a journey in the management of research knowledge, remembering the first struggle in my first research protocol. Thirdly and mostly, a journey of personal development and insights - a journey that is not yet complete.

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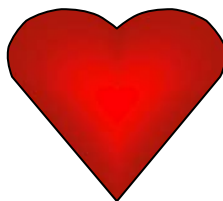
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