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Borghammar, Camilla

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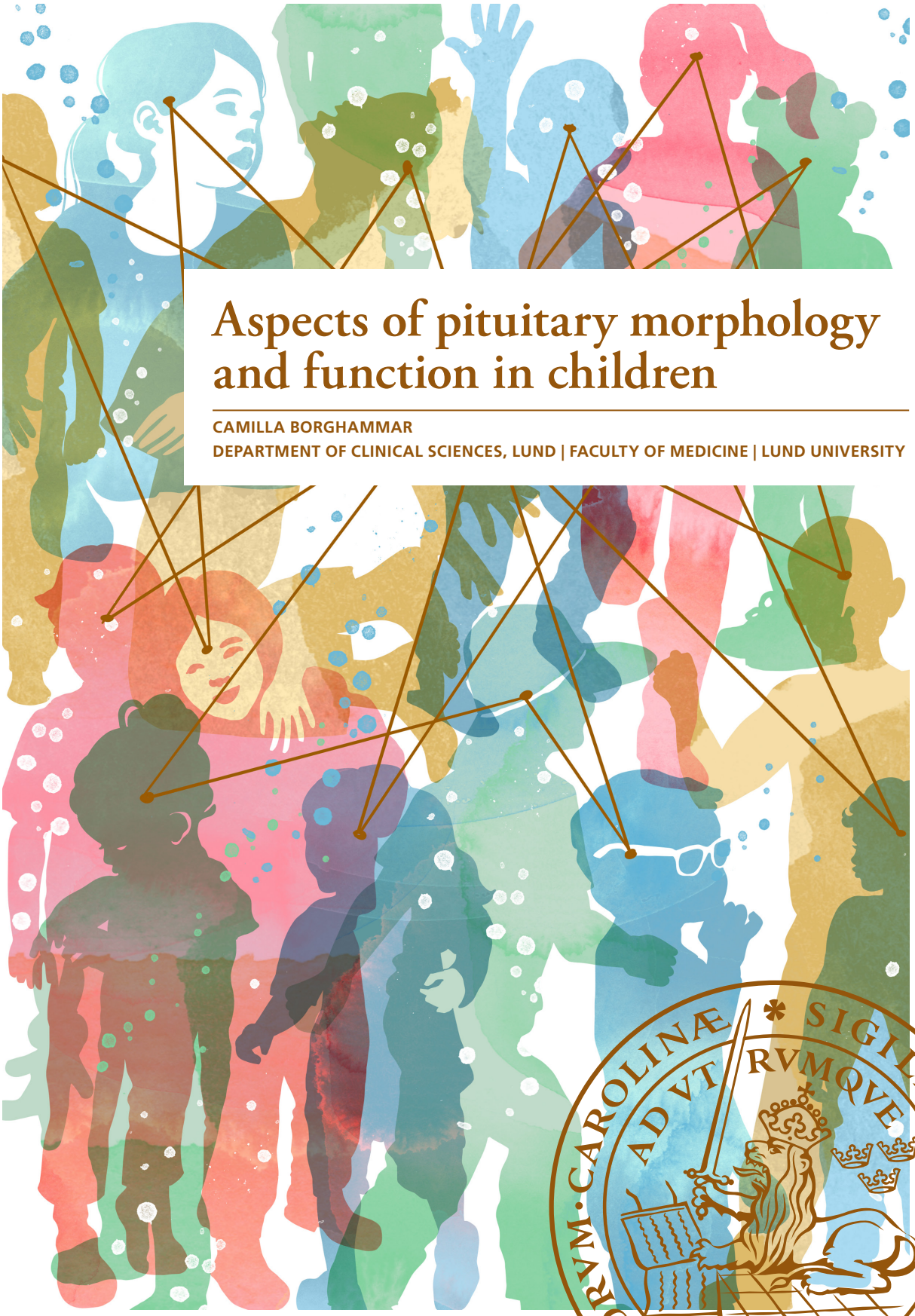

Aspects of pituitary morphology and function in children

CAMILLA BORGHAMMAR
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

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Camilla Borghammar

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 19th of December at 09.00 in Belfragesalen BMC, Klinikgatan 32, Lund

Faculty opponent
Professor Ola Nilsson
Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

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Abstract

Background: Evidence-based guidelines for assessing pituitary function in children remain limited. Improved understanding of paediatric pituitary diseases may improve clinical management and patient counselling.

Aim: This thesis aimed to evaluate diagnostic aspects of paediatric pituitary diseases, focusing on radiological follow-up of pituitary microadenomas and cysts, pituitary refractoriness to growth hormone (GH) secretion, and plasma cortisol and GH levels during stimulation tests.

Methods: Studies I–III were retrospective reviews of medical records. Study I included 74 children with a non-functioning pituitary microadenoma or cyst <10 mm on magnetic resonance imaging (MRI). Study II assessed GH levels in 257 children with short stature using a short spontaneous nocturnal profile (SSNP) and an arginine insulin tolerance test (AITT). Study III evaluated cortisol levels in 171 short-statured children using a glucagon stimulation test (GST). Study IV was a prospective study of adrenal function in 26 children treated with high-dose dexamethasone for acute lymphoblastic leukaemia (ALL), assessed with extended glucocorticoid analysis and low-dose ACTH tests two days and two weeks post treatment.

Results: Study I revealed that no microadenoma or cyst increased significantly in size during follow-up in children without hormonal abnormalities. In Study II, 21.9% of the children had a GH peak below the cut-off (≥7 μg/L) in the AITT, despite a normal GH peak during SSNP. In Study III, peak cortisol during the GST negatively correlated with age (r_s = -0.26, p <0.001). Females had higher peak cortisol than males (667.5 nmol/L vs. 602 nmol/L, p = 0.005), a finding that remained after adjustment for age (β (95% confidence interval) 65.3 (15.9–114.6), p = 0.01). In Study IV, peak cortisol during low-dose ACTH testing correlated with cortisol/cortisone ratio (r_s = 0.39, p = 0.0497) and increased significantly in the second test among children who exceeded the cortisol cut-off (≥450 nmol/L) in both tests (p = 0.018).

Conclusion: The likelihood of size progression in non-functioning pituitary microadenomas or cystic lesions appears small. Moreover, in GH stimulation tests, pituitary refractoriness to GH production after a prior spontaneous peak should be considered. Concerning plasma cortisol evaluation, sex- and age-specific reference intervals may be necessary. Further research is needed to determine cortisol cut-offs and the role of the cortisol/cortisone ratio in evaluating adrenal function in children.

Key words: pituitary microadenoma, pituitary cyst, MRI follow-up, growth hormone refractoriness, childhood cortisol levels, cortisol/cortisone ratio, spontaneous nocturnal GH profile, insulin arginine tolerance test, glucagon stimulation test, low-dose ACTH test.

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To my family

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Populärvetenskaplig sammanfattning

Bakgrund och syfte

Hypofysen är en liten körtel, med stor betydelse för kroppens hormonproduktion. Den sitter centralt i hjärnan och styr bland annat produktionen av könshormoner, tillväxthormon, sköldkörtelhormon och kortisol - vårt viktigaste stresshormon. Hypofysen är också viktig för kontrollen av kroppens vätskebalans. I många avseenden saknas tydliga, vetenskapligt underbyggda riktlinjer för hur vi på bästa sätt undersöker hypofysens funktion hos barn. Att få mer kunskap om detta är viktigt för att barnen ska kunna få rätt diagnos och behandling, utan onödiga undersökningar eller felaktiga läkemedel.

När man undersöker hypofysens hormonproduktion är det sällan tillräckligt att bara ta ett blodprov. I många fall behöver också så kallade stimuleringstester genomföras, där barnet får ett läkemedel som stimulerar till hormonproduktion från hypofysen. I vissa fall kan man även behöva göra röntgenundersökningar, oftast magnetkameraundersökningar (MR), för att upptäcka om det finns strukturella förändringar, som till exempel tumörer, som påverkar hypofysens funktion.

Tack vare modern MR-teknik kan vi upptäcka mycket små förändringar i hypofysen. Detta har dock också en baksida och innebär att vi ibland ser förändringar som sannolikt saknar betydelse för barnets hälsa. Sådana bifynd kan även vara artefakter, det vill säga ett bildtekniskt fel som kan misstolkas som en verklig anatomisk struktur. Exempel på bifynd i hypofysen är små cystor eller mikroadenom som inte producerar hormoner och är godartade förändringar. Det är osäkert hur vanliga dessa förändringar är hos barn, men studier uppger att mikroadenom utgör 3–15 % av alla hjärntumörer hos barn. Det finns även begränsade fakta kring tillväxtmönstret hos dessa förändringar hos barn, men ett fåtal tidigare studier talar för att risken för tillväxt är liten.

När ett mikroadenom, eller en liten cysta, upptäcks vid MR-undersökning av hypofysen görs ofta upprepade MR-undersökningar för att kontrollera om det sker någon tillväxt. Dessa upprepade MR-undersökningar är inte utan risk; små barn måste ofta sövas för att klara av undersökningen och kontrastmedlet som används vid undersökningen kan inlagras i hjärnan. MR bör därför bara utföras när det är absolut nödvändigt, både för barnets skull och för att prioritera undersökningstillfällena för de barn som har potentiellt elakartade hjärntumörer.

Även när det gäller beslut kring behandling med tillväxthormon är prioriteringar och utredningsgång viktigt. Behandlingen är dyr, innebär dagliga sprutor, regelbundna sjukhusbesök och kan medföra en risk för biverkningar. Det är därför viktigt att tillväxthormon endast ges till de barn som bedöms ha tydlig nytta av behandlingen, både av hänsyn till individens bästa och för ett ansvarfullt utnyttjande av sjukvårdsresurser.

Att utreda möjlig tillväxthormonbrist är komplext. Hormonet utsöndras naturligt i korta pulsatila toppar, vilket gör att dess nivå inte kan mätas med ett vanligt blodprov. För att utvärdera tillväxthormontopparna genomförs istället stimuleringstester, men även här finns det fallgropar. Bland annat finns det en risk att barnet har haft en naturlig hormontopp innan testet, och att hypofysen därför är tillfälligt ”uttröttad” och oförmögen att svara på stimuleringen. Detta fenomen kallas för refraktäritet och kan medföra att barnet felaktigt får diagnosen tillväxthormonbrist och onödig behandling med tillväxthormon.

Kortisol är ett livsviktigt hormon som behövs framför allt när kroppen utsätts för stress, såsom vid allvarliga infektioner, olyckor eller kirurgi. Kortisol produceras i binjurarna, som in sin tur styrs av hypofysen. Barn som behandlas med läkemedel som innehåller kortison riskerar att binjurarna förtvinar och slutar bilda kroppseget kortisol. Sådan binjuresvikt kan vara ett mycket allvarligt och ibland potentiellt livshotande tillstånd. Det är därför viktigt att kunna identifiera de barn som har risk för att utveckla sviktande binjurefunktion, vilket även detta kan göras via stimuleringstester. Idag finns det inga tydliga riktlinjer för hur höga kortisolnivåer barn normalt har vid sådana tester. Det är också fortsatt oklart hur faktorer såsom ålder och kön påverkar den normala kortisolnivån, och vilket simuleringstest som är mest tillförlitligt.

Syftet med denna avhandling var att undersöka strukturella och diagnostiska aspekter av hypofyssjukdomar hos barn. Fokus var särskilt på att utvärdera om små förändringar i hypofysen (mikroadenom och cystor <10 mm) behöver följas upp med upprepade MR-undersökningar, hur vanligt det är med refraktäritet när tillväxthormon mäts vid stimuleringstester, samt kortisolnivåer vid stimuleringstester hos kortvuxna barn och hos barn som behandlats med höga doser kortisoninnehållande läkemedel.

Metod

Denna avhandling innefattar fyra olika vetenskapliga studier (Studie I–IV). Studie I–III genomfördes som journalgranskningsstudier där vi identifierade och inkluderade barn som:

- hade små förändringar i hypofysen som identifierats via MR-undersökning,
- hade genomgått nattlig mätning av spontan tillväxthormonproduktion och efterföljande stimuleringstest av tillväxthormonproduktionen via arginin-insulin-toleranstest (AITT), eller
- hade genomgått stimuleringstest av kortisolproduktionen via glukagontest.

I Studie IV inkluderades fortlöpande barn med nydiagnostiserad akut lymfatisk leukemi (ALL). I den initiala behandlingen mot ALL ingår höga doser kortisoninnehållande läkemedel. För att bedöma binjurarnas förmåga att producera

kortisol efter avslutad kortisonbehandling genomgick dessa barn blodprovstagning och stimuleringstester (lågdos ACTH-test).

Alla studier har godkänts av etikprövningsnämnden.

Resultat och slutsatser

Resultaten från våra studier visade att:

- Risken för tillväxt av små, icke hormonproducerande, godartade hypofystumörer (mikroadenom och cystor <10 mm) är liten. Tät och långvarig uppföljning med MR av hypofysen är därför sällan nödvändig om barnets hormonnivåer är normala.
- Vid stimuleringstest (AITT) för bedömning av tillväxthormonproduktion kunde en femtedel av barnen inte uppnå normala tillväxthormontoppar, trots att de hade normala nivåer under den föregående nattliga registreringen av spontana tillväxthormonnivåer. Detta fenomen, när hypofysen blir tillfälligt oförmögen att utsöndra tillväxthormon efter en tidigare spontan hormontopp, är viktigt att känna till eftersom det annars kan leda till en felaktig tillväxthormonbrist-diagnos.
- Glukagontest bedöms vara ett bra alternativ till andra typer av stimuleringstester (som till exempel ACTH-test eller insulintolerans-test) för att bedöma binjurens funktion och kortisolproduktionen hos barn.
- Kortisolnivåer i blodet påverkades av både ålder och kön. Äldre barn hade lägre toppnivåer än yngre, och flickor hade generellt högre kortisolnivåer än pojkar. Detta talar för att referensvärden anpassade efter barnets ålder och kön kan behövas; sådana finns inte tillgängliga idag.
- Ett möjligt samband noterades mellan toppnivåer av kortisol vid stimuleringstest (lågdos ACTH-test) och kroppens omvandling mellan biologiskt inaktivt och aktivt kortisol. Barn som hade högre kortisolnivåer hade högre andel aktivt kortisol, och lägre andel av den biologiskt inaktiva formen. Detta kan ha betydelse för att bättre förstå omständigheterna kring sviktande binjurefunktion hos barn.

Genom detta projekt hoppas jag kunna bidra till det viktiga arbetet med att ta fram vetenskapligt förankrade riktlinjer för utredning av barn med misstänkta sjukdomar i hypofysen. Min förhoppning är att våra resultat kan leda till färre onödiga MR-undersökningar, minskad risk för felaktig diagnostisering av tillväxthormonbrist, och förbättrad utredning och diagnostik av binjuresvikt och kortisolbrist. Jag hoppas även att arbetet skall kunna bidra till att spara sjukvårdsresurser och fördela dem till de som behöver dem mest. Målet är att varje barn ska få både rätt diagnos och behandling, varken mer eller mindre.

List of papers

This thesis includes the following papers, which are referred to throughout the text by their Roman numerals:

I. **Borghammar C**, Tamaddon A, Erfurth EM, Sundgren PC, Siesjö P, Elfving M*, Nilsson M*. Non-functioning pituitary microadenoma in children and adolescents: Is follow-up with diagnostic imaging necessary? Endocrine. 2023 Jan;79(1):152-160. doi: 10.1007/s12020-022-03212-7. Epub 2022 Oct 17. Erratum in: Endocrine. 2024 Jul;85(1):454. doi: 10.1007/s12020-023-03541-1. PMID: 36251115; PMCID: PMC9813011. **These authors share last authorship.*

II. **Borghammar C**, Boije V, Becker C, Lindberg B, Elfving M. Prevalence of refractoriness when testing growth hormone levels in children. Growth Horm IGF Res. 2023 Aug;71:101549. doi: 10.1016/j.ghir.2023.101549. Epub 2023 Jul 24. PMID: 37562165.

III. **Borghammar C**, Svensson J, Tidblad A, Elfving M. Sex and age differences in cortisol levels during glucagon stimulation test in children. BMC Pediatr. 2025 May 31;25(1):440. doi: 10.1186/s12887-025-05784-5. PMID: 40450209; PMCID: PMC12125804.

IV. **Borghammar C**, Holmqvist AS, Romerius P, Becker C, Malmros J, Elfving M. Cortisol/cortisone ratio and early peak cortisol levels in low-dose ACTH stimulation tests after treatment with dexamethasone in children with acute lymphoblastic leukaemia. Manuscript.

Abbreviations

11ß-HSD	11ß-hydroxysteroid dehydrogenase
ACTH	adrenocorticotropic hormone
ADH	antidiuretic hormone
AITT	arginine-insulin-tolerance test
ALL	acute lymphoblastic leukaemia
ALS	acid-labile subunit
AST	arginine stimulation test
AVP	arginine vasopressin
BMI	body mass index
CAH	congenital adrenal hypoplasia
CBG	corticosteroid-binding globulin
CI	confidence interval
CL	cystic lesion
CRH	corticotropin-releasing hormone
CT	computer tomography
CV	coefficient of variation
FSH	follicle-stimulating hormone
GH	growth hormone
GHBP	growth hormone-binding protein
GHD	growth hormone deficiency
GHIH	growth hormone-inhibiting hormone (somatostatin)
GHR	growth hormone receptor
GHRH	growth hormone-releasing hormone
GnRH	gonadotrophin-releasing hormone
GST	glucagon stimulation test
HPA	hypothalamic-pituitary-adrenal
ICD	International Statistical Classification of Diseases and Related Health Problems

IGF-1	insulin growth factor-1
IGFBP	IGF-binding proteins
ITT	insulin tolerance test
KVÅ	klassifikation av vårdåtgärder [classification of care actions]
LH	luteinizing hormone
MA	microadenoma
MPH	mid-parental height
MPHD	multiple pituitary hormone deficiency
MRI	magnetic resonance imaging
MSH	melanocyte-stimulating hormone
NFPAs	non-functioning pituitary adenomas
PA	probable microadenoma
PACS	Picture Archiving and Communication system
PIH	prolactin-inhibiting hormone (dopamine)
POMC	proopiomelanocortin
PRL	prolactinoma
rhGH	recombinant human growth hormone
SDS	standard deviation score
SGA	small for gestational age
SSNP	short spontaneous nocturnal profile
T	tesla
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone

Abstract

Background: Evidence-based guidelines for assessing pituitary function in children remain limited. Improved understanding of paediatric pituitary diseases may improve clinical management and patient counselling.

Aim: This thesis aimed to evaluate diagnostic aspects of paediatric pituitary diseases, focusing on radiological follow-up of pituitary microadenomas and cysts, pituitary refractoriness to growth hormone (GH) secretion, and plasma cortisol and GH levels during stimulation tests.

Methods: Studies I–III were retrospective reviews of medical records. Study I included 74 children with a non-functioning pituitary microadenoma or cyst <10 mm on magnetic resonance imaging (MRI). Study II assessed GH levels in 257 children with short stature using a short spontaneous nocturnal profile (SSNP) and an arginine insulin tolerance test (AITT). Study III evaluated cortisol levels in 171 short-statured children using a glucagon stimulation test (GST). Study IV was a prospective study of adrenal function in 26 children treated with high-dose dexamethasone for acute lymphoblastic leukaemia (ALL), assessed with extended glucocorticoid analysis and low-dose ACTH tests two days and two weeks post treatment.

Results: Study I revealed that no microadenoma or cyst increased significantly in size during follow-up in children without hormonal abnormalities. In Study II, 21.9% of the children had a GH peak below the cut-off (≥7 μg/L) in the AITT, despite a normal GH peak during SSNP. In Study III, peak cortisol during the GST negatively correlated with age (r_s = -0.26, p <0.001). Females had higher peak cortisol than males (667.5 nmol/L vs. 602 nmol/L, p = 0.005), a finding that remained after adjustment for age (β (95% confidence interval) 65.3 (15.9–114.6), p = 0.01). In Study IV, peak cortisol during low-dose ACTH testing correlated with cortisol/cortisone ratio (r_s = 0.39, p = 0.0497) and increased significantly in the second test among children who exceeded the cortisol cut-off (≥450 nmol/L) in both tests (p = 0.018).

Conclusion: The likelihood of size progression in non-functioning pituitary microadenomas or cystic lesions appears small. Moreover, in GH stimulation tests, pituitary refractoriness to GH production after a prior spontaneous peak should be considered. Concerning plasma cortisol evaluation, sex- and age-specific reference intervals may be necessary. Further research is needed to determine cortisol cut-offs and the role of the cortisol/cortisone ratio in evaluating adrenal function in children.

Overview of this thesis

Study	Aim	Method	Results	Conclusions
I	To evaluate the growth potential of pituitary solid and cystic lesions <10 mm and to evaluate the accuracy of MRI measurements.	Retrospective study of 74 children <18 years old, diagnosed with a pituitary microadenoma or cyst <10 mm in MRI. All MRI examinations were re-evaluated.	None of the pituitary lesions in children without hormonal disturbances increased significantly in size during follow-up.	The likelihood of size progression in small non-functioning pituitary microadenoma or cystic lesion is very low.
II	To evaluate the GH response during an AITT after a GH peak during a short spontaneous nocturnal profile.	Retrospective study of 257 short statured children, ≤18 years old, who had undergone an AITT following a short spontaneous nocturnal GH profile. A peak GH of ≥7 µg/L was considered normal.	Of the children who had a normal GH peak in either of the tests (201/257), 21.9% were refractory and could not produce a GH peak ≥7 µg/L in the AITT, despite having a GH peak ≥7 µg/L in the short spontaneous nocturnal profile.	Refractoriness of the pituitary to produce GH in a stimulation test after a previous spontaneous peak needs to be considered when evaluating the GH response in children.
III	To evaluate cortisol levels in GST and to assess how clinical parameters correlate to cortisol levels.	Retrospective study of 171 children, ≤18 years old, with short stature who had undergone a GST. Both GH levels- and cortisol levels were evaluated. A peak cortisol of ≥450 nmol/L was considered normal.	Girls and younger children had higher peak cortisol levels in the GST. The GST is a reliable method for assessing the HPA axis in children.	There may be a need for sex- and age-weighted reference intervals for plasma cortisol in children.
IV	To evaluate cortisol/ cortisone ratio, serum dexamethasone and peak plasma cortisol levels during low-dose ACTH tests in children with ALL, after treatment with dexamethasone.	Prospective study of 26 children <18 years, diagnosed with ALL and treated with dexamethasone. Adrenal function was evaluated with repeated low-dose ACTH tests and an extended glucocorticoid analysis. A peak cortisol of ≥450 nmol/L was considered normal.	A correlation was found between peak cortisol and cortisol/cortisone ratio. Cortisol levels in the low-dose ACTH test increased between the first and second test in children with two normal tests.	The correlation between peak cortisol levels and cortisol/cortisone ratio suggests that a 11β-HSD-mediated conversion of cortisone to cortisol is important for higher cortisol levels during testing.

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; ACTH, adrenocorticotropic hormone; AITT, arginine insulin tolerance test; ALL, acute lymphoblastic leukaemia; GH, growth hormone; GST, glucagon stimulation test; HPA, hypothalamic-pituitary-adrenal; MRI, magnetic resonance imaging.

Author's contribution to the studies

I am the first author of all four papers included in this thesis. A description of my contributions to each project is provided below.

Study I

I initiated this project during my residency as part of the mandatory scientific project. It was only partially completed during that period and was finalised after I had been admitted as a doctoral student. My contributions to this project were as follows:

- participated in discussions on the study design and contributed to the development of the study database,
- identified and selected eligible patients through a review of medical records,
- collected clinical data from medical records,
- performed descriptive statistical analyses,
- drafted the initial manuscript and performed the subsequent revisions, and
- served as the corresponding author during the publication process.

Study II

In this project, I had a more prominent role and was involved from the outset. I served as a co-supervisor for a medical student who contributed to the data collection from medical records as part of a master's thesis. I subsequently finalised the work as a published article. The following were my contributions to the project:

- contributed to the study design and development of the study protocol,
- participated in writing the application for ethical approval,
- established the database for data management and reviewed medical records to identify eligible patients,
- initiated and finalised the collection of clinical data from medical records,
- participated in planning and executing the statistical analyses,
- wrote the first draft of the manuscript and revised the manuscript, and
- was responsible for journal communication, review, and manuscript submission.

Study III

During this project, I worked more independently compared with in the previous studies and maintained a more self-directed role throughout the manuscript preparation and publication process. I contributed to the project in the following ways:

- contributed to the design of the study and developed the research programme,
- wrote the application for ethical approval,
- identified eligible patients and developed the database for data storage,
- extracted all clinical data from medical records,
- performed all statistical analyses,
- wrote the manuscript's first draft and led the revision process, and
- handled all correspondence with the journal during the submission and review.

Study IV

This prospective, ongoing project presented entirely different challenges compared with the previous studies. I was involved from the outset and participated in all discussions concerning the planning and development of the study. Below is a description of how I contributed to this project:

- participated in study design and formulation of the research plan,
- assisted in writing the ethics application and designed the patient information sheets and informed consent forms,
- participated in drafting collaboration agreements between Lund University and the Childhood Cancer Centres in Lund and Stockholm,
- contributed to the structure of the REDCap database for data storage,
- provided interpretation of laboratory results and informed the families of the participating children (this responsibility remains ongoing), and
- extracted data for analysis, conducted statistical analyses, and wrote the first draft of the manuscript for the study presented as Study IV in this thesis.

Introduction

The pituitary gland

Morphology and embryology

The pituitary gland is centrally located in the brain, within a bone cavity of the sphenoid bone called the sella turcica [1, 2]. Positioned superior to the pituitary gland and inferior to the third ventricle is the hypothalamus, which is connected to the pituitary gland via the pituitary stalk (infundibulum) [2, 3].

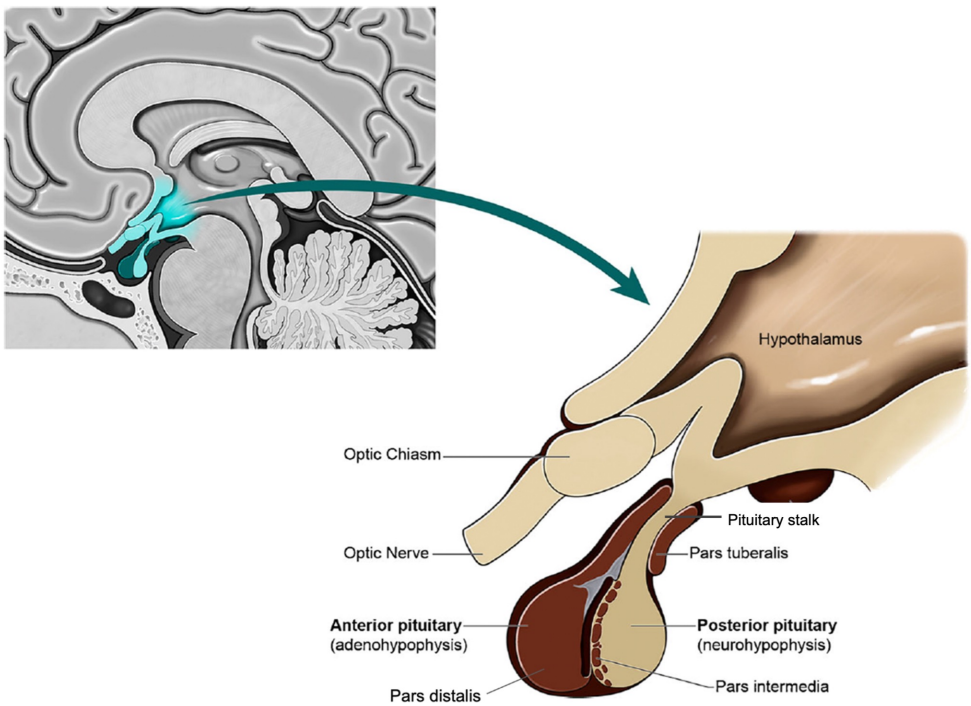

Fig. 1. 'Normal pituitary anatomy' by Shields et al., licensed under CC BY 4.0 [2]. Modified: Text elements have been both removed and added.

Located in close proximity to the pituitary gland is the optic chiasm, which is the region where the optic nerves cross, essential to the visual pathway (Fig. 1) [4].

The pituitary gland consists of two main parts: the anterior lobe (adenohypophysis), and the posterior lobe (neurohypophysis) (Fig. 1) [5]. Hormone secretion from the anterior lobe is regulated by hormones derived from the hypothalamus via the hypothalamic-hypophyseal portal vascular system, whereas the posterior lobe's hormonal secretion is controlled by axons projecting from neurons in the paraventricular or supraoptic nuclei of the hypothalamus. Both the vascular and the axonal pathways run through the pituitary stalk [1, 6].

The anterior pituitary gland is further subdivided into three regions: pars tuberalis, pars intermedia, and pars distalis (Fig. 1). Of these, the pars distalis is primarily responsible for the hormone production from the anterior pituitary gland [1, 5].

The complex embryological development of the pituitary is well-characterised in animal models, and the human process is believed to follow a similar pattern. This process involves both oral and neural ectoderm, giving the pituitary gland a dual embryological origin [1, 7].

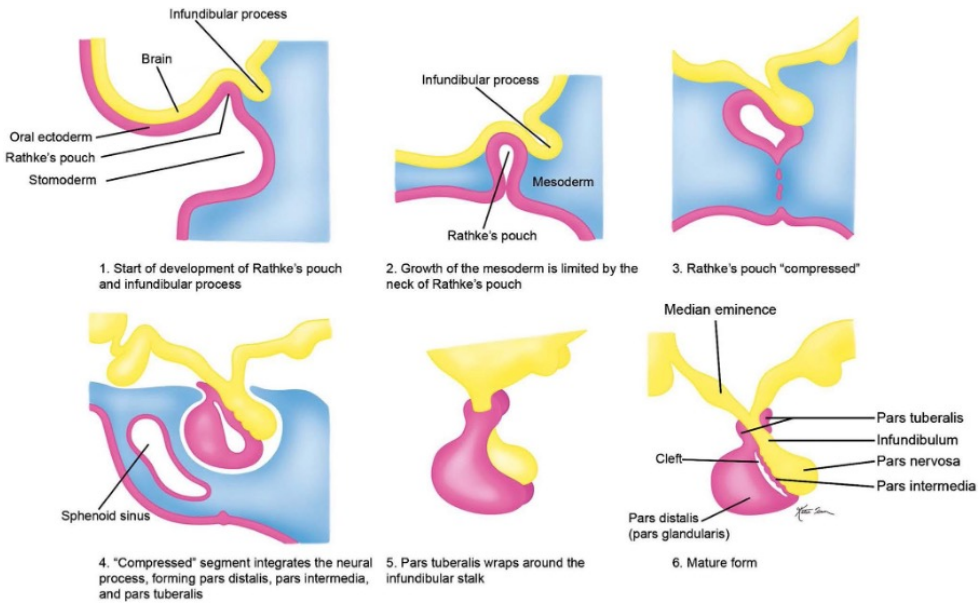

Fig. 2. 'Normal development of the pituitary gland' by Shields et al., licensed under CC BY 4.0 [2]. No changes were made.

By gestational week five, the anterior and intermediate lobes begin to develop from a thickening of the oral ectoderm, which invaginates to form the rudimentary Rathke's pouch. Simultaneously, the infundibular process begins to form from the

diencephalon. Eventually, Rathke's pouch detaches from the oral ectoderm, forming the definitive Rathke's pouch which connects with the infundibular process. Further invagination of the diencephalon gives rise to the infundibulum and the posterior pituitary gland (Fig. 2) [1, 2, 6].

The proliferation and differentiation of the pituitary cell types is tightly regulated by a complex interplay of signalling proteins, transcription factors, and growth factors [8, 9].

Hormonal axes of the pituitary

Hormonal secretion of the anterior pituitary gland is regulated by the hypothalamus which secretes both stimulatory hormones—such as gonadotrophin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH), growth hormone-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH)—and inhibitory hormones including somatostatin (also known as growth hormone-inhibiting hormone, GHIH) and dopamine (also known as prolactin-inhibiting hormone, PIH) [6, 7]. The hypothalamic hormones are released into the primary capillary network of the hypothalamo-hypophyseal portal system and are then transported to the capillary network of the anterior pituitary lobe via veins in the pituitary stalk (Fig. 3) [10].

The anterior pituitary gland, which includes pars distalis, pars tuberalis and pars intermedia, contains six distinct cell types that secrete hormones in response to hypothalamic stimulation. The pars distalis consists of somatotrophs, lactotrophs, gonadotrophs, corticotrophs, and thyrotrophs which secrete growth hormone (GH), prolactin, follicle-stimulating hormone (FSH)/luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and thyroid-stimulating hormone (TSH), respectively. The pars intermedia contains melanotrophs which secrete proopiomelanocortin (POMC), a precursor molecule which produces both melanocyte-stimulating hormone (MSH) and endorphins [6, 7]. No specific hormonal function has been described for the pars tuberalis, although it contains a small number of gonadotrophs and corticotrophs [5, 10].

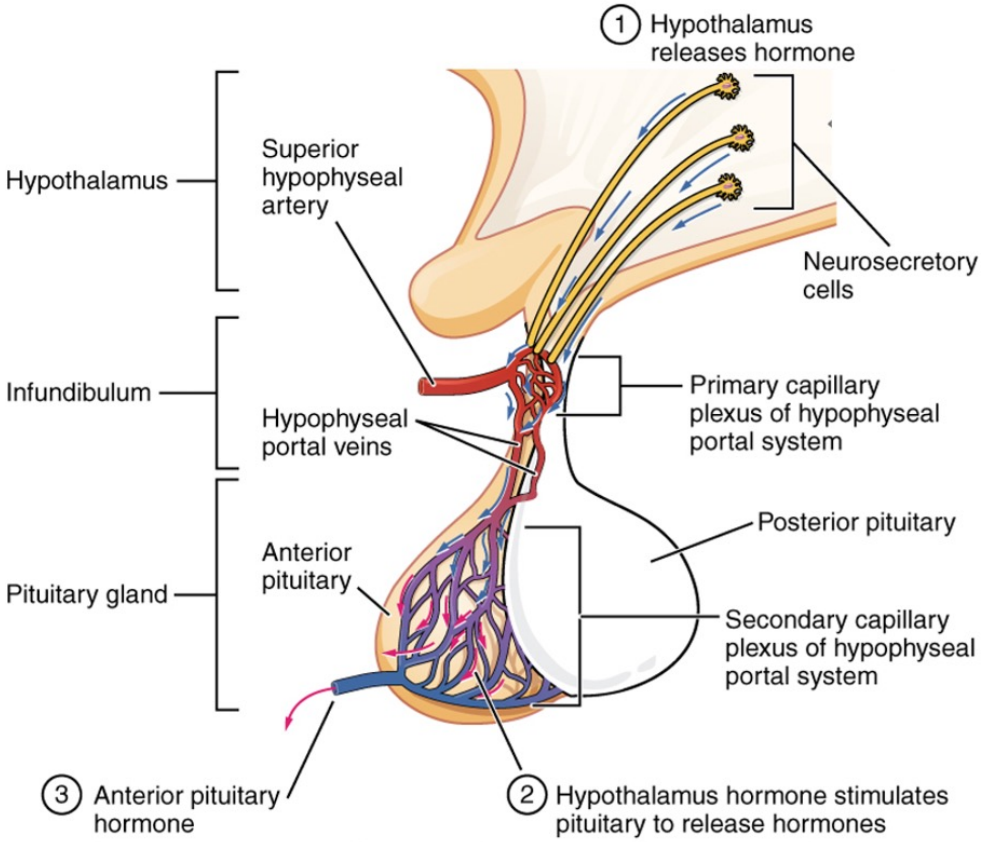

Fig. 3. The release of hypothalamic hormones from the neurosecretory cells into the hypothalamo-hypophyseal portal system. Adapted from 'The anterior pituitary complex' by OpenStax College, licensed CC BY 3.0 [11]. Modified: Cropped from original.

Hormonal secretion from the posterior pituitary originates from hypothalamic axonal projections arising in the supraoptic and paraventricular nuclei, which release antidiuretic hormone (ADH), also known as arginine vasopressin (AVP), and oxytocin. Short axons release the hormones in the primary capillary bed of the hypothalamo-hypophyseal portal system, whereas longer axons terminate in the posterior pituitary lobe and generate the hypothalamo-neurohypophyseal tract [6, 10].

Figure 4 describes the relationship between the hypothalamic and pituitary hormones as well as their major effect on the target organs. In line with the main focus of this thesis, the GH and insulin growth factor-1 (IGF-1) axis and the hypothalamic-pituitary-adrenal (HPA) axis are elaborated on in the following sections.

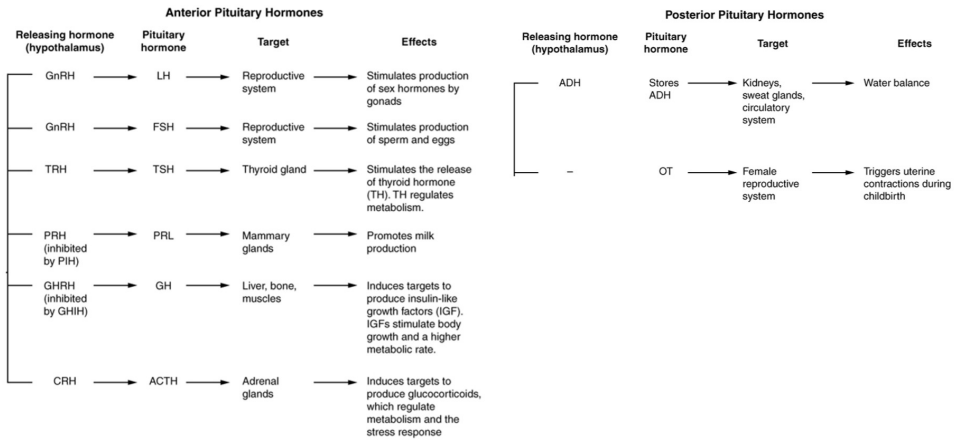

Fig. 4. Overview of the hormonal axes of the anterior and posterior pituitary gland. Abbreviations: ACTH, adrenocorticotrophic hormone; ADH, antidiuretic hormone; CRH, corticotropin-releasing hormone; GH, growth hormone; GHIH; growth hormone-inhibiting hormone (somatostatin); GHRH, growth hormone-releasing hormone; GnRH, gonadotrophin-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; IGF-1, insulin growth factor-1; LH, luteinizing hormone; OT, oxytocin; PIH, prolactin-inhibiting hormone (dopamine); PRH, prolactin-releasing hormone; PRL, prolactin; TRH, thyroid-releasing hormone; TSH, thyroid-stimulating hormone. Adapted from 'Major pituitary hormones' by OpenStax College, licensed CC BY 3.0 [12]. Modified: cropped from original.

The GH-IGF-1 axis

GH is a polypeptide that exists in two forms with different molecular weights: the predominant 22 kDa variant and the less common isoform of 20 kDa [13-15]. Typically, GH is secreted in a pulsatile manner, with peak levels occurring during the night. The secretion is stimulated by GHRH and inhibited by somatostatin, both produced by the hypothalamus [13].

Approximately half of the circulating GH is bound to a growth hormone-binding protein (GHBP), which prolongs its half-life in the circulation [13, 16]. GH mediates its effects by binding to the GH receptor (GHR), which is expressed in various tissues such as the liver, muscles, bone, and adipose tissue (Fig. 5). Binding to a GHR activates transcription of several genes, including those encoding for IGF-1, IGF-binding proteins (IGFBP), and the large protein acid-labile subunit (ALS) [13, 16].

GH exerts its physiological effects both directly and indirectly through IGF-1. IGF-1 is mainly produced in the liver and circulates in the bloodstream in binary complexes with IGF-binding proteins, IGFBP1–6 [13, 16, 17]. Together with IGFBP3 and ALS, IGF-1 forms large ternary complexes (150 kDa), which account for approximately 80% of circulating IGF-1 and extends its half-life by providing a stable structure [17, 18].

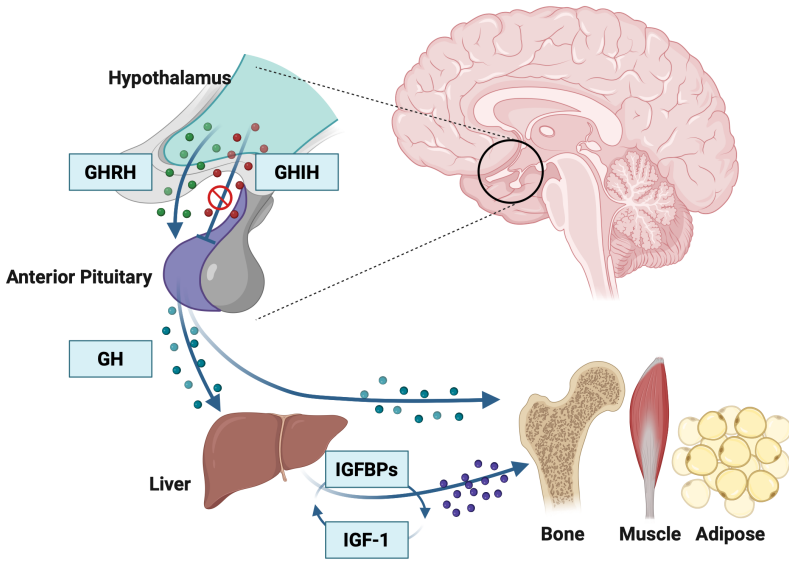

Fig. 5. Illustration of the GH-IGF-1 axis. GH secretion from the anterior pituitary is stimulated by GHRH and inhibited by GHIH (also known as somatostatin), both produced in the hypothalamus. GH acts on target tissues, including the liver, bone, muscle, and adipose tissue, by binding to GHRs. IGF-1 is mainly produced in the liver, circulates bound to IGFBPs, and mediates growth-promoting effects on peripheral tissues. Abbreviations: GH, growth hormone; GHIH, growth hormone-inhibiting hormone; GHR, growth hormone receptor; GHRH, growth hormone-releasing hormone; IGFBPs, insulin growth factor binding proteins; IGF-1, insulin growth factor 1.
Created in BioRender. Borghammar, C. (2025) https://BioRender.com/q8m4twl

The GH-IGF-1 axis plays a central role in the regulation of bone metabolism and linear growth, modulation of adipose tissue and body composition, influence on metabolic profile and promotion of muscle development [16, 19]. Although GH is essential for the regulation of IGF-1 concentrations, circulating IGF-1 levels are influenced by other factors such as malnutrition, oestrogen and androgen levels, thyroid hormone levels, and chronic inflammatory conditions, such as inflammatory bowel disease [20]. Under good health and nutritional conditions, IGF-1 promotes cell proliferation and exhibits anti-apoptotic effects in the target tissues [20]. IGFBP3 modulates IGF-1 activity by both inhibitory and potentiating mechanisms but also exerts IGF-1-independent actions on cell proliferation and survival, although these actions are yet not fully understood [17, 21].

The HPA axis

Corticotrophs in the anterior pituitary lobe produce the prohormone POMC, which is cleaved into other peptide hormones, including ACTH and MSH. ACTH secretion is regulated by the HPA axis via a negative feedback mechanism: it is inhibited by circulating cortisol and synthetic glucocorticoids and stimulated by CRH from the hypothalamus. ACTH stimulates the synthesis and secretion of cortisol in the zona

fasciculata of the adrenal cortex [22, 23]. The secretion of CRH, ACTH, and cortisol follows a circadian rhythm with peak levels in the early morning and the lowest levels around midnight. Although the exact timing of circadian rhythm establishment in children is not completely understood, most studies suggest that a diurnal pattern of cortisol secretion is present by a few months of age [24-29].

Cortisol is a glucocorticoid steroid hormone, and more than 90% of circulating cortisol is transported bound to proteins. Approximately 80% is carried by corticosteroid-binding globulin (CBG, also known as transcortin), whereas 10–15% is bound to albumin [23, 30]. CBG is synthesised in the liver, and the production is upregulated by oestrogens, resulting in elevated total cortisol levels in both pregnant women and individuals receiving oestrogen therapy. However, only the free cortisol fraction is biologically active [30]. In a study of adults, males were found to have lower CBG levels than females, and CBG levels declined with age in both sexes [31]. In contrast, a study of children reported that CBG levels did not vary with age but tended to be higher in girls than in boys at all ages [32]. Cortisol bioavailability is further modulated by interconversion between the biologically active form cortisol and the inactive form cortisone. This interconversion is mediated by the enzyme 11ß-hydroxysteroid dehydrogenase (11ß-HSD), which is expressed in peripheral tissues and affects the glucocorticoid activity both locally and in the systemic circulation [33, 34]. The biological effect of cortisol is mediated through intracellular glucocorticoid receptors, which are found in nearly all cells and organ systems throughout the body [29, 35].

Cortisol is an essential hormone in the physiological stress response. During stressful events, such as severe infections or trauma, cortisol plays a critical role in maintaining an adequate blood pressure through enhanced vasoconstriction, increased cardiac output, and reduced capillary permeability. Additionally, cortisol exerts mineralocorticoid effects, leading to water retention by the kidneys. It also has catabolic effects on lipids and proteins, increases plasma glucose through enhanced gluconeogenesis, and acts as an antagonist to insulin. Furthermore, glucocorticoids possess immunosuppressive and anti-inflammatory actions and influence both behaviour, emotional state, and cognition [29, 36-38].

Pituitary adenomas and cysts

Pituitary adenomas

Pituitary adenomas are benign tumours that originate from the hormone-secreting cells of the pituitary gland. They are classified based on size (microadenoma <10 mm and macroadenoma ≥10 mm), cell type, and hormonal secretion. Non-functioning pituitary adenomas (NFPAs) are lesions that do not secrete any hormones and can present as either a micro- or macroadenoma [39, 40]. Non-

functioning pituitary lesions discovered accidentally in asymptomatic individuals without any laboratory deviations are referred to as pituitary incidentalomas. These include not only adenomas, but also a variety of other incidental findings of the pituitary, such as pituitary hypertrophy or hypoplasia, hamartoma, and thickened pituitary stalk [41, 42]. Although a range of abnormalities can be observed, the most common pituitary incidentaloma is an adenoma [43].

Prevalence of pituitary adenomas

The exact prevalence of pituitary adenomas in children is not well-established, but they have been reported to constitute 3–15% of all intracranial tumours in childhood [44-46]. The incidence is reported to be higher in adolescence compared with children under 15 years of age [39]. As for pituitary incidentalomas, there is a lack of data in children, but autopsy studies have reported a prevalence of 1.5% in children, compared with 10.6–14.4 % in adults [43, 47-49]. With increasing use and more advanced technology of diagnostic imaging techniques such as magnetic resonance imaging (MRI), the detection of small pituitary lesions has become more common [41]. In studies on adults, pituitary micro incidentaloma have been identified in 10–38% of patients undergoing MRI and in 4–20% of those undergoing computer tomography (CT) [43]. In children, pituitary incidentaloma has been reported with an incidence of 22 per 1,000 children on MRI [41].

Natural history and size progression of pituitary adenomas

Evidence on the natural course and size progression of pituitary microadenoma is limited. The following data are based on studies including mainly adults, with some inclusion of paediatric cases.

Concerning accidental, non-functioning findings on MRI pituitary microadenoma is a far more frequent finding (>99% of all cases), compared with macroadenoma [50]. In a review of autopsy studies, only 0.4% of the incidentally discovered adenomas were macroadenomas [49]. These data suggest that it is rare for a microadenoma to progress to a macroadenoma over time. Moreover, studies indicate that the risk of size progression is greater for lesions ≥10 mm [51, 52]. Size progression of a nonfunctioning pituitary microadenoma has been estimated to occur in 1.8% of patients per year [52]. Other studies have reported size progression in 10% of non-functioning pituitary microadenoma during long-term follow-up of maximum 8 years [53]. During a follow-up period of 18 months, small increases in size (1–2 mm) were observed in nonfunctioning pituitary microadenoma [50]. These small changes in size are near the resolution limits of the MRI scanners; their reliability is therefore difficult to interpret. Interestingly, some studies report a reduction in size in 6–9.5% of microadenoma during long-term follow-up [49, 50].

Data on paediatric cases remain scarce. Studies involving a total of 145 children with incidentally discovered pituitary lesions on MRI report a size progression in 0.7% of the cases during the follow-up [42, 54-56]. Notably, one study reported that

25% of the microadenomas decreased in size during a mean follow-up period of 4.5 years [56]. Another study of children showed that more than half (58–67% depending on indication for MRI) of the non-functioning pituitary microadenoma had disappeared at follow-up MRI [54].

Clinical features, genetic syndromes, and hormonal secreting pituitary adenomas

Paediatric pituitary adenomas are predominantly sporadic, arising without a known genetic predisposition. However, they may also be associated with a tumour-predisposing genetic syndrome such as multiple endocrine neoplasia type 1 (MEN-1), familial isolated pituitary adenoma syndrome (FIPAs), Carney complex, or McCune Albright syndrome [44, 47].

The frequency of the different types of hormone-secreting adenomas varies by age groups in children. Overall, prolactin-producing prolactinoma is the most common hormone-producing pituitary adenoma, accounting for approximately 50% of all cases. Prolactinoma is also the most frequent hormone-producing pituitary adenoma in adolescence. In contrast, in prepubertal children, ACTH-producing corticotroph adenomas which cause Cushing's disease are the most frequent, although still very uncommon. Approximately 5–15% of all hormone-secreting pituitary adenomas in children are GH-secreting somatotropinomas. Gonadotropin- and TSH-secreting adenomas are also observed, but are notably rare [40, 44, 47].

Management and follow-up of pituitary adenomas

The treatment and follow-up of a pituitary adenoma is dependent on several factors including tumour size, compression of adjacent structures, and effects on endocrine function or the visual field. Management decisions are made by a multidisciplinary team and may include both expectation and follow-up, medical and surgical intervention, and/or radiation treatment [39, 40].

For non-functioning pituitary microadenoma in children, no consensus currently exists regarding optimal follow-up protocols. Most children are enrolled in a local or national follow-up programme with repeated diagnostic imaging. A recent consensus statement suggests that nonfunctioning microadenoma in children generally follow a benign course and that follow-up imaging can be reduced over time and eventually be discontinued. However, no specific suggestion for this procedure is stated [40]. In a study of children with non-functioning pituitary microadenoma, it was concluded that repeated MRI may not be necessary in the absence of hormonal abnormalities or visual symptoms [56]. Similarly, a review article, including both children and adults, concluded that the follow-up should be individual and that a less rigorous follow-up of non-functioning microadenomas is appropriate [52].

In adults, follow-up recommendations vary. Some MRI recommendations are dependent on the size of the microadenoma, with more rigorous follow-up for

lesions >5 mm, whereas others recommend MRI follow-up regardless of lesion size. Nonetheless, they agree that initial clinical and hormonal evaluation should be done, and that morphological assessment can be discontinued if there is no size progression over the first few years of follow-up [43, 50].

Pituitary cysts

Rathke's cleft cysts are benign remnants of embryological development, originating from Rathke's pouch (Fig. 2). They are most commonly located in the midline, specifically within the pars intermedia of the pituitary, and are therefore also referred to as pars intermedia cysts [57-59].

In contrast, pituitary adenomas are typically located in the anterior lobe of the pituitary and are surrounded by normal pituitary tissue [60]. Pituitary adenomas with cystic components are called cystic pituitary adenomas, which can be challenging to distinguish from Rathke's cleft cysts on MRI [61]. Cystic pituitary adenomas are recognised by a cyst wall generated by tumour tissue which is thickened and irregular, which is not characteristic of Rathke's cleft cysts [59, 60]. Additionally, cystic pituitary adenomas are most frequently located off-midline, whereas Rathke's cleft cysts are typically positioned in the midline of the pituitary [60, 61].

Prevalence of pituitary cysts

As for pituitary adenomas, it has been suggested that pituitary cysts are increasingly detected in children as a result of the growing use and improvement of radiological imaging techniques [59, 62, 63]. The prevalence of Rathke's cleft cysts in children is not completely known. In retrospective studies of children, <15 years old, without related symptoms or hormonal deviations, the frequency of Rathke's cleft cysts was 1.2–3% on MRI [60, 64]. In another retrospective study of children <18 years, the authors found pituitary cysts or cyst-like lesions in 57.7% (n = 232) of asymptomatic children who had undergone MRI [59]. An autopsy study found incidental Rathke's cleft cysts in 1.7% of individuals aged 10–29 years old [65]. The frequency seems to be higher in adults, with an incidence of 4.2% in autopsies of individuals aged ≥30 years, suggesting a slow growth rate of these cysts [60, 65].

Natural history and size progression of pituitary cysts

The natural history of pituitary cysts remains incompletely understood [58, 66]. In a study of children, the majority (73.3%) of Rathke's cleft cysts without pressure-related symptoms were found to remain stable or decrease in size during a follow-up period of two years [63]. Similarly, in an adult cohort, cysts <10 mm had a stable size during the follow-up of five years [66]. As previously mentioned, the growth of these cysts appears to be slow, which is supported by the discrepancy in the frequency of Rathke's cleft cysts between younger and older individuals [60].

Clinical features of pituitary cysts

Rathke's cleft cysts are non-hormone-secreting lesions. Clinical manifestations, when present, are primarily due to size effects, and may include headaches, visual field defects, and hormonal disturbances [57].

Management and follow-up of pituitary cysts

The management and follow-up of Rathke's cysts depend on their size, the size effect on adjacent structures and the presence of clinical symptoms. For larger lesions (≥10 mm) that cause symptoms, surgical intervention is the treatment of choice. In contrast, asymptomatic lesions are usually managed conservatively with MRI follow-up [57, 67].

Much like nonfunctioning pituitary microadenomas, small Ratkhe's cleft cysts (<10 mm) can be discovered as incidental findings on MRI and rarely give any symptoms. In a study of adults, radiological follow-up was recommended after five years in cysts with a size <10 mm [66]. Some studies in children have suggested a conservative approach; one study concluded that small Rathke's cysts in children, with no symptoms or hormonal abnormalities, should be considered incidental findings [60]. Another article proposed that no follow-up is necessary for small, asymptomatic pituitary cystic lesions [59].

Evaluation of pituitary morphology, diagnostic imaging

When potential pituitary gland pathology is investigated, for example in cases of suspected multiple pituitary hormone deficiency (MPHD), growth hormone deficiency (GHD), or precocious puberty, MRI of the pituitary and hypothalamus is performed to identify potential underlying structural deviations [55, 68, 69]. Incidental findings suggestive of pituitary pathology may also be noted when brain MRI is performed in investigations of symptoms unrelated to the pituitary gland. As mentioned, the growing use of MRI and the improved technique of the MRI scanners have made these incidental findings more frequent [41, 58, 62].

MRI scanners employ different magnetic field strengths, such as 1.5 tesla (T) and 3 T. A higher magnetic field strength, like 3 T, gives a better quality and spatial resolution of the image. However, the higher field strength is more susceptible to artefacts related to movements of the patient and vascular flow [68].

MRI protocols include T1- and T2-weighted sequences. T1-weighted images are useful to describe anatomical structures, whereas T2-weighted images are commonly used to visualize fluid and pathological structures [70].

The standard MRI protocol for imaging of the pituitary gland typically involves thin-section images (2–3 mm) in both sagittal and coronal planes [68, 71]. Solid

pituitary adenomas are commonly visualised on conventional (non-dynamic) T1-weighted images, where they show a delayed contrast enhancement, relative to the normal pituitary tissue, on post-contrast images [68, 71]. Dynamic contrast-enhanced T1-weighted imaging can also be applied to detect pituitary microadenomas. In this technique, images are acquired before and at multiple timepoints after contrast administration. Microadenomas appear as an area with diminished contrast enhancement compared with pituitary tissue [68, 72]. As for cystic pituitary lesions, the most common MRI finding is a fluid-filled lesion on T2-weighted images. On T1-weighted images, the pituitary cysts typically do not show any enhancement following contrast administration [57].

Gadolinium-based contrast agents are commonly used in pituitary MRI [72]. Studies have shown that there is a risk of deposition of gadolinium in the brain after repeated use. The clinical significance of this gadolinium accumulation remains unknown, but the risk of neurotoxicity should be considered during follow-up with repeated MRI scans [72, 73].

Deviations in pituitary function

Hormonal deviations may affect any of the pituitary hormonal axes (Fig. 4). In line with the primary focus of this thesis, deviations in the GH-IGF-1 axis as well as in the HPA axis, are described below.

The GH-IGF-1 axis

Congenital and acquired GHD

GHD can be either congenital or acquired and may present as an isolated condition or in combination with other pituitary hormonal deficiencies, as in MPHD [16].

Congenital GHD may be genetic and associated with structural abnormalities of the pituitary, such as pituitary hypoplasia or aplasia, pituitary stalk abnormalities, empty sella and ectopic posterior pituitary. Moreover, it can be a result from perinatal events [16, 74]. Isolated GHD is the most common form, with an estimated incidence of 1:4,000–10,000 live births. It may be inherited in an autosomal recessive, autosomal dominant or X-linked manner, and is often associated with mutations in the genes encoding GH or the GHRH receptor [16]. The genetic forms of GHD may also involve mutations in genes encoding for transcription factors and signalling molecules involved in the embryological development of the pituitary gland (e.g., PROP1 or POU1F1 mutations). These cases are typically associated with MPHD [7, 74].

MPHD, including GHD, should be considered and investigated in children with a neonatal history of hypoglycaemia, prolonged jaundice, and/or micropenis. In

childhood, the most pronounced clinical signs of GHD are reduced growth velocity and short stature. In more severe cases, children with GHD may also present with characteristic physical features, including frontal bossing, midfacial hypoplasia, and truncal adiposity [75]. Bone age assessment, performed via hand and wrist X-ray, typically show a delayed bone maturation in children with GHD [69].

Acquired GHD can be a result of various conditions affecting the HPA axis, such as brain tumours (e.g., craniopharyngioma), cranial irradiation therapy, physical brain trauma or surgery, brain infections (meningitis, encephalitis), inflammation (hypophysitis, autoimmune polyglandular syndrome), or infiltrative disorders such as Langerhans cell histiocytosis or sarcoidosis [16]. Childhood cancer survivors with tumours in the hypothalamic-pituitary regions and those who have received cranial irradiation often experience several pituitary hormonal deficiencies. The risk is higher in children diagnosed at a younger age [76]. The GH-producing cells in the pituitary are sensitive to irradiation, and GHD may occur at relatively low doses of cranial irradiation (≥18 Gy) [76]. Moreover, it has been shown that 100% of adults who reived cranial irradiation (37.5–42.5 Gy) as treatment of a pituitary tumour developed GHD within 5 years [77]. The acquired GHD presents as impaired growth, which can be masked by growth stimulated by sex hormones in these patients, who are also at risk of precocious or early puberty [76].

Definition of short stature and criteria for evaluating potential GHD

When a child meets the criteria for short stature, an investigation should be initiated after other reasons for growth failure, such as Turner syndrome, hypothyroidism, and chronic or inflammatory systemic disease, have been excluded [15]. Short stature is defined based on both absolute height, height related to target height and growth velocity, and generally includes following criteria [15, 78]:

1) Height >3 standard deviation score (SDS) below the population mean.
2) Height >1.5 SDS below the mid-parental height (MPH).
3) Height >2 SDS below the mean, combined with either:
    - Growth velocity >1 SDS below mean for age over 1 year, or
    - Decrease in height >0.5 SDS over 1 year in children >2 years.
4) Normal height, combined with either:
    - Growth velocity >2 SDS below the mean over 1 year, or
    - Growth velocity >1.5 SDS below the mean over 2 years.

Additionally, the following children should be evaluated for GHD [15, 78]:

- Children presenting with neonatal symptoms of GHD.
- Children with signs of MPHD.
- Children suspected to have an intracranial lesion.

The HPA axis

Primary and secondary adrenal insufficiency

Adrenal insufficiency may be either congenital or acquired and classified as primary or secondary. It is a potentially life-threatening condition that must be recognised and treated to prevent cardiovascular collapse [36].

In primary adrenal insufficiency, the adrenal glands fail to produce glucocorticoids, and mineralocorticoid secretion may also be impaired. In children, the most common cause of primary congenital adrenal insufficiency is congenital adrenal hypoplasia (CAH), whereas in adults, autoimmune adrenalitis (Addison's disease) is the predominant cause [79, 80]. CAH has an incidence of 1:10,000–18,000 live births. It is most often recognised after birth in girls due to virilisation, whereas boys present within the first weeks of life with a salt-wasting crisis. CAH is included in many national newborn screening programmes, allowing early diagnosis before the onset of severe symptoms [36, 81].

In secondary adrenal insufficiency, impaired secretion of CRH and/or ACTH leads to reduced cortisol production by the adrenal glands. This may be a result of tumours in the hypothalamic-pituitary region or from traumatic brain injury [79]. However, the most common cause of secondary adrenal insufficiency is iatrogenic suppression of the HPA axis following treatment with exogenous glucocorticoids (Fig. 6) [82, 83].

In children with adrenal insufficiency, glucocorticoid replacement therapy is given at a dose corresponding to hydrocortisone 8–12 mg/m^2/day [84, 85]. Treatment with supraphysiological doses of glucocorticoids, for more than 2–4 weeks, exerts negative feedback on the HPA axis and inhibits CRH and ACTH production. This may result in adrenal cortex atrophy and secondary adrenal insufficiency upon discontinuation of the glucocorticoid treatment [36, 86].

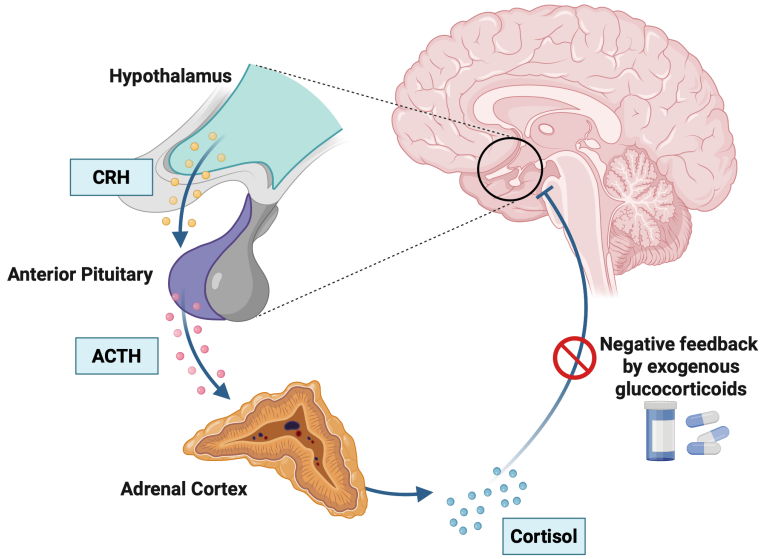

Fig. 6. The negative feedback of exogenous glucocorticoids on the hypothalamic-pituitary-adrenal axis. Abbreviations: ACTH, adrenocorticotrophic hormone; CRH, corticotropin-releasing hormone. Created in BioRender. Borghammar, C. (2025) https://BioRender.com/it4qpgp

Some risk factors for developing secondary adrenal insufficiency are identified, such as the glucocorticoid dose, potency and the duration of treatment. Glucocorticoids with a longer half-life give less time for adrenal recovery, which increase the risk of secondary adrenal insufficiency (Table 1) [87]. Conversely, glucocorticoid regimes such as pulse therapy or alternate-day dosing allow more time for adrenal recovery and decrease the risk of adrenal insufficiency [86, 88].

Table 1. Equivalent doses and effect durations for exogenous glucocorticoids.

Glucocorticoid	Equivalent dose (mg)	Duration of action (h)
Short-acting		
Hydrocortisone	20	8–12
Intermediate-acting		
Prednisolone	5	12–36
Methylprednisolone	4	12–36
Long-acting		
Betamethasone	0.6	36–72
Dexamethasone	0.75	36–72

Table adapted from Liu et al. Allergy, Asthma & Clinical Immunology 2013 [87].

Concomitant use of other medications can also influence the risk of developing secondary adrenal insufficiency. For example, CYP3A4 inhibitors such as azole-based antifungal agents (e.g., fluconazole, itraconazole) impair the breakdown of many glucocorticoids, thereby increasing the exposure of glucocorticoids and

enhancing the risk of suppression of the HPA axis [88]. In children, the duration of adrenal insufficiency following treatment with glucocorticoids is not well-established. In a review including 298 children with acute lymphoblastic leukaemia (ALL), treated with supraphysiological doses of glucocorticoids, the majority had adrenal insufficiency in the first days after completion of glucocorticoid treatment. Although adrenal recovery occurred within a few weeks for most children, 11% had sustained adrenal insufficiency at retesting 12–34 weeks after discontinuation of glucocorticoid treatment [88, 89].

Symptoms of secondary adrenal insufficiency are often non-specific, with examples being fatigue, abdominal discomfort, and muscle pain, and can be difficult to recognise. The symptoms can be mistaken for those of the underlying condition, potentially leading to a delayed diagnosis [82, 90]. In a meta-analysis, only 10% (n = 98) of patients with biochemical secondary adrenal insufficiency reported symptoms, indicating that the experience of symptoms does not correspond to the laboratory findings [90].

Acute adrenal insufficiency (adrenal crisis) may occur in patients treated with glucocorticoids, for example during withdrawal or tapering of glucocorticoid therapy, during episodes of prolonged vomiting or diarrhoea, or during states of physiological stress such as fever and severe infections, trauma, surgery, and other procedures requiring anaesthesia. In these situations, the adrenal cortex may be unable to produce sufficient cortisol levels, resulting in an impaired stress response with hypotension or hypovolemic shock. Other associated symptoms of an adrenal crisis may include severe fatigue, abdominal pain, vomiting or nausea, and laboratory deviations (e.g., hyponatremia, hypoglycaemia) [79, 86, 88].

Evaluation of pituitary function, stimulation tests

Various methods, including pharmacological stimulation tests, are available to assess the hormonal functions of the pituitary gland. This thesis focuses specifically on the GH-IGF-1 axis, and the HPA axis, and the evaluation of these axes is described below.

The GH-IGF-1 axis

Plasma GH-IGF-1

Due to the pulsatile secretion pattern, diurnal variation, and short half-life of GH, random measurements of GH levels are generally unreliable for diagnostic purposes. One exception exists in neonates, where a random GH value of <7 μg/L is considered indicative of GHD [16, 78].

Unlike GH, IGF-1 and its major binding protein, IGFBP3, are secreted in a stable manner without pulsatility or obvious circadian rhythm. Their concentrations correlate positively with GH levels and are thus used as markers for GH secretion. IGF-1 levels vary with age, sex, and pubertal status, and are also affected and reduced by nutritional status and chronical diseases such as diabetes, renal and liver failure, and hypothyroidism. Although IGFBP3 is less sensitive to nutritional status, it has not been shown to have any clear advantage over IGF-1 in assessing GHD [16, 78, 91].

Several articles conclude that IGF-1 has a moderate to good specificity but low sensitivity in diagnosing GHD, and that IGF-1 values ≤2 SDS are predictive of an abnormality of the GH axis, and values >0.0 SDS, adjusted for age, sex, and pubertal status, make a diagnosis of GHD unlikely [16, 20]. In children <3 years of age, IGF-1 is less reliable as a marker for GHD, as normal levels and the levels seen in GHD overlap [69, 78].

Pharmacological stimulation tests of the GH/IGF-1 axis

To improve diagnostic accuracy and reduce the risk of false positive results, it is recommended to perform two different pharmacological GH stimulation tests to evaluate GH levels, as the tests have low reproducibility [69, 78, 92]. However, in patients with MPHD, a history of central nervous system pathology, cranial irradiation or a known genetic defect associated with GHD, one test is considered sufficient [78].

When interpreting the results of a GH stimulation test, several factors must be considered. Obese children typically show lower peak GH levels, and sex hormones are known to increase activity in the GH axis. Hypothyroidism and hypogonadism can result in reduced GH levels [69]. Additionally, pituitary refractoriness, the temporary inability to produce another GH peak after a recent spontaneous one, can give rise to falsely low GH values [93-95].

Sex steroids enhance GH secretion and action [96]. Among children of peripubertal age, prepubertal children may show low GH responses compared with pubertal children of the same age, which can lead to a false positive diagnosis of GHD. To improve the test specificity and reduce false positive test result, it is therefore suggested that sex steroid priming should be considered for prepubertal children of peripubertal age [97]. The recommended age and protocol for sex steroid priming varies between different centres [96].

It is also important to consider the isoforms of GH – 22 and 20 kDa – when interpreting results. Different assays may have varying immunoactivity for the two isoforms, which can affect the choice of cut-off level for a normal GH response and makes it challenging to compare different studies [13-15]. A GH peak of ≥7 μg/L is commonly used as the diagnostic threshold, although values of 5 and 10 μg/L are also applied [13, 78].

Several pharmacological tests are used to evaluate the GH axis, including the insulin tolerance test (ITT), glucagon stimulation test (GST), arginine stimulation test (AST), the L-dopa test and the GHRH test. All the tests should be performed after an overnight fast, to reduce metabolic variability [69].

The ITT induces hypoglycaemia, which stimulates GH secretion as a counter-regulatory hormonal response to hypoglycaemia [75]. The use of ITT is limited due to the risk of severe hypoglycaemia in children [69, 78]. In the GST, glucagon initially raises blood glucose, followed by an increase in insulin levels. The resulting fluctuations in glucose and insulin levels are believed to stimulate GH secretion [75, 98]. Arginine stimulates GH secretion by inhibiting somatostatin secretion and enhancing GHRH release from the hypothalamus [99]. It does not induce hypoglycaemia and is therefore a safer and more appropriate test, for instance for children with diabetes or epilepsy [75].

In addition to pharmacological testing, spontaneous GH secretion can be assessed using 24-hour secretion curves or 12-hour nocturnal profiles [78]. These methods require hospitalisation and frequent blood sampling every 20–30 minutes but provide information of the natural pulsatile GH secretion [100, 101]. The nocturnal profile can be valuable in evaluating the risk of pituitary refractoriness, which may affect the GH response in a subsequent simulation test [93].

The HPA axis

Evaluation of the HPA axis involves both basal plasma cortisol measurements and dynamic, pharmacological stimulation tests.

Plasma cortisol

Due to the diurnal variation in cortisol secretion, blood samples for basal plasma cortisol should be taken in the early morning, preferably at 8:00 am [25, 86, 88]. When interpreting the results, it is important to consider factors that affect CBG and albumin levels, which influence the total cortisol concentration. Examples of such factors are treatment with oestradiol and conditions such as liver or kidney disease [30, 86]. Moreover, the clinical context must be considered. The cut-offs for cortisol values suggested below apply only to patients who are not experiencing physiological stress and who have a normal sleep-wake cycle [85, 86]. In patients receiving glucocorticoid therapy, the last dose should be taken 24 hours before evaluation of cortisol levels [86, 88].

For patients at risk of secondary adrenal insufficiency, the normal range for morning cortisol levels is not clearly established. The proposed cut-off levels vary and are influenced by the specific assay used [80, 86, 88, 102]. In guidelines from the European Society of Endocrinology and Endocrine Society, the following interpretation of morning plasma cortisol values are suggested [86]:

- Cortisol <150 nmol/L: high risk of adrenal insufficiency.
- Cortisol 150–300 nmol/L: intermediate risk of adrenal insufficiency.
- Cortisol >300 nmol/L: low risk of adrenal insufficiency.

For patients in the high-risk group, continued glucocorticoid replacement therapy is recommended, with reassessment after a few months. For those at intermediate risk, continued treatment and retesting in a few weeks, or testing with a pharmacological stimulation test, is recommended. In patients with low risk, glucocorticoid treatment can generally be discontinued. However, stress doses should be considered in situations with physiological stress for individuals with cortisol values close to 300 nmol/L, and testing with a pharmacological stimulation test should be considered [86].

Providing proper information to the patient and their family about the risk of adrenal insufficiency is of great importance. To support this, a medical alert card has been developed by the Swedish Endocrine Society for these patients [79, 103].

Pharmacological stimulation tests of the HPA axis

When there is uncertainty regarding whether an individual has an adequate adrenal cortex function to secrete cortisol, a dynamic stimulation test is recommended [86, 88]. Several methods are available to assess the HPA axis and examples of the pharmacological stimulation tests used include the standard-dose and low-dose ACTH stimulation tests, the GST and the ITT. In children, no gold standard for evaluating the HPA axis exist. In adults, the ACTH test is recommended by the Endocrine Society Guidelines [104]. Moreover, in children, there is no universally accepted cut-off value for a normal cortisol peak during stimulation tests. Previously used thresholds in paediatric studies range between 400 and 500 nmol/L [105-112]. As mentioned, the cortisol response may vary between different assay methods [102, 113]. Additionally, studies show inconsistent findings regarding the influence of clinical variables, such as age, sex, pubertal status, and body mass index (BMI), on cortisol levels [106, 108, 111, 112, 114-117].

Even when cortisol levels exceed the cortisol cut-off, the possibility of relative adrenal insufficiency should be considered [36]. This is because during episodes of physiological stress, significantly elevated cortisol levels may be required. A review of children with normal adrenal function found that children with sepsis (n = 129), without shock, had a mean cortisol value of 1,021 nmol/L [118].

The ACTH test assesses adrenal responsiveness to synthetic ACTH, whereas the GST assesses the HPA axis [119, 120]. In the standard-dose ACTH test protocol, 250 µg of synthetic ACTH is used (for infants 15 µg/kg up to the maximum 250 µg), whereas the low-dose protocol includes 1 µg of synthetic ACTH [85]. The choice between the two approaches is debated [85]. Some argue that there is a risk of false negative results with the standard dose as the supraphysiological dose might elicit a cortisol response over cut-off even in patients with impaired adrenal

function. Several studies suggest that the low-dose ACTH test increases sensitivity and more accurately reflects the function of the HPA axis [80, 110].

As mentioned, unlike the ACTH test, the GST indirectly assesses the HPA axis. The exact mechanism for the stimulation of the GH and HPA axes remains uncertain, but proposed explanations include glucose variability triggered by glucagon, and glucagon-stimulated release of arginine vasopressin/copeptin and noradrenalin, which may contribute to HPA axis activation [98, 121, 122].

The ITT has traditionally been considered the gold standard for evaluating the HPA axis [123]. However, as for GH testing, the use of this test is limited in children due to the risks associated with hypoglycaemia [123, 124].

Hormone substitution therapy

When necessary, deficient pituitary hormone axes can be substituted with hormone replacement therapy. Below is a summary of hormonal therapies used in children with GHD and cortisol deficiency.

Growth hormone therapy

Children with GHD are treated with recombinant human growth hormone (rhGH) to support growth, metabolic function, and both physiological and psychological health [69]. rhGH is also indicated in several other conditions, including Turner syndrome, Noonan syndrome, chronic kidney disease, children born small for gestational age (SGA) without catch-up growth, and Leri Weill dyschondrosteosis (SHOX gene deletion) [125, 126].

The recommended dose of rhGH varies across guidelines and is also dependent on the indication for rhGH. For children with GHD, the typical starting dose is 0.022–0.035 mg/kg/day [16]. To titrate to the lowest effective dose, and to evaluate the response to therapy, parameters such as growth velocity, height SDS, and IGF-1 levels should be assessed regularly [69]. Although concerns about the safety of rhGH therapy have been raised, data from a large European cohort (the SAGhe Study) have found no elevated risk for malignancy in otherwise healthy children with GHD treated with rhGH [127].

Glucocorticoid substitution therapy

In patients with adrenal insufficiency, a daily dose of 8–12 mg/m^2/day of hydrocortisone corresponds to the physiological cortisol production. The total daily dose is often divided into three doses, with the largest administered in the morning [84, 85].

During physiological stress, cortisol requirements increase, and the dose must be elevated. Slightly different regimes for stress dosing have been proposed [82, 85, 86, 118]. Ahmet et al. recommend hydrocortisone doses of 30 mg/m^2/day, divided into three doses, for children with fever >38.5 °C. In cases of more severe stress, such as high fever or symptoms such as fatigue, doses of 30–50 mg/m^2/day are recommended. If the patient experiences vomiting or diarrhoea, absorption may be impaired, and the glucocorticoid must be given intravenously or intramuscularly. In such cases, the patient should seek medical care. For surgical procedures, an intravenous hydrocortisone dose of 50–100 mg/m^2 is recommended. In the event of an adrenal crisis, a bolus of 100 mg/m^2 should be administered. In both cases, the bolus should be followed by 100 mg/m^2 over the next 24 hours, either as a continuous infusion or divided into four doses [85].

When discontinuing long-term glucocorticoid therapy, a structured tapering strategy is important. When the therapy consists of long-acting glucocorticoids (e.g., dexamethasone), it is recommended to convert to an equivalent dose of a short-acting glucocorticoid, such as hydrocortisone [86]. The rate of tapering should be individualised based on the underlying condition, with observance of withdrawal symptoms. Once tapering has reached a physiological glucocorticoid level (8–12 mg hydrocortisone/m^2/day), the HPA axis should be assessed and decisions on continued glucocorticoid therapy should be made thereafter (see page 38) [85, 86].

Rationale

As a paediatrician specialising in paediatric endocrinology, I frequently encounter situations where evidence-based guidelines for evaluation and interpretation of diagnostic tests and laboratory results are lacking. This gap in knowledge was a key motivation for developing this research project together with my supervisors.

In our clinical practice, MRI of the pituitary gland is performed as part of the investigation of various medical conditions, such as precocious puberty and growth deviations. Moreover, colleagues in other paediatric specialities conduct brain MRIs in the investigation of, e.g., neurological conditions unrelated to the pituitary. Through collaboration with the neuroradiologists, we observed a notable number of incidental findings in the pituitary. It also became evident that the follow-up of these incidental findings was not standardised at our department, and after a review of the literature we found that there was a lack of clear evidence or consensus-based recommendations on how these children should be managed. Our impression was that a substantial number of follow-up MRIs were conducted, raising concerns with respect to the known risk of brain deposition of gadolinium contrast. Additional considerations included the psychological burden of repeated imaging, as well as the risks associated with general anaesthesia in young children. Moreover, at Skåne University Hospital, access to general anaesthesia is extremely limited. These resources are prioritised daily and allocated to the children considered to be in greatest need. Based on this, we wanted to investigate the natural course and growth potential of these findings, as well as to evaluate methodological challenges related to size assessment of small pituitary lesions on MRI.

The idea of conducting Study II emerged when a change in the clinical protocol for evaluation of GH levels was implemented at our clinic. The procedure of a short spontaneous nocturnal profile (SSNP) and a subsequent arginine insulin tolerance test (AITT) requires hospital admission. Due to increasing limitations on inpatient capacity, and with the knowledge of a well-established, less resource-intensive protocol using the arginine-glucagon stimulation test, our clinic changed procedure to this alternative. However, a few previous studies pointed out the risk of pituitary refractoriness following a previous spontaneous GH peak, which could affect the reliability of the subsequent stimulation test. Based on this, we identified a potential risk of false positive results in children undergoing GH stimulation testing without knowledge of prior spontaneous GH peaks. Therefore, we wanted to gain knowledge concerning the phenomenon of pituitary refractoriness, to better

understand its clinical relevance and implications for diagnostic accuracy in evaluation of GH levels with stimulation test in children.

The rationale for Study III was based on the need to evaluate alternative methods for assessing the HPA axis in children. Although both the standard-dose and low-dose ACTH test have traditionally been used at our clinic, we wanted to investigate the GST as a potential alternative. We hypothesised that this test might offer advantages in the case of secondary adrenal insufficiency, as it evaluates the entire HPA axis, unlike the ACTH tests, which act directly on the adrenal gland.

In paediatric endocrinology, there are often discussions around the appropriate cut-off levels of hormonal assays in relation to sex, age, and pubertal status. Given the physiological heterogenicity of children, it is reasonable to assume that reference values may differ between a toddler and an adolescent approaching adulthood. In the light of this, we wanted to explore if clinical parameters, such as sex and age, had an impact on peak cortisol levels in the GST. Currently, there is no gold standard for cortisol cut-off levels in simulation testing. By examining clinical variables in the context of the GST, we wanted to contribute evidence to support the development of more appropriate reference standards.

Secondary adrenal insufficiency is a serious medical condition but easily overlooked due to vague symptoms. It is known that there is a risk of developing the condition after treatment with long-acting glucocorticoids such as dexamethasone. Children with ALL are treated with high doses of dexamethasone. Currently, there are no guidelines on follow-up and substitutional therapy for secondary adrenal insufficiency in these children. To investigate this further, we sent out a questionnaire to the Childhood Cancer Centres in Sweden. It became apparent that guidelines are missing and requested. To gain knowledge on this subject, we set up a prospective study with the main goal to investigate the prevalence and duration of secondary adrenal insufficiency in children with ALL treated with dexamethasone. As presented in this thesis in Study IV, we conducted a sub-analysis within this study to investigate the relevance of an extended glucocorticoid analysis in diagnosing secondary adrenal insufficiency. This is a relatively new analysis available in Lund, which gives the opportunity to detect both cortisol and biologically inactive cortisone, as well as exogenous glucocorticoids including dexamethasone. As the method was new for us, we wanted to explore the clinical use for this analysis in diagnosing secondary adrenal insufficiency.

By conducting this research project, I hope to have contributed with a small piece to the larger puzzle of developing evidence-based guidelines for evaluation and interpretation of diagnostic procedures in paediatric pituitary diseases. I further hope that our findings will help to reduce overuse of MRI in cases of small incidental pituitary lesions, decrease the overdiagnosis of GHD, and improve the accuracy of diagnosing secondary adrenal insufficiency, minimising both over- and underdiagnosis.

Aims

The overall aim of this PhD project was to evaluate diagnostic aspects of pituitary diseases in children, thereby enhancing the understanding of these conditions and contributing to improved investigation, treatment, and follow-up.

The specific aims were:

- To evaluate the growth potential of pituitary solid and cystic lesions <10 mm in children and to evaluate the accuracy of MRI measurements.
- To evaluate the GH response during an AITT after a GH peak during a short spontaneous nocturnal profile in children with short stature or low growth velocity.
- To evaluate cortisol levels in the GST and to evaluate how clinical parameters, such as age, sex, pubertal status, and BMI, correlate with cortisol levels in children.
- To evaluate the utility of an extended glucocorticoid analysis, including cortisol/cortisone ratio and serum dexamethasone levels, when assessing adrenal function after high-dose dexamethasone treatment in children with ALL, and to identify any correlation between cortisol/cortisone ratio and peak plasma cortisol levels during the low-dose ACTH test.

Methods

Study design and populations

The studies included in this thesis are based on four different Swedish cohorts. Studies I–III were retrospective observational studies based on reviews of medical records, whereas Study IV was a prospective, longitudinal data collection study. An overview of the study designs and included children is presented in Table 2.

Table 2. An overview of the study designs and populations across the included studies.

Study	Study design	Sample size (n)	Median age (years)	Females/Males (%)
I	Retrospective observational, medical record review, reassessment of MRI	74*	12 (3–17)	43 (58)/31 (42)
II	Retrospective observational, medical record review	257	8 (4–18)	119 (46)/138 (54)
III	Retrospective observational, medical record review	171	8 (1–18)	60 (35)/111 (65)
IV	Prospective, longitudinal data collection	26	4 (1–16)	9 (35)/17 (65)

*Additionally, 12 children with prolactinoma (median age 16 (12–17) years; 8 (67%) females) were included in the part of the study evaluating the accuracy of MRI measurements. Values presented as number (%) and median (range).

Study I

A retrospective review including re-evaluation of MRI scans

Study I retrospectively assessed the growth potential of small (<10 mm) solid and cystic pituitary lesions, as well as measurement accuracy on MRI scans. All the available MRI examinations were independently re-evaluated by two radiologists.

The study population consisted of children <18 years of age who had undergone one or more pituitary MRI examinations (1.5 T or 3 T) at Skåne University Hospital, between 2007 and 2017. Eligible children had original MRI reports describing a pituitary microadenoma, probable microadenoma, or a cystic lesion <10 mm. The term probable adenoma referred to lesions that were considered uncertain in the initial report but had a planned radiological follow-up due to suspicion of pathology.

Study participants were identified through the Picture Archiving and Communication system (PACS), as well as through the national patient register and

the BORISS database (Paediatric Oncology Register in the South Region of Sweden) using ICD-10 codes (International Statistical Classification of Diseases and Related Health Problems) (ICD-10 codes used: D352, D443).

Exclusion criteria included: a diagnosis of craniopharyngioma, macroadenoma, hormone-producing adenoma (ACTH, GH, TSH, LH, or FSH), normal or inconclusive imaging results, and unavailable medical records.

Children diagnosed with prolactinoma were included for the evaluation of the accuracy of MRI measurements.

Data were collected from medical records concerning: clinical characteristics (sex, age at first MRI scan, pubertal status), information related to the MRI scans (indication for MRI scan, probable diagnosis at first and most recent MRI scan, planned follow-up, and total number of MRI examinations), laboratory results (pituitary hormone levels at the time of the first MRI), medical history, and treatment details (ophthalmological examination, brain surgery, histopathological findings, and hormone replacement therapy).

The MRI protocols varied somewhat between examinations. In general, both T1-weighted sequences with gadolinium contrast and T2-weighted sequences were performed, with a slice thickness of 2–3 mm.

Definition of size change

A progression or reduction in lesion size was defined as a change of >2 mm. In statistical analyses, the largest lesion dimension measured was used.

MRI lesions size review

Because lesion size was not consistently reported in the original MRI interpretations, both the first and the most recent MRI scan for each patient were re-evaluated by a neuroradiologist. The revised measurements were used to assess changes in lesion size over time.

MRI measurement accuracy

All MRI scans were independently reassessed by two neuroradiologists in a random order, blinded to all clinical and diagnostic information. Each radiologist evaluated whether a pituitary lesion could be identified and determined the lesion location within the pituitary gland (using quarters of the sagittal and coronal plane).

Inter-observer agreement on lesion location was compared across subgroups based on the original MRI report diagnoses (microadenoma, probable microadenoma, cystic lesion, or prolactinoma), as well as based on MRI field strength (1.5 T or 3 T).

For lesions where both radiologists identified the same location, the lesion dimensions (height, width, depth) were compared between the two observers.

Studies II and III

Retrospective reviews of medical records

In Studies II and III, medical records of children, evaluated for short stature and/or slow growth velocity, were retrospectively reviewed. Height SDS, weight SDS, BMI SDS, mid-parental height (MPH) SDS, and sitting height SDS were evaluated based on Swedish standards [128].

Study II

Study II was a retrospective study designed to evaluate GH peak levels in the AITT, in relation to spontaneous GH peaks observed during a prior SSNP.

Children eligible for inclusion were younger than 18.99 years, had been evaluated for short stature or slow growth velocity, and had undergone both a SSNP and a subsequent AITT between 2 May 2011 and 30 November 2020 at Skåne University Hospital, Helsingborg Hospital, or Kristianstad Central Hospital, Sweden. Participants were identified through serial serum GH values registered in the Laboratory Information Management System of the Department of Clinical Chemistry, through registration in the National Swedish GH registry or by codes in the national patient register (ICD-10 codes: E23.0, E34.3, and KVÅ [Klassifikation av vårdåtgärder, classification of care actions] codes, procedure code numbers: AB007, AB013, AB017, AB034, AB036).

Children were excluded if the GH tests were incomplete, contained missing GH values, or were performed as a second testing procedure after finishing GH therapy.

Data were collected from medical records and included: clinical parameters (sex, age, pubertal status, height SDS, BMI SDS, MPH SDS), laboratory results (IGF-1, glucose during AITT, GH values ≥5, ≥7 and ≥10 µg/L in SSNP and AITT, value and timing of GH_{max} in AITT, value and timing of GH ≥7 in SSNP, $cortisol_{max}$), and details on medical treatment and history (priming with sex steroids, previous cranial radiotherapy or MRI of the hypothalamus/pituitary, diagnosis of GHD, and/or treatment with rhGH or other hormone replacement therapy).

Study III

Study III was a retrospective study investigating cortisol levels, as well as correlations between cortisol levels and clinical parameters, in a GST.

Children younger than 18.99 years were included if they had been evaluated for short stature or slow growth velocity and had completed a GST during the period 2018–2023 at Karolinska University Hospital, Stockholm, Sweden or between 2020–2023 at Skåne University Hospital, Malmö, Sweden. Participants were identified by KVÅ codes from the national patient register (KVÅ code used: AB013).

Children with a previous cancer diagnosis or pathology of the hypothalamic pituitary region at MRI were excluded, as well as children with more than one missing cortisol value after glucagon administration.

All participants were assessed due to short stature and initially completed an AST. If the peak GH level was below the cut-off (6 or 7 μg/L depending on GH method), they proceeded to undergo a GST, during which both GH and cortisol levels were evaluated. The same protocol for the AST and the following GST was used at Karolinska University Hospital and at Skåne University Hospital.

Data was obtained from medical records regarding clinical parameters (sex, age, weight SDS, height SDS, BMI SDS, sitting height ratio SDS, MPH SDS, birth weight and height, pubertal status, SGA at birth), laboratory results (cortisol, GH and glucose levels at testing, baseline cortisol, IGF-1), and information om medical history (priming with sex steroids before test, use of any exogenous steroids or any hormonal substitutional therapy, diagnosis of GHD or treatment with rhGH, prior MRI of the sella region).

Study IV

Prospective, longitudinal data collection

Study IV was designed as a prospective study to assess the utility of an extended glucocorticoid analysis, including cortisol/cortisone ratio and dexamethasone concentrations, in evaluating adrenal function following high-dose dexamethasone therapy in children diagnosed with ALL. The analysis was conducted as a sub-study within a larger ongoing prospective, longitudinal study aimed at assessing the prevalence and duration of secondary adrenal insufficiency in the same cohort of children.

Children included were 1–17.99 years old, diagnosed with ALL and treated according to the ALLTogether protocol [129] between 7 October 2022 and 20 January 2025 at the Childhood Cancer Centres at Karolinska University Hospital, Stockholm, and Skåne University Hospital, Lund, Sweden. Written informed consent was obtained prior to participant inclusion. The collaboration with Karolinska University Hospital required establishment of several legal agreements, including a data processing agreement and a clinical site agreement.

ALL treatment in accordance with the ALLTogether protocols includes dexamethasone, which is administered at a dose of 6 mg/m^2/day throughout the induction phase (treatment days 1–28), followed by a 9-day tapering period. In this study, analysis of extended glucocorticoids and a low-dose ACTH test were conducted 24–48 hours after the last dexamethasone dose. Thereafter, the same testing procedure was repeated approximately two weeks later, see Figure 7.

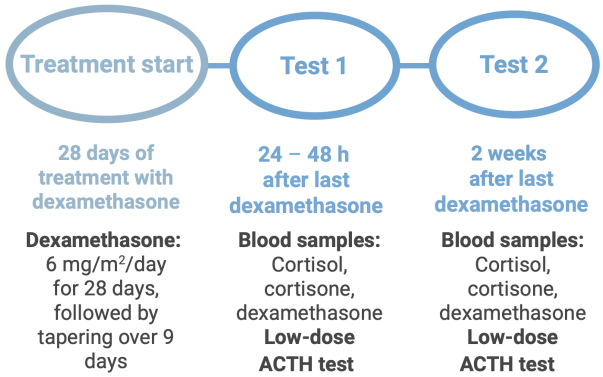

Fig. 7. Overview of the evaluation for adrenal function in children with acute lymphoblastic leukaemia treated with dexamethasone. Abbreviations: ACTH, adrenocorticotrophin. Created in BioRender. Borghammar, C. (2025) https://BioRender.com/zmqb4xn

Overall eligibility criteria for inclusion in the study were in accordance with those specified in the ALLTogether protocol [129]. All children included had undergone two low-dose ACTH tests. Children were excluded from participation in this study if they had any missing values in either the analysis of extended glucocorticoids or the low-dose ACTH tests.

Data on clinical information (age, sex, height, weight, BMI), laboratory results (peak cortisol in low-dose ACTH tests, cortisol, cortisone, and dexamethasone levels, timing of testing relative to last dexamethasone dose), and medical history (other glucocorticoid treatment, treatment with azole based antifungal agents, history of disease of the HPA axis) were collected from medical records and managed using REDCap (Research Electronic Data Capture). REDCap is a secure web-based software platform, hosted at Lund University, designed to support data capture for research studies [130].

All children with suspected adrenal insufficiency (cortisol peak <450 nmol/L on the low-dose ACTH test) got an alert in the medical journal indicating that they were at risk of an adrenal crisis. These children were recommended to receive stress doses of hydrocortisone in the event of severe illness. Additionally, they were provided with a medical alert card containing information about the study and their medical condition.

Although the study was not an intervention study, stress doses of hydrocortisone were recommended for children with suspected secondary adrenal insufficiency at events of infections, trauma, or surgery. The recommended hydrocortisone dosages for fever were as follows: temperature >38 °C, hydrocortisone 30 mg/m^2/day, and for >39 °C, 45 mg/m^2/day. Once the fever disappeared, the hydrocortisone dose was tapered over 2–3 days. In case of severe infections or other life-threatening events,

hydrocortisone 50–100 mg/m^2 was recommended as a bolus dose, followed by 100 mg/m^2 divided into four doses over the subsequent 24 hours. Moreover, for children with very low peak cortisol levels and/or symptoms of adrenal insufficiency, substitution therapy was considered.

Biochemical analyses

Cortisol

Plasma cortisol levels in Studies II–IV were assessed with the cortisol assay from Roche Diagnostics (Mannheim, Germany) and analysed at the Department of Clinical Chemistry and Pharmacology at the hospital where the testing was conducted (Helsingborg Hospital, Kristianstad Central Hospital, Karolinska University Hospital or Skåne University Hospital). For details on the method, detection limit, functional detection limit, and coefficient of variation see Table 3.

GH

In Study II, one GH assay method, Cobas from Roche Diagnostics, was used consistently throughout the study period. All samples were analysed at the Department of Clinical Chemistry and Pharmacology at Skåne Regional and University Laboratories.

In Study III, three different methods for analysing GH were used, Cobas Roche, IDS-iSYS, and Immulite 2000 XPi, and the analyses were performed at either Karolinska University Hospital or Skåne University Hospital. Due to the difficulties in comparing GH levels analysed with different methods, no absolute figures for GH levels were reported in Study III.

Glucocorticoids

Extended glucocorticoid measurements, including cortisol, cortisone, prednisolone, betamethasone and dexamethasone, were made in Study IV. Blood samples taken in Stockholm were sent for analysis in Lund, as the method is only available at the Department of Clinical Chemistry and Pharmacology at Skåne Regional and University Laboratories, Lund.

Glucose

For analyses of glucose levels in Studies II and III, point-of-care plasma glucose instrument were used.

IGF-1

For analyses of IGF-1, two different methods (IDS-iSYS and Immulite) with high concordance ($r^2 = 0.963$; linear coefficient = 0.919) were used in Study II. In Study

III, IDS-iSYS and Immulite 2000XPi were used. As IGF-1 reference values are age- and sex-specific, the results were reported as percentiles specific for age and sex.

Table 3. Methods, detection and functional detection limit, coefficient of variation (CV) for the analyses.

Blood sample	Method	Detection limit/ Functional detection limit	CV
Cortisol	Cobas e601 from Roche Diagnostics (Mannheim, Germany) standardised against IRMM/IFCC 451 (ID-GC/MS).	1.5 nmol/L / 3.0 nmol/L	3% at 38 nmol/L and 2% at 550 nmol/L
GH	Cobas e601 from Roche Diagnostics (Mannheim, Germany) standardised against WHO 98/574.	0.03 µg/L / 0.05 µg/L	3% at 2.0 µg/L and 3% at 9 µg/L
GH	Immulite 2000XPi from Siemens Healthcare Diagnostics, Gwynedd, UK standardised against WHO 98/574.	N/A	6.5% at 2.6 µg/L and 4.2% at 8 µg/L
GH	iSYS from IDS® (Immunodiagnostic Systems Ltd., England), standardised against WHO 98/574.	0.015 µg/L / 0.05 µg/L	10% at 1.5 µg/L and 9% at 10 µg/L
Glucocorticoids	In-house method, LC-MS/MS, on a Sciex 5500 QTrap system (Sciex, Framingham, MA, USA).	0.5–1.0 nmol/L/N/A	4.8–10.1% at 3 nmol/L and 5.7–8.5% at 400–600 nmol/L
Glucose	Point-of- care plasma glucose instruments, for example ACCU-CHEK Inform II from Roche Diagnostics (Mannheim, Germany).	0.6 mmol/L* / N/A	5.2% at 2.5 mmol/L and 6.0% at 17 mmol/L*
IGF-1	Immulite 2000XPi from Siemens Healthcare Diagnostics, Gwynedd, UK, standardised against WHO 02/254.	13.3 µg/L / 24.9 µg/L	7.6% at 56 µg/L, 3.9% at 200 µg/L, and 3.4% at 621 µg/L
IGF-1	Immulite from Siemens Healthcare Diagnostics, Tarrytown, USA, standardised against WHO 87/518.	20 µg/L / N/A	5% at 74 µg/L, 6% at 210 µg/L.
IGF-1	iSYS from IDS® (Immunodiagnostic Systems Ltd., England) standardised against WHO 02/254.	10 µg/L / N/A	6% at 63 µg/L, 6% at 260 µg/L, and 6% at 770 µg/L

*For ACCU-CHEK. Abbreviations: CV, coefficient of variation; GH, growth hormone; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; IGF-1, insulin growth factor 1; IRMM, Institute for Reference Materials and Measurements; N/A, not available; WHO, World Health Organization.

Evaluation of hormonal secretion, stimulation tests

Short spontaneous nocturnal profile, SSNP

In Study II, the spontaneous nocturnal GH secretion was evaluated. Children were admitted to the hospital on the day preceding the tests and were fitted with intravenous access. On the following morning, blood samples of GH were collected at intervals of 30 minutes over a period of 180 or 210 minutes, starting at approximately 04:00 a.m. For data registration and evaluation of the timing of GH peaks ≥7 μg/L, 04:00 a.m. was defined as time zero (0 minutes). Subsequent time points for GH peaks ≥7 μg/L were recorded and rounded to the nearest half hour.

Arginine insulin tolerance test, AITT

In Study II, an AITT was initiated after the completion of the SSNP, see Figure 8. Arginine (500 mg/kg) was administered as an intravenous infusion, and GH levels were measured at 0, 30, 60, 90, 120, 135, 150, and 180 minutes.

At 90 minutes, intravenous insulin (0.1 U/kg) was administered. In addition to the GH sampling, blood glucose levels were measured at the same timepoints, as well as at 105, 110, and 115 minutes. Plasma cortisol was assessed concurrently with GH from 90 to 180 minutes.

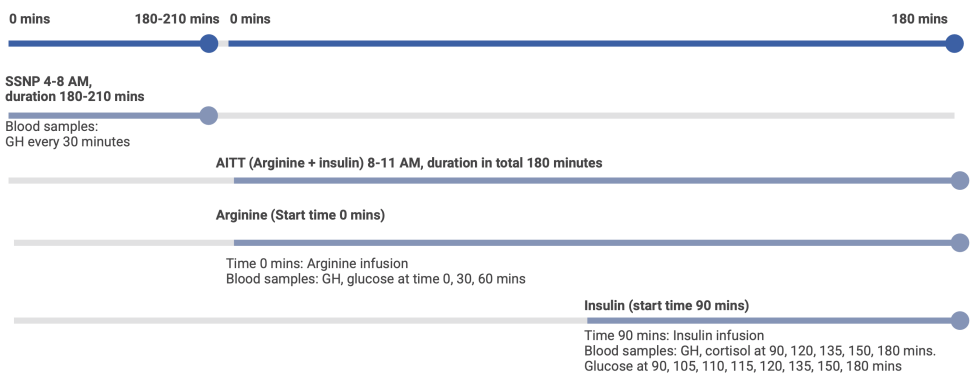

Fig. 8. How blood samples of GH, cortisol, and glucose were taken during the SSNP and the following AITT. Abbreviations: AITT, arginine insulin tolerance test; GH, growth hormone; mins, minutes; SSNP, short spontaneous nocturnal profile. Created in BioRender. Borghammar, C. (2025) https://BioRender.com/hpsp5k2

For each child, the timing of the peak GH during the AITT was documented in minutes relative to the initiation of the test (e.g., 0, 30, etc.). For the arginine test, timepoints were approximated to the nearest 30 minutes. During more intense insulin testing, they were adjusted to the closest 15-minute interval.

All children were fasting from midnight on the day of testing. The fast was interrupted if the child experienced severe symptoms of hypoglycaemia or if the blood glucose level was <2.0 mmol/L.

Sex steroid priming was not routinely implemented during the study period.

GH cut-off

In Study II, a peak GH value of ≥7 µg/L was used as the cut-off to define a normal GH response during testing, which was the recommended cut-off for the GH method used (Cobas e601 from Roche Diagnostics). In addition, statistical analyses were also made for a GH cut-off level of ≥5 and ≥10 µg/L.

Definition of refractoriness and classification of participants

Refractoriness refers to the phenomenon in which the pituitary gland fails to produce a GH peak during a stimulation test due to a preceding spontaneous GH peak. In Study II, refractoriness was defined as a GH peak ≥7 µg/L during the SSNP, followed by no GH peak ≥7 µg/L during the AITT.

Participants were divided into subgroups based on GH response (Table 4). The group HIGH-LOW was classified as refractory, LOW-HIGH and HIGH-HIGH as non-refractory, and LOW-LOW group as probable GHD.

Table 4. Classification of study participants based on GH response during SSNP and AITT.

Group	Definition
LOW-LOW	No GH peak ≥7 µg/L during the SSNP or the AITT
LOW-HIGH	No GH peak ≥7 µg/L during the SSNP, GH peak ≥7µg/L during the AITT
HIGH-LOW	GH peak ≥7 µg/L during the SSNP, no GH peak ≥7 µg/L during the AITT
HIGH-HIGH	GH peak ≥7 µg/L during both the SSNP and AITT

Abbreviations: AITT, arginine insulin tolerance test; GH, growth hormone; SSNP, short spontaneous nocturnal profile.

Glucagon stimulation test, GST

In Study III, cortisol levels in the GST were evaluated. For the GST, no hospitalisation was needed, and the test was initiated in the morning (between 8:00 a.m. and 9:00 a.m.) at the outpatient clinic.

Intravenous access was typically established the day before the GST. Glucagon (1 mg/ml, 30 µg/kg, maximum 1 mg) was administered intramuscularly or subcutaneously. Blood samples for plasma glucose, GH, and cortisol were collected at -30, 0, 30, 60, 90, 120, and 180 minutes relative to the time of the glucagon administration.

As in the AITT protocol, all participants fasted from midnight prior to testing.

Sex steroid priming was routinely considered before the GST, with pubertal development evaluated using Tanner staging. Girls with a Tanner breast stage of 1 and boys with a Tanner genital stage of 1 were classified as prepubertal. Prepubertal girls older than 8 years, and prepubertal boys older than 9 years were eligible for sex steroid priming (17-beta-estradiol (Progynon) 1 mg/day if <20 kg and 2 mg/day if >20 kg given for three days, with the final dose the day before the GST).

Cortisol cut-off

In Study III, a cortisol level of ≥450 nmol/L in the GST was interpreted as indicative of an adequate cortisol secretion. This cut-off was recommended for the cortisol method used (Roche Cortisol II) by the Swedish Endocrinological Association, Swedish Society for Clinical Chemistry, and Equalis (2017) [131].

Low-dose ACTH stimulation test

The low-dose ACTH stimulation test in Study IV was started between 07:30 a.m. and 08:30 a.m. A diluted solution of Synachten (tetracosactide 0.25 mg/ml) at a concentration of 1 μg/ml was used, and 1 μg was administered intravenously. Plasma cortisol levels were subsequently measured at baseline (0 minutes), and at 30, 45, 60, and 90 minutes.

In Study IV, the test was carried out at 24 to 48 hours after the final dose of dexamethasone and repeated two weeks later for all children. If the scheduled test date fell on a weekend or was delayed due to the child's clinical condition, the procedure was rescheduled to the earliest possible opportunity.

Cortisol cut-off

The same cortisol cut-off value (≥450 nmol/L) and assay method (Roche Cortisol II) used in Study III, were applied in the low-dose ACTH test in Study IV.

Statistical analyses

Statistical analyses were conducted using Stata SE 16.0 (Study I) and in IBM® SPSS® Statistics, versions 27 and 29 (Studies II–IV).

A p-value of <0.05 was considered statistically significant, and confidence intervals (CIs) were set at 95%.

Non-parametric statistical methods were used in all studies included in this thesis. Descriptive data were presented as counts, percentages of valid patients, and medians with minimum and maximum values.

To compare continuous variables, the Mann-Whitney U test or the Kruskal Wallis test were used (Studies II–IV). For comparison of two related samples, the Wilcoxon test was applied (Study IV). The chi-squared test and Fisher's exact test were used to compare the distribution of categorical values (Studies II, III). Correlations between continuous variables were assessed using Spearman's rho correlation coefficient (r_s) (Studies III, IV).

In Study III, linear regression analysis was performed to assess factors influencing the peak cortisol value in the GST.

Moreover, in Study I, Cohen's kappa (κ) was used to compare the level of agreement between two reviewers. The interpretation of Cohen's kappa was as follows: <0.2 poor agreement, 0.21–0.4 fair agreement, 0.41–0.6 moderate agreement, 0.61–0.8 good agreement and, >0.8 very good agreement.

Ethical approvals

Below the ethical approvals for each study are listed:

Study I

Approved by the Regional Ethics Committee, Medical Faculty, Lund University, Sweden (Dnr 2017/849).

Study II

Approved by the Swedish Ethical Review Authority (Dnr 2019-06416).

Study III

Approved by the Swedish Ethical Review Authority (Dnr 2023-05588-01).

Study IV

Approved by the Swedish Ethical Review Authority (Dnr 2021-06133-01).

Main results

Study I

Study participants

A total of 707 children who had undergone pituitary MRI were identified through searches in PACS, the national patient register, and in the BORISS database. Of these, 621 did not meet the inclusion criteria, resulting in a final study cohort of 86 children. Among them, 74 had a primary radiological diagnosis of a pituitary microadenoma, probable microadenoma, or cystic lesion (Rathke's cleft cyst or off-midline cyst) <10 mm. Of these 74 children, 55 underwent repeated MRI examinations and constituted the main population of the study. Additionally, 12 of the 86 children were diagnosed with a microprolactinoma.

The 74 children with a suggested diagnosis of a microadenoma, probable microadenoma, or cystic lesion <10 mm had a median age of 12 years; 48 (65%) were pubertal and 43 (58%) were female. Table 5 shows clinical characteristics by initial diagnosis and number of MRI examinations. Among the 55 children who had repeated MRI scans, the median number of scans was 3 (2–7) over a follow-up period of 37 (4–189) months (Table 5).

The most common indications for MRI in the group of children with a diagnosis of a pituitary microadenoma, probable microadenoma, or cystic lesion (n = 74) were evaluation of precocious puberty (n = 27, 36%) and growth disturbances (n = 22, 30%). Of these children, 32 (43%) received hormone treatment, with rhGH being the most common therapy (n = 10), followed by GnRH analogues (n = 9).

Among the 12 children diagnosed with a microprolactinoma, the most common presenting symptom was galactorrhoea (n = 5, 42%), and all these children were treated with a dopamine agonist.

Table 5. Clinical characteristics and MRI follow-up for children with microadenoma, probable microadenoma, cystic lesion, or prolactinoma.

	≥2 MRIs MA, PA, CL n = 55	1 MRI PA, CL n = 19	Prolactinoma n = 12
Age at first MRI scan, years	12 (4–17)	10 (3–17)	16 (12–17)
Female	31 (56%)	12 (63%)	8 (67%)
Pubertal	35 (64%)	13 (68%)	12 (100%)
Ophthalmological examination	3 (5%)*	1 (5%)*	7 (58%)*
Operation	1 (2%)	0	1 (8%)
Hormone treatment	22 (40%)	10 (53%)	12 (100%)
Number of MRI scans	3 (2–7)	NA	5.5 (2–11)
Follow-up time, months	37 (4–189)	NA	75.5 (26–136)

Values presented as median (range) and number (%). *Normal result of examination. Abbreviations: CL, cystic lesion; NA, not applicable; MA, microadenoma; MRI, magnetic resonance imaging; PA, probable microadenoma.

Original MRI reports and clinical management

Review of the original MRI reports and medical records showed that the proposed diagnoses of several pituitary lesions changed over time, and that the follow-up procedures were not standardised.

Single MRI

In 19 cases with an initial MRI diagnosis of a probable microadenoma or cystic lesion, only a single MRI scan was performed. These images were re-evaluated at a multidisciplinary conference, which concluded the following: in seven cases, the MRI was assessed as normal, in nine cases, the lesion was diagnosed as a cystic lesion with no need for follow-up, and in three cases, the findings were considered as a probable microadenoma requiring clinical follow-up only.

Repeated MRI

Among the 55 children who underwent repeated MRI scans, 19 were assessed as normal with no need for follow-up at the most recent MRI, 13 had a scheduled MRI follow-up, 10 were recommended for clinical follow-up, and 9 had no planned follow-up in the medical records. The remaining four children were lost to follow-up.

At the time of the initial MRI, eight lesions were diagnosed as a microadenoma, 35 as a probable microadenoma, and 12 as a cystic lesion. However, by the time of the most recent MRI, five lesions were classified as a microadenoma, 15 as a probable microadenoma, 16 as a cystic lesion, and 19 as normal.

MRI lesion size review

Reassessed lesion sizes are presented in Table 6. Comparison of the initial and the most recent MRI showed that none of the lesions initially classified as a microadenoma (n = 8) or probable microadenoma (n = 35) increased significantly in size (defined as >2 mm). Of the 12 cystic lesions, one increased in size. This occurred in a child who initially had one pituitary cystic lesion, with a size of 4 mm. During follow-up, a new, small cyst was noted, and the original cyst was now measured to 3 mm. Five years later, the child developed multiple pituitary hormone deficiencies, and a new follow-up MRI revealed that one of the cysts had enlarged to 6 mm.

Of all the lesions included, 10 (18%) decreased in size.

Table 6. Reassessed MRI size measurements of pituitary lesions.

Diagnosis at first MRI	Size, first MRI (mm)	Size, most recent MRI (mm)	Size progression (n)
Single MRI			
PA, n = 10	2.2 (1.5–4)	NA	NA
CL, n = 9	2.0 (0–2.6)	NA	NA
Repeated MRI			
MA, n = 8	4.5 (2–6)	4.5 (0–6)	0
PA, n = 35	3.0 (2–7)	3.0 (0–7)	0
CL, n = 12	3.6 (1–7)	4.0 (1–7.2)	1

Values presented as median (range) and number. Abbreviations: CL, cystic lesion; MA, microadenoma; MRI; magnetic resonance imaging; NA, not applicable, PA, probable microadenoma.

MRI measurement accuracy

A total of 269 MRI examinations were reassessed by the two radiologists. In 38 cases (14%), they agreed that no lesion could be identified. Among the remaining 231 MRI examinations disagreement on location occurred in 51 cases (22%). For the remaining 180 examinations, where both radiologists identified a lesion at the same location, size measurement (height, width, and depth) were compared. In 34 out of 460 size measurements (7%), there was a discrepancy of >2 mm in at least one dimension.

The interobserver agreement was further analysed according to the original diagnosis, microadenoma (MA), probable microadenoma (PA), cystic lesion (CL), or prolactinoma (PRL), as well as MRI field strength (1.5 T or 3 T) (Table 7). Agreement was lower for examinations performed on 3 T MRI. Additionally, 40 children (46.5%) had at least one MRI examination where the reviewers and/or the original report disagreed on lesion location.

Table 7. Interobserver agreement by initial diagnosis (n = 269) and MRI field strength (n = 256).

	MRI examinations, n (%)	% agreement (95% CI)	κ (95% CI)
MA	34 (12.6)	0.85 (0.73–0.98)	0.48 (0.09–0.86)
PA	122 (45.4)	0.76 (0.69–0.84)	0.42 (0.25–0.60)
CL	45 (16.7)	0.91 (0.83–1.0)	0.72 (0.46–0.99)
PRL	68 (25.3)	0.81 (0.71–0.91)	0.36 (0.08–0.65)
3 T	73 (28.5)	0.74 (0.64–0.84)	0.26 (0.00–0.52)
1.5 T	183 (71.5)	0.83 (0.77–0.88)	0.51 (0.36–0.66)

Values presented as n (%), % agreement, and Cohen's kappa (κ). Abbreviations: CI, confidence interval; CL, cystic lesion; MA, microadenoma; MRI; magnetic resonance imaging, PA, probable microadenoma; PRL, prolactinoma; T, tesla.

Study II

Study participants

In all, 602 children were initially identified based on serial GH values, with 246 excluded due to stimulation test other than the AITT, and two due to missing medical records. An additional 55 children were identified in the GH registry, 51 of whom overlapped with the initial group, and additional three being excluded due to no AITT performed. National patient register searches identified 270 children, 269 of whom were duplicates with previous identified children, and one who was excluded due to the wrong type of stimulation test. In total, 355 unique children were identified and 257 met the inclusion criteria and were included in the study.

The 257 included children had a median age of 8 years (4–18), 119 (46.3%) were female, and 45 (17.5%) were in puberty. Seven (2.7%) children received sex steroid priming prior to the AITT. Overall, 85 children (33.1%) had any condition that could affect the GH-IGF-1 axis, e.g., born SGA (n = 29, 34.1%), pubertal disorders, such as delayed puberty (n = 15, 17.6%), or specific syndromes (n = 10, 11.8%), such as Turner or Noonan syndrome. Hormonal therapy was administered to 12 (4.7%) of the children, most commonly testosterone or oestrogen (n = 7, 58.3%).

The study participants were divided into four subgroups based on GH response above (HIGH) or below (LOW) the GH cut-off (≥7 µg/L) in the SSNP and AITT tests, respectively (Table 4); LOW-LOW (n = 56, 21.8%), LOW-HIGH (n = 78, 30.4%), HIGH-LOW (refractory group, n = 44, 17.1%), and HIGH-HIGH (n = 79, 30.7%). Clinical characteristics for the subgroups of children are shown in Table 8.

Table 8. Clinical characteristics for the subgroups of children based on GH peaks during testing.

Groups	LOW-LOW	LOW-HIGH	HIGH-LOW	HIGH-HIGH
Number of patients	56 (21.8)	78 (30.4)	44 (17.1)	79 (30.7)
Female	22 (39.3)	44 (56.4)	15 (34.1)	38 (48.1)
Age, years	8.0 (4.0–18.0)	8.0 (4.0–18.0)	8.0 (4.0–16.9)	8.0 (4.0–17.9)
Pubertal	8 (14.3)	12 (15.4)	8 (18.2)	17 (21.5)
Height SDS, prepubertal	-2.9 ((-4.2)–(-1.8))	-2.9 ((-4.3)–(-1.8))	-2.9 ((-4.1)–(-1.8))	-2.9 ((-4.2)–(-0.4))
BMI SDS	-0.3 (-2.1–2.3)	-0.6 (-3.1–2.5)	-0.2 (-2.6–2.6)	-0.7 (-4.6–2.2)
GH_{max} during SSNP, µg/L	4.4 (0.4–6.9)	3.7 (0.3–6.9)	10.0 (7.2–22.0)	11.0 (7.0–46.0)
GH_{max} during AITT, µg/L	4.4 (0.2–6.8)	10.0 (7.1–41.0)	5.2 (1.1–6.9)	11.0 (7.4–50.0)
IGF-1 above 50th percentile	4 (7.7)	12 (16.9)	11 (27.5)	19 (28.8)
IGF-1 50th–25th percentile	9 (17.3)	14 (19.7)	6 (15.0)	15 (22.7)
IGF-1 below 25th percentile	39 (75.0)	45 (63.4)	23 (57.5)	32 (48.5)
Priming with sex steroids	1 (1.8)	1 (1.3)	1 (2.3)	4 (5.1)
Hypoglycaemia <2.2 mmol/L	47 (85.5)	50 (64.9)	34 (79.1)	53 (67.1)

Values presented as median (range) and number (%). Group LOW-LOW: no GH-peak ≥7 µg/L during the SSNP or the AITT; group LOW-HIGH: no GH-peak ≥7 µg/L during the SSNP but a GH-peak ≥7 µg/L during the AITT; group HIGH-LOW (refractory group): GH-peak ≥7 µg/L during the SSNP but no GH-peak ≥7 µg/L during the AITT; group HIGH-HIGH: a GH-peak ≥7 µg/L during both the SSNP and the AITT. Abbreviations: AITT, arginin insulin tolerance test; GH, growth hormone; IGF-1, insulin like growth factor-1; SSNP, short spontaneous nocturnal profile; SDS, standard deviation score.

Refractoriness at GH cut-off ≥7 µg/L

In total, 44 of the 257 (17.1%) children had a GH peak ≥7 µg/L in the SSNP, but no GH peak ≥7 µg/L in the AITT and were classified as refractory (HIGH-LOW group). When excluding children with potential GHD (LOW-LOW group), the proportion of refractory children was 21.9% (n = 44/201).

The refractory group (HIGH-LOW) had significantly higher proportion of males (p = 0.033) and higher BMI SDS (p = 0.037) compared with the non-refractory children (LOW-HIGH and HIGH-HIGH groups). No significant differences were observed in age, height SDS, puberty, or presence of comorbidities. Similarly, IGF-1 levels or occurrence of hypoglycaemia <2.2 mmol/L did not differ between refractory and non-refractory children.

Timing and magnitude of GH peaks

Maximal GH levels during the SSNP and AITT for both refractory and non-refractory children are presented in Table 9. Among refractory children (HIGH-

LOW), the maximal GH value in the SSNP did not differ significantly from that of the HIGH-HIGH group (p = 0.783, Table 10).

In the HIGH-HIGH group, the maximal GH peak at AITT occurred 30 minutes later than in the LOW-HIGH group (p = 0.004, Table 10). No significant difference was observed in the timing of the last GH peak ≥7 µg/L in the SSNP between the refractory group (HIGH-LOW) and the HIGH-HIGH group. The median time between the last GH peak ≥7 µg/L in the SSNP and the maximal GH peak in the AITT in the HIGH-HIGH group was 210 minutes.

Table 9. Maximal GH values in SSNP and AITT for refractory and non-refractory children.

Groups **n (%)**	**Refractory children (HIGH-LOW)** **44 (21.9)**	**Non-refractory children (LOW-HIGH, HIGH-HIGH)** **157 (78.1)**	**P-value**
GH_{max} SSNP, µg/L	10.0 (7.2–22.0)	7.0 (0.3–46.0)	<0.001
GH_{max} AITT, µg/L	5.2 (1.1–6.9)	11.0 (7.1–50.0)	<0.001

Values presented as median (range). Abbreviations: AITT, arginine insulin tolerance test; GH, growth hormone; SSNP, short spontaneous nocturnal profile.

Table 10. Timing and magnitude of GH peaks in SSNP and AITT.

Time-point of GH-peak **n (%)**	**LOW-HIGH** **78 (38.8)**	**HIGH-LOW** **44 (21.9)**	**HIGH-HIGH** **79 (39.3)**	**P-value**
Magnitude of GH-peaks				
Last GH-peak ≥7 µg/L during SSNP	–	9.5 (7.2–22.0)	9.1 (7.0–27.0)	0.523
GH_{max} during SSNP, µg/L	–	10.0 (7.2–22.0)	11.0 (7.0–46.0)	0.783
GH_{max} during AITT, µg/L	10.0 (7.1–41.0)	–	11.0 (7.4–50.0)	0.214
SSNP				
Time from start of SSNP to last GH-peak ≥7 µg/L, minutes	–	135 (0–210)	120 (0–210)	0.303
AITT				
Time from start of AITT to GH_{max}, minutes	60 (0–180)	–	90 (0–180)	0.004
Refractory period				
Time between last peak ≥7 µg/L in SSNP and GH_{max} AITT, minutes	–	–	210 (30–390)	–

Values presented as median (range). Abbreviations: AITT, arginine insulin tolerance test; GH, growth hormone; SSNP, short spontaneous nocturnal profile.

IGF-1 levels

In the LOW-LOW group, 39 children (75%) had IGF-1 below the 25th percentile and 4 (7.7%) had levels above the 50th percentile. In contrast, within the HIGH-HIGH group, 32 (48.5%) children had IGF-1 levels below the 25th percentile and 19 (28.8%) children had levels above the 50th percentile.

Refractoriness at GH cut-offs ≥5 and ≥10 μg/L

Among all included children (n = 257), 35 (13.6%) were classified as refractory using a GH cut off-of ≥5 μg/L. When a higher GH cut-off of ≥10 μg/L was used, 34 (13.2%) children met the criteria for refractoriness.

Study III

Study participants

In total, 149 children were identified from the national patient registries at Karolinska University Hospital and 64 at Skåne University Hospital. Of these, 19 were excluded due to age >18.99 years, having undergone a test other than the GST, or having unavailable medical records. Additionally, 23 children with pathological MRI of the pituitary and/or a history of child cancer were excluded. Ultimately, 171 children were included in the study.

The median age of the participants was 7.8 (1.0–18.0) years, with a median BMI SDS of -0.2 (-3.6–3.3). Of the 171 children, 60 (35.1%) were female, and 23 (13.5%) had entered puberty. Priming with sex steroids prior to the GST was performed in 50 (29.4%) of the children. Additionally, 15 (8.8%) children had reported use of inhalation steroids.

When comparing boys and girls, no statistically significant differences in clinical parameters were observed (Table 11).

Table 11. Clinical characteristics by sex (girls and boys).

	Girls	Boys	P-value
Number of patients	60 (35.1)	111 (64.9)	
Pubertal	6 (10.0)	17 (15.3)	0.331
rhGH treatment	29 (48.3)	56 (50.5)	0.792
Inhalation steroids treatment	5 (8.3)	10 (9.0)	0.882
Age, years	7.5 (1.8–14.5)	8.7 (1.0–18.0)	0.102
BMI, SDS	-0.1 (-3.1–3.3)	-0.4 (-3.6–2.8)	0.928
Height, SDS	-2.9 (-4.2–0.8)	-2.8 (-9.0–1.2)	0.537
Stimulated peak cortisol, nmol/L	667.5 (400–995)	602 (202–1,008)	0.005

Values presented as median (range) and number (%).

Cortisol levels during GST

For all included children, the median peak cortisol level during the GST was 628 (202–1,008) nmol/L. A peak cortisol level of ≥450 nmol/L was reached by 145 children (84.8%), including 93.3% of girls (56/60) and 80.2% of boys (89/111). Figure 9a presents the cortisol values during the GST for the children included in this study, whereas Figure 9b displays cortisol values for the excluded children who had pituitary MRI abnormalities and/or history of childhood cancer (unpublished data).

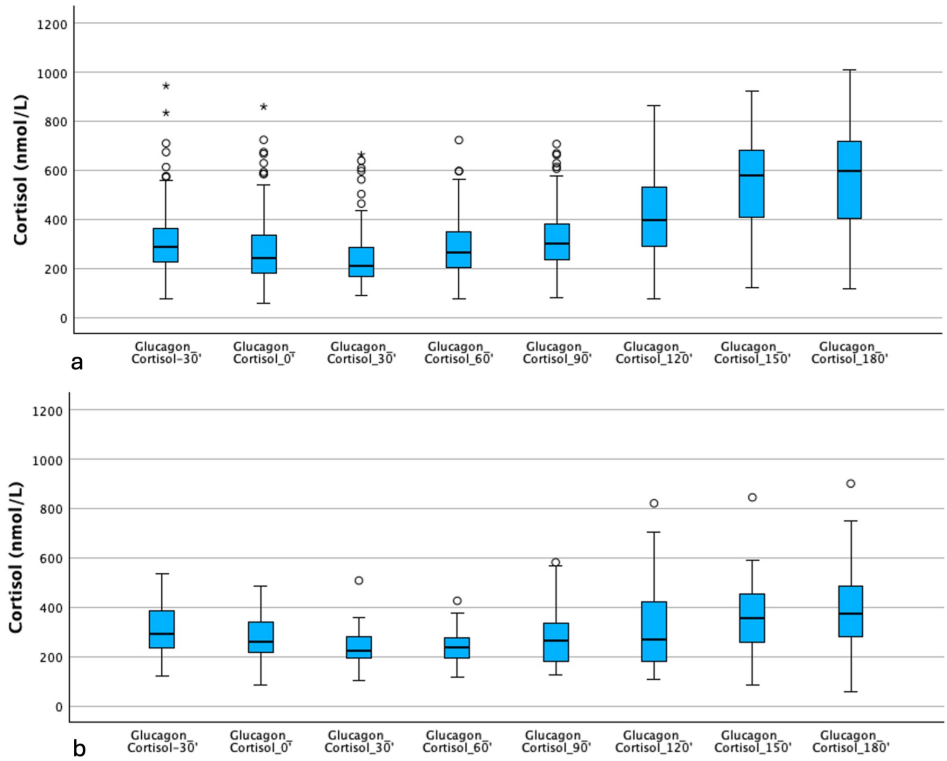

Fig. 9a-b. Panel a: Boxplots illustrating cortisol levels (nmol/L) during the GST for the children included in this study (n = 171). Panel b: Boxplots illustrating cortisol levels (nmol/L) during the GST in children excluded from this study with MRI pituitary pathology and/or a history of childhood cancer (n = 23) (Unpublished data).

Correlations between peak cortisol in GST and clinical variables

As shown in Figure 10, peak cortisol levels in the GST were negatively correlated with age (Spearman's rho = -0.26, $p<0.001$, $R^2 = 0.079$). Moreover, there was a positive correlation between peak cortisol and the change in glucose (delta glucose)

during the GST (Spearman's rho = 0.19, p = 0.014, R^2 = 0.029). However, no significant correlations were found between peak cortisol and lowest glucose in the GST (p = 0.94), basal cortisol (p = 0.17), or BMI (p = 0.29).

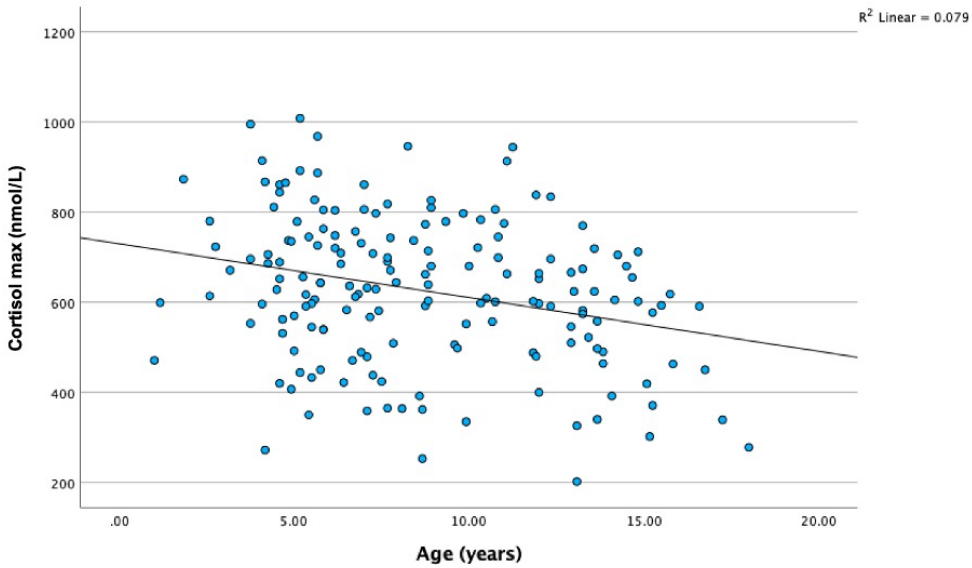

Fig. 10 Negative correlation between peak cortisol and age in the glucagon stimulation test. Spearman's rho = -0.26, p <0.001, R^2 = 0.079.

Sex differences in cortisol levels in GST

Cortisol values during the GST are presented in Figure 11 for girls and boys respectively. Among all included children, girls had significantly higher median peak cortisol values than boys (667.5 nmol/L vs. 602 nmol/L, p = 0.005, Table 11). This difference was also observed in children who had entered puberty, with girls showing higher median peak cortisol levels than boys (672 nmol/L vs. 490 nmol/L, p = 0.002).

The influence of sex on peak cortisol levels during the GST was also assessed using a linear regression analysis. This demonstrated that girls had significantly higher stimulated cortisol levels than boys (β (95% CI) 78.1 nmol/L (27.7–128.4) p = 0.003). The sex difference persisted after adjusting for age (β (95% CI) 65.3 (15.9–114.6), p = 0.01).

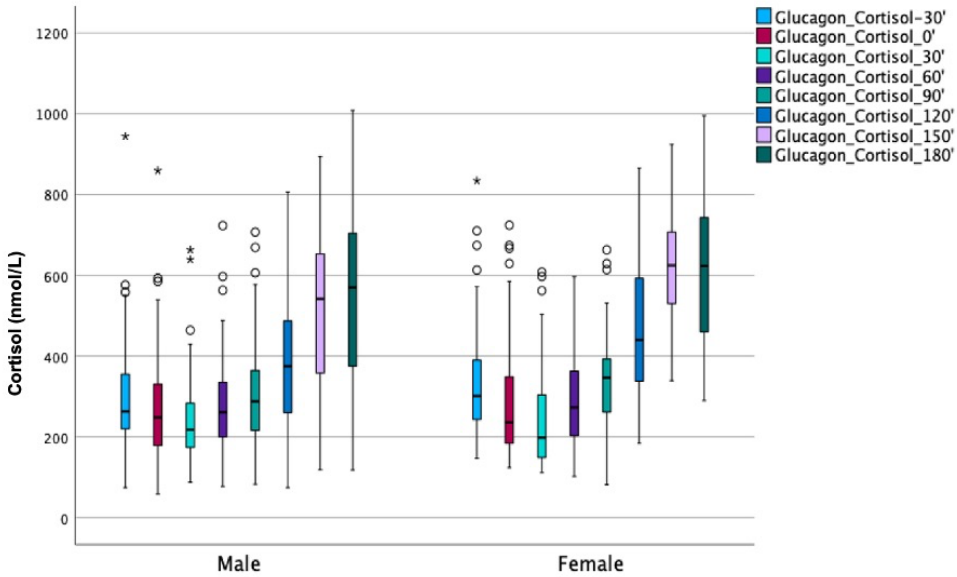

Fig. 11. Boxplots showing cortisol levels (nmol/L) during the glucagon stimulation test at timepoints from -30 to 180 minutes, presented separately for boys and girls.

Peak cortisol level related to puberty and sex steroid priming

The median peak cortisol level was higher in prepubertal children than in the 23 (13.5%) children who had entered puberty (median: 643 nmol/L vs. 577 nmol/L, p = 0.005). When comparing the peak cortisol levels between the 50 children who received sex steroid priming and those who did not, no significant difference in peak cortisol level was observed (602.5 nmol/ vs. 643 nmol/L, p = 0.335, n = 170).

Study IV

Study participants

A total of 26 children with ALL, treated in accordance with the ALLTogether protocol at either Skåne University Hospital or Karolinska University Hospital were included in this study. All participants had undergone low-dose ACTH tests and extended glucocorticoid analyses approximately two days and two weeks after completing dexamethasone treatment.

The median age at the time of inclusion was 4 (1–16) years, and 9 (34.6%) children were female. Median BMI (SDS) was -0.2 (-2.2–1.4). None of the children had a history of any HPA axis disorder.

No child had received glucocorticoid treatment prior to the ALL diagnosis. However, two (7.7%) children had received a glucocorticoid, betamethasone (3.4 mg/m^2), and intravenous hydrocortisone (90 mg/m^2), 6 and 11 days, respectively, before the second low-dose ACTH test. One child (3.8%) had been treated with an azole (fluconazole 3 mg/kg for 10 days) prior to the first test. This child had cortisol values below the cut-off, whereas the two children who received glucocorticoids had peak cortisol above the cut-off.

Peak cortisol levels in low-dose ACTH test

The median number of days after discontinuation of dexamethasone was 2.5 (1–6) days for performing the first low-dose ACTH test and 16 (14–22) days for the second test.

Peak cortisol levels for both tests are presented in Table 12. Children with two normal tests (peak cortisol ≥450 nmol/L) had significantly higher peak cortisol in the second test. The cortisol values for this subgroup (Normal/Normal, n = 7) are illustrated in Figure 12.

Table 12. Peak cortisol levels (nmol/L) in first and second low-dose ACTH test for all patients and in subgroups by test result.

Test result, First/second low-dose ACTH test	n (%)	First test, peak cortisol (nmol/L)	Second test, peak cortisol (nmol/L)	P-value
All patients	26 (100)	249.5 (14.9–976)	639 (304–1,152)	<0.001
Normal/Normal	7 (26.9)	632 (457–976)	800 (568–1,152)	0.018
Low/Normal	16 (61.5)	183.5 (40–375)	629 (489–981)	<0.001
Low/Low	3 (11.5)	261 (14.9–333)	326 (304–390)	0.109

Values presented as median (range) and number (%). Normal refers to peak cortisol ≥450 nmol/L and low to peak cortisol <450 nmol/L at the low-dose ACTH test.

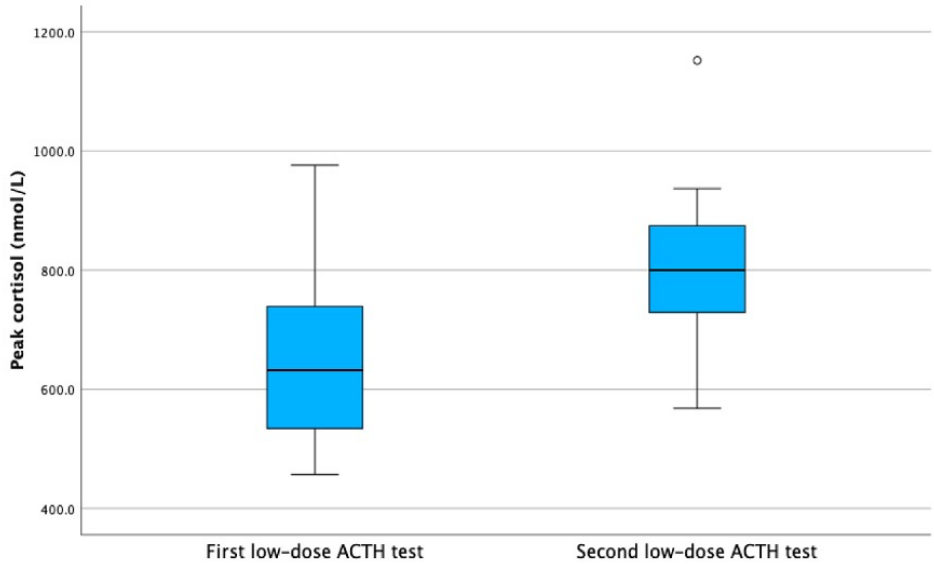

Fig. 12. Cortisol levels in the first and second low-dose ACTH test for children with two normal test results (peak cortisol ≥450 nmol/L, n = 7). The first test was performed approximately two days after the final dexamethasone dose and the second test approximately two weeks later.

Correlation between peak cortisol and cortisol/cortisone ratio

A positive association was observed between peak cortisol levels and cortisol/cortisone ratio during the initial low-dose ACTH test (Fig. 13; Spearman's rho = 0.39, p = 0.0497, R^2 = 0.099). In the second test, the correlation between cortisol and cortisol/cortisone ratio was non-significant (Spearman's rho = 0.37, p = 0.061, R^2 = 0.047).

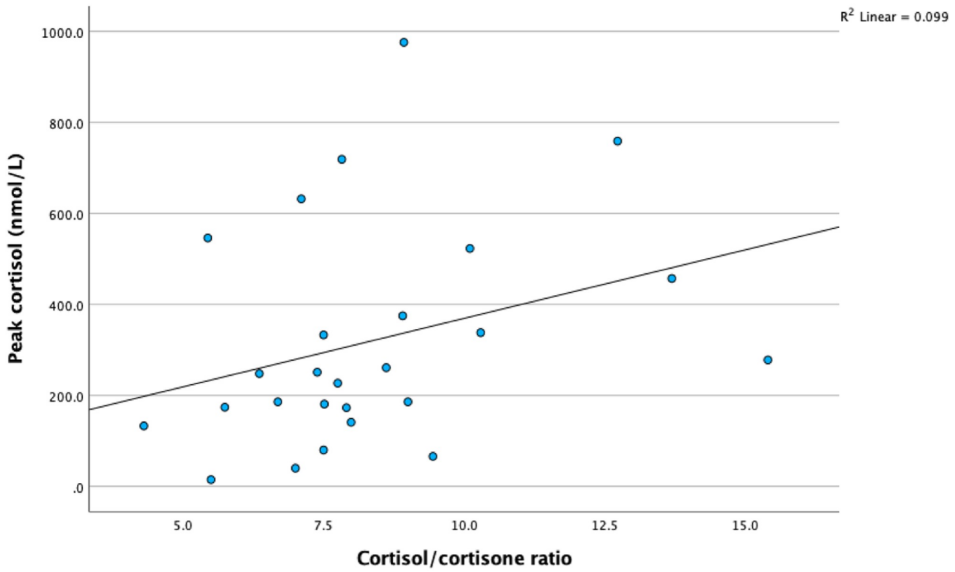

Fig. 13. Correlation between peak cortisol levels (nmol/L) during the initial low-dose ACTH stimulation test and cortisol/cortisone ratio, n = 26 (Spearman's rho = 0.39, p = 0.0497, R^2 = 0.099). The first test was performed approximately two days after the final dexamethasone dose, and the second approximately 14 days later.

Cortisol/cortisone ratio, first and second low-dose ACTH test

The cortisol/cortisone ratio in the first and second low-dose ACTH tests is illustrated in Fig. 14. No significant changes in cortisol/cortisone ratio were observed between the two timepoints in the overall group of children (n = 26, p = 0.64), nor within subgroup of children with two normal test results (n = 7, p = 0.31) or those with a Low/Normal result (n = 16, p = 0.57) (Table 13). Additionally, no significant difference in the change in cortisol/cortisone ratio between the two tests was seen when comparing the Normal/Normal and Low/Normal groups (p = 0.34).

Table 13. The cortisol/cortisone ratio in the low-dose ACTH tests. The first test was conducted two days after last dexamethasone dose and the second test two weeks later.

Test result, First/second low-dose ACTH test	n (%)	First test, cortisol/cortisone ratio	Second test, cortisol/cortisone ratio	P-value
All patients	26 (100)	7.8 (4.3–15.4)	8.5 (5.3–18.2)	0.64
Normal/Normal	7 (26.9)	8.9 (5.4–13.4)	10.3 (5.4–17.9)	0.31
Low/Normal	16 (61.5)	7.6 (4.3–15.4)	7.4 (5.3–13.6)	0.57
Low/Low	3 (11.5)	7.5 (5.5–8.6)	8.7 (6.0–18.2)	0.59

Values presented as n (%) and median (range). ACTH: adrenocorticotropic hormone. Normal = peak cortisol ≥450 nmol/L, Low = peak cortisol <450 nmol/L.

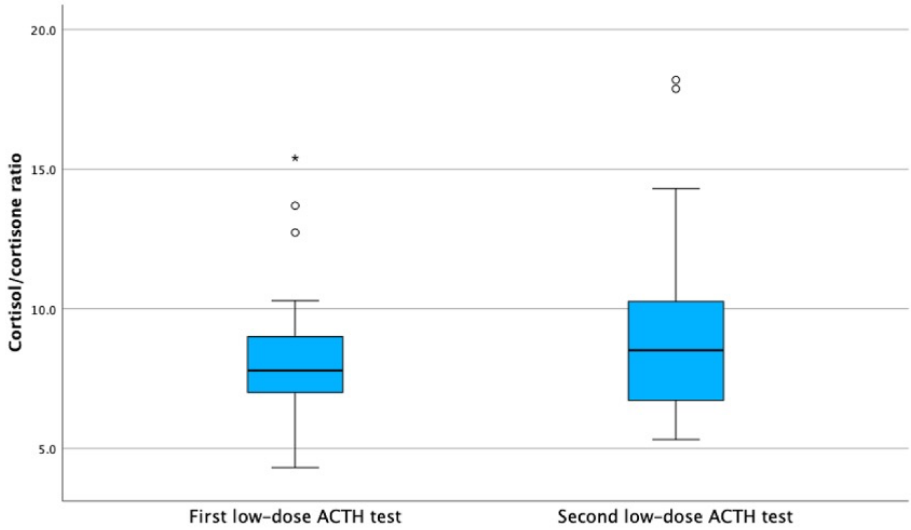

Fig. 14. Cortisol/cortisone ratio in first and second low-dose ACTH test, n = 26. The first test was performed approximately two days after the final dexamethasone dose, and the second approximately two weeks later.

Plasma levels of dexamethasone

At the time of the first low-dose ACTH test, performed a median of 2.5 days after the last dexamethasone dose, detectable levels of serum dexamethasone were found in four children (15.4%), with concentrations in the range 0.57–3.4 nmol/L. The median peak cortisol among these four was 243 nmol/L (14.9–759), and three had a peak cortisol below the cut-off (≥450 nmol/L). In the second test, performed a median of 16 days after the last dexamethasone dose, serum dexamethasone was undetectable in all children.

Discussion

Several aspects of the assessment and validation of pituitary function in children currently lack evidence-based guidelines. Enhancing our knowledge of how to evaluate pituitary diseases in children will better equip clinicians to make evidence-based decisions aligned with the principles of 'choosing wisely'. This may reduce the use of low-yield diagnostic tests that provide limited clinical value, thereby contributing to improved patient care through more effective diagnostic and therapeutic strategies. Ultimately, it will also help to allocate healthcare resources to those most in need [132, 133].

To improve the understanding of different aspects of pituitary morphology and function in children, we designed the studies included in this thesis. We investigated the growth potential and need for radiological follow-up in small non-functioning pituitary microadenomas and cysts. Additionally, we evaluated pharmacological stimulation tests used to assess the GH-IGF-1 axis (including SSNP, AITT) and the HPA axis (using GST, low-dose ACTH test). Our aim was to evaluate potential diagnostic pitfalls, such as pituitary refractoriness and the influence of clinical parameters on test results, which may affect interpretation and clinical decision-making. Furthermore, we wanted to enhance the understating of secondary adrenal insufficiency to enable early identification of affected children, thereby reducing the risk of serious outcomes and need for advanced medical care.

The role of MRI follow-up in the evaluation of non-functioning pituitary lesions

Overuse of diagnostic examinations can cause stress and anxiety for paediatric patients and their families and may also contribute to delays in care for those in more urgent need. Moreover, there might be a risk that excessive examinations are harmful for the patient [132]. In the context of repeated pituitary MRI examinations in children, potential physiological risks include those associated with general anaesthesia [134], as well as concerns regarding brain deposition of potentially neurotoxic gadolinium-based contrast agents [73]. Based on these concerns, we designed Study I, to evaluate the necessity for follow-up imaging of non-functioning pituitary microadenoma and small pituitary cysts. As outlined in the introduction, radiologists increasingly encounter incidental findings in the pituitary as a result of a broader use of MRI and advancements in MRI techniques [41, 58, 62].

A challenge in the assessment of repeated MRI examinations is the risk of anchoring bias, where a radiologist may be influenced by prior interpretations [135]. To avoid

this bias, all MRI scans in our study were reassessed by neuroradiologists blinded to the previous statements. This approach differed from that in most earlier studies, which primarily relied on the original MRI statements [54-56].

As previously mentioned, knowledge of the natural course of pituitary microadenomas and cysts is limited, and the development of endocrine dysfunction or visual field deficits for incidental pituitary adenomas seem to be uncommon [136]. In our study, we investigated size variations of non-functioning pituitary microadenomas or cystic lesions in 55 children over a median follow-up time of three years. One child had a significant enlargement of a cystic lesion over a time period of five years. In this case, the follow-up MRI was conducted because the child developed hormonal abnormalities. Our findings align with those of Thaker et al., who evaluated 40 children with non-functioning pituitary microadenomas over an average follow-up time of 4.5 years. Their study reported that one lesion increased in size by 4 mm but remained stable and <10 mm after six years [56]. Derrick et al. found no size progression in 54 children with pituitary microadenoma on repeated MRIs for 1.8–3.6 years [54]. Similarly, studies by Pedicelli et al. and Souteriou et al. evaluating the growth potential of pituitary incidentalomas in 28 and 23 children, respectively, found no enlargement of any lesions in repeated MRI [42, 55]. Taken together, studies assessing the growth potential of non-functioning pituitary incidentalomas, including ours, indicate that significant size enlargement occurred in 2 out of 200 (1%) children [42, 54-56].

Moreover, in Study I, 7% of MRI measurements differed >2 mm between the two reviewers; an inter-observer variation that, in a clinical setting, could have been interpreted as tumour progression. These minor size variations are close to the spatial resolution limits of MRI and should therefore be interpreted with caution. Additionally, in our study, as well as in previous studies, several pituitary lesions were no longer visible during follow-up [54, 56]. This leads one to consider whether these lesions truly resolved or whether they were initially artefactual findings arising from the sensitive modern MRI protocols [54]. In line with this, our study found lower inter-observer agreement with 3 T than with 1.5 T, likely due to increased susceptibility to imaging artefacts [68]. Inter-observer agreement has not been assessed in previous studies [42, 54-56], and our evaluation provided information regarding the methodological limitations of MRI-based measurements.

As previously mentioned, Korbonits et al. recently presented consensus guidelines for management of pituitary adenomas in children. They noted that non-functioning pituitary microadenoma generally follows a benign course and recommend follow-up MRI after one year, followed by imaging at 1–2-year intervals for the next three years, with gradual extension of the interval if the lesion remains stable. However, no more specific guidelines are presented [40]. This is in line with a systematic review by Rikvold et al., which suggests individualized follow-up of non-functioning pituitary adenomas in children and adults, with less rigorous monitoring for microadenomas [52]. Nevertheless, this review also lacks concrete guidance on

how the follow-up should be structured. The Swedish National Treatment Program for Pituitary Tumours currently recommends MRI follow-up after 1 and 3 years, and thereafter every 3 years until after puberty, in addition to clinical monitoring of growth and pubertal development in children with non-functioning pituitary adenomas <10 mm [137]. For adults, the same programme only advises MRI follow-up for lesions ≥4 mm [137].

In Study I, consistent with previous research, we found that the risk of lesion growth was low. Considering existing evidence suggesting a higher risk of growth in larger lesions [51, 52], along with the measurement variability related to slice thickness and resolution limitations, we proposed follow-up strategies based on lesion size. This is consistent with recommendations in the French consensus document by Galland et al. [50]. To assist clinicians, we suggest the following follow-up protocol:

Lesions <4 mm: clinical monitoring only.

Lesions 4–6 mm: MRI after 2 years, with consistent clinical follow-up.

Lesions ≥7 mm: MRI at 1 and 3 years, with consistent clinical follow-up.

We further suggest that subsequent MRIs should only be performed if there is clinical evidence of new symptoms or hormonal disturbances. Follow-up MRIs can be conducted without gadolinium contrast, as relevant changes in size and morphology, as well as cystic lesions, can be assessed using non-contrast imaging.

The role of pituitary refractoriness in the evaluation of the GH axis

Assessment of pituitary GH secretion is essential for evaluating suspected GHD and for guiding decisions regarding rhGH therapy. Treatment with rhGH involves daily subcutaneous injections and regular hospital visits, leading to financial, logistical, and emotional challenges for families, as well as strain on healthcare resources [138, 139]. To provide the best possible care, avoid initiating an expensive treatment without clear clinical benefit, and allocate healthcare resources wisely, it is important to accurately identify the children most likely to benefit from rhGH therapy. To enhance our understanding of the challenges in diagnosing GHD, we designed Study II to investigate the phenomenon of pituitary refractoriness, which may lead to a false diagnosis of GHD and unnecessary rhGH treatment.

In Study II, we found that 21.9% of all children who achieved a GH peak ≥7 μg/L in either SSNP or AITT, failed to reach the cut-off during the AITT when it followed a GH peak ≥7 μg/L during the SSNP. These children, defined as refractory in our study, would have been at risk of being misdiagnosed with GHD if only the AITT had been used for evaluation. The observed frequency of refractory children was similar to the results of Lennartsson et al., who reported that 19% of the children in their study (n = 102) were refractory in the AITT using the same cut-off [93]. A few other studies have also suggested that spontaneous GH peaks may influence the GH response to stimulation tests in children [94, 140].

No clinical parameters that could predict refractoriness were identified. The refractory group included a higher proportion of males and had a somewhat higher BMI, although the median BMI SDS remained below average for Swedish children.

We evaluated possible biochemical differences between the refractory and non-refractory group. Concerning hypoglycaemia during the ITT, glucose <2.2 mmol/L was used as the threshold for an adequate hypoglycaemia in our protocol. No universally accepted cut-off for the lowest glucose value in the ITT exists; thresholds ranging from 2.2 to 2.8 mmol/L are commonly employed [141]. We found no difference concerning occurrence of adequate hypoglycaemia between the refractory and non-refractory group and, unfortunately, we had no information concerning the duration of the hypoglycaemia. Concerning the magnitude of the GH peaks over cut-off in the SSNP and AITT, we found no differences when comparing the subgroups (LOW-HIGH, HIGH-LOW, HIGH-HIGH). However, the timing of the GH peak in the AITT differed, and the HIGH-HIGH group had the GH peak 30 minutes later in the AITT than the LOW-HIGH group. This may indicate that the timing of a spontaneous GH peak, prior to a stimulation test, plays a greater role than its magnitude for the ability to achieve another peak during a subsequent stimulation test.

The period of possible refractoriness was estimated to 210 minutes for the group with two GH peaks above the cut-off (HIGH-HIGH group). The blood sampling in the AITT ended after 180 minutes, and we therefore had no information concerning the length of the refractory period in the refractory group (HIGH-LOW group). However, the time between the median last GH peak ≥7 μg/L in the SSNP and the end of the AITT was 225–255 minutes. Lennartsson et al. found a similar duration of the refractory period, median 202.5 minutes [93].

It has been shown that a high proportion of children, up to 81%, with GH values below the cut-off during the initial stimulation test demonstrate normal GH levels upon retesting after reaching final height [142]. This finding raises the possibility that many diagnoses of GHD may be false positive. Several previous studies have shown that priming with oestrogens reduces the proportion of children unresponsive in GH stimulation tests [143-147]. Furthermore, a recent study found that priming also increased spontaneous nocturnal GH pulses and reduced discrepancies between spontaneous nocturnal GH levels and stimulated GH levels [148]. Sex steroid priming was not routinely included in the testing protocol during the period of Study II. However, at out clinic, priming was introduced at a later stage. It had become standard practice for prepubertal children undergoing evaluation of GH and cortisol levels using arginine and glucagon stimulation tests, by the time Study III was conducted (see Methods section for details). In Study II, the median age of the children was 8 years, and 212 (82.5%) were prepubertal. Among these, only seven received sex steroid priming. In the group of children with two GH peaks ≥7 μg/L (HIGH-HIGH), four (5.1%) received priming, compared with just one child (1.3–

2.3%) in each of the other subgroups. It is reasonable to assume that, under our current guidelines, a larger proportion would have received priming, and it is possible that fewer children would have been classified as refractory if priming had been routinely implemented.

Given the many pitfalls in diagnosing GHD using GH stimulation tests, including refractoriness, it has been suggested that children who are unresponsive to these tests should be classified as 'Short Stature Unresponsive to Stimulation test (SUS)'. According to this proposal, a diagnosis of GHD would be limited to those with a genetic condition causing GHD, MPHD, hypothalamic or pituitary abnormalities on MRI, and/or acquired pituitary/hypothalamic damage [149]. Such a reclassification could spare many children the anxiety and burden of being labelled with a potentially serious illness, though still allowing continued diagnostic evaluation during follow-up and assessment of treatment response.

Regardless of whether such a reclassification is adopted, a very important factor in the diagnosis and management of affected children is clinicians' awareness of factors that may contribute to low GH values during stimulation tests. These include, e.g., refractoriness, obesity, hypothyroidism, hypogonadism, and individual GH sensitivity [69, 93, 150]. By conducting Study II, we hope to have contributed to increased understanding and awareness GH refractoriness, thereby helping to prevent the overdiagnosis of GHD and the unnecessary treatment with rhGH therapy.

The role of stimulation tests and clinical parameters in the evaluation of the HPA axis

Secondary adrenal insufficiency can present with vague, non-specific symptoms, or in severe cases, lead to an acute cardiovascular collapse [36]. To reduce both morbidity and mortality, and to prevent the suffering associated with potential intensive care unit admission, as well as the related costs and resource burden, it is of great importance to identify and manage this condition at an early stage. Moreover, overdiagnosis of the condition, leading to potential unnecessary treatment with glucocorticoids, should also be avoided. To achieve this, clinicians must not only be aware of the condition but also have reliable testing procedures and an understanding of the potential pitfalls in the interpretation of test results.

In Study III, we explored this topic by evaluating the GST as a tool for assessing HPA axis function, as well as clinical parameters that might influence test results. Additionally, in Study IV, we further investigated possible biochemical factors that could help to identify children at risk of developing secondary adrenal insufficiency. In the same cohort of children with ALL treated with dexamethasone from which Study IV originated, we are also conducting an ongoing study to investigate the prevalence and duration of secondary adrenal insufficiency. This study also aims to

evaluate the necessity of HPA axis testing using stimulation tests, and to determine whether glucocorticoid substitution therapy may be required.

During the GST, the entire HPA axis is indirectly assessed, unlike in the commonly used ACTH stimulation test, which directly stimulates the adrenal glands with synthetic ACTH, thereby bypassing the hypothalamus and pituitary [119, 120]. Although the GST is primarily used to evaluate the GH axis rather than the HPA axis, we propose that assessment of the HPA axis with the GST may be an advantageous approach, particularly in early stages of secondary adrenal insufficiency, when atrophy of the adrenal cortex may still be partial [151].

To investigate this further, we evaluated cortisol responses during the GST in Study III. Cortisol levels were also analysed in the group of excluded children who were at higher risk of adrenal insufficiency due to history of childhood cancer and/or deviations at pituitary MRI (unpublished data). These unpublished data, together with cortisol levels of the children included in Study III, are illustrated in Figure 9. The children at higher risk of adrenal insufficiency seemed to consistently show lower median cortisol levels (median <400 nmol/L) between 120 to 180 minutes during the GST. Moreover, among the children included in Study III, we found that the median peak cortisol value reached 628 nmol/L, with 93% of the girls and 80% of the boys achieving peak cortisol levels above the cut-off of the study (≥450 nmol/L). The ITT, despite its high risk of hypoglycaemia, has been considered a gold standard for the evaluation of the HPA axis [123], and as a comparison to our results, O'Grady et al. concluded that 82% of the children in their study (n = 223) had peak cortisol above cut-off (defined as ≥500 nmol/L at ITT) [111].

Taken together, we believe that these results support the GST as a reliable and safe test for evaluating the complete HPA axis, with a lower likelihood of severe hypoglycaemia compared with the ITT.

Regarding the use of the ACTH test in evaluating the HPA axis, it is, as mentioned in the introduction, debated whether the standard-dose or low-dose ACTH test is most appropriate for diagnosing secondary adrenal insufficiency [85]. In Study IV, the low-dose ACTH test was used to assess adrenal function. None of the children who had a peak cortisol level above the cut-off in the first test demonstrated a value below the threshold in the second test. This finding may support the usefulness of the low-dose ACTH test as a reliable method for assessing secondary adrenal insufficiency in children.

The cortisol cut-off level for stimulation tests is not well established in children. As previously mentioned, different cut-off levels have been proposed, with values typically ranging from 400–500 nmol/L [105-112]. The threshold of 450 nmol/L, used in our studies (Studies III and IV), is based on adult reference values for the specific biochemical assay applied [131].

In Study IV, we observed that children at risk of developing secondary adrenal insufficiency but with peak cortisol levels ≥450 nmol/L in their initial low-dose ACTH test, had significantly higher median cortisol levels in the second test conducted two weeks later (median 632 nmol/L vs. 800 nmol/L). This finding raises the possibility that a state of partial adrenal insufficiency may have gone undiagnosed in the first test. If so, it could be hypothesised that that these children might not have been able to generate an adequate cortisol response under physiological stress at the time of the initial assessment.

Moreover, in Study III, we aimed to investigate whether clinical parameters affected cortisol levels, and consequently the appropriate cortisol cut-off. We found that peak cortisol levels during the GST were negatively correlated with age, and that girls had significantly higher peak cortisol levels than boys, also after adjusting for age. Previous studies have also reported age- and sex-related differences in cortisol levels [108, 109, 115, 152-154]. Concerning age, our finding of a negative correlation between peak cortisol levels and age is consistent with the results of some earlier studies assessing cortisol levels during the GST [108, 109, 115, 154]. Similarly, studies using the ACTH test and the ITT have demonstrated that peak cortisol responses tend to decrease with increasing age [111, 112, 117, 155]. Interestingly, Weintrob et al. reported that only total cortisol, not free serum cortisol, correlated negatively with age during the GST [115]. Regarding sex differences, some previous studies have also found that girls have higher peak cortisol levels in the GST [108, 115, 154]. However, other studies have reported no significant differences in cortisol responses with respect to either age or sex [106, 114, 156], and research on sex differences in salivary cortisol levels in children has shown inconsistent findings [153, 157, 158]. Our results suggest that for more reliable diagnosis based on stimulation tests, age- and sex-specific cortisol cut-off values may be necessary. However, more studies are needed to validate these findings. Moreover, when interpreting test results, it should be considered if a stimulation test result close to the cut-off might fail to identify children with partial adrenal insufficiency.

Weintrob et al. observed that girls tended to have higher levels of total cortisol than boys, whereas no difference was found in serum free cortisol levels between the sexes [115]. When considered together with the previously mentioned finding that CBG levels seem to be lower in males than in females [31, 32], this raises the possibility that the sex differences in CBG concentrations may contribute to the observed differences in cortisol levels. Moreover, oestrogens are known to increase CBG concentrations, for example during pregnancy or oestrogen replacement therapy, which in turn leads to elevated total cortisol levels [30].

In Study III, sex steroid priming was part of the GST protocol, and 29% of the children had received oestradiol as part of the priming regimen. Given that oestradiol can raise both CBG and total cortisol levels, this may have influenced our results. However, to our knowledge, there are no studies defining the duration of

oestrogen treatment required to alter CBG levels. As the oestradiol priming in Study III was limited to three days, we consider it unlikely that this brief oestrogen exposure substantially affected CBG levels.

We found no difference in cortisol levels between children who received priming and those who did not. However, it should be noted that there was a difference in age between unprimed and primed groups, which may have influenced this finding. In addition, the use of oestrogen-based oral contraceptives can increase total cortisol levels, although information on contraceptive use was not available in our data. Altogether, these findings suggest that measuring free cortisol, rather than total cortisol, may potentially provide a more accurate assessment of adrenal function. Unfortunately, we did not have the opportunity to evaluate free cortisol levels in our studies.

Through Studies III and IV, we hope to promote recognition of the GST and low-dose ACTH test as reliable methods for evaluating the HPA axis, while also highlighting the need for age- and sex-specific reference intervals in children.

The role of glucocorticoid analyses in the evaluation of the HPA axis

Predictors for identifying children at risk of developing secondary adrenal insufficiency are not completely understood [89]. To address this, Study IV aimed to evaluate the usefulness of an extended glucocorticoid analysis including cortisol, cortisone, and dexamethasone levels in the assessment of secondary adrenal insufficiency.

We observed a positive correlation between peak cortisol levels during the low-dose ACTH test and cortisol/cortisone ratio. As described in the introduction, the enzyme 11β-HSD mediates the reversible conversion between biologically active cortisol and inactive cortisone [33, 34]. An indirect assessment of 11β-HSD function can be made using the cortisol/cortisone ratio [159]. Our findings may indicate that an altered cortisol metabolism, reflected by a lower cortisol/cortisone ratio, may affect cortisol metabolism in children with impaired adrenal function. Although this topic is not well-studied, previous reports have described changes in the cortisol/cortisone ratio under physiological stress and in adrenal disorders [160, 161]. Additional large-scale studies are required to validate the role of 11β-HSD in the context of secondary adrenal insufficiency.

Moreover, we proposed, in Study IV, that reduced peak cortisol responses could be associated with a slower metabolic clearance of dexamethasone. However, our results did not support plasma dexamethasone levels as a useful marker for identifying those at risk of developing secondary adrenal insufficiency.

The role of decision complexity and responsible resource use in paediatrics

In paediatric medicine, clinicians frequently encounter situations were evidence-based guidance is limited, largely due to the challenges of conducting large clinical

trials in children [162]. This project aimed to investigate diagnostic challenges in paediatric pituitary diseases, with the goal to provide knowledge to support evidence-based strategies for diagnosis and management. This is of importance not only for the best interest of individual patients, but also for responsible and effective use of limited health care resources. This overall aim aligns with the principles of the 'Choosing Wisely' initiative, encouraging a greater awareness of the risks associated with overdiagnosis and overtreatment, promoting high-value care [163]. Studies indicate that 25% of health care expenditures in the United States are due to interventions that offer no benefit to patients [164]. This issue of low-value care is also a recognised concern in Sweden [165].

Clinical decision-making is a complex process, influenced by many factors. In paediatric care, parental anxiety may play a role, as well as the doctor's personal concern about missing a serious condition [132]. A European survey of 1,416 physicians treating children found that 83% identified expectations from families and patients as the most influential factor contributing to medical overuse. Other important factors included worry for parental reactions, the desire to reduce clinical uncertainty, and adherence to national guidelines [166]. Such medical overuse may lead to excessive diagnostic investigations, with risks of both false positive test results and the detection of incidentalomas [132].

Additionally, clinical decisions are sometimes based on outdated routines, as established practice patterns can be difficult to change even when new, evidence-based recommendations become available [167, 168]. In situations where evidence is lacking, the concept of 'eminence based' medicine has been described, defined as a model of 'making the same mistakes with increasing confidence over an impressive number of years' [169]. Cognitive biases further influence medical decision-making. For example, 'personal bias', may occur when a clinician's own past experiences influence current decisions, potentially leading to biased decisions for investigation or follow-up. Another relevant bias in this context is 'group think', a psychological phenomenon in which the desire for consensus within a group overrides critical evaluation, potentially resulting in irrational decision-making [170]. Moreover, cognitive biases such as 'anchoring bias' (over-reliance on initial clinical assessments), 'framing bias' (influencing how a clinical problem is presented), and 'attribution bias' (assessments based on stereotypes related to the patient's characteristics and demographics) can all affect clinical judgement and decisions [135].

An awareness of the complexities involved in clinical decision-making, together with a focus on providing high-value care, is something every clinician should actively reflect on in daily practice to improve care and support the responsible allocation of healthcare resources.

Methodological considerations, limitations, and strengths

The main limitations of Studies I–III are primarily associated with their retrospective design, which for example limits the ability to standardise testing procedures.

In Study I, MRI protocols were somewhat heterogenous, and the follow-up time was both limited and non-standardised, which may have influenced our findings. Furthermore, the statistical assessment of the inter-observer agreement using Cohen's kappa had limited generalisability, as it was based on evaluations by only the two reviewers involved in the study. To improve the reliability and reproducibility of future studies, a prospective study design with standardised imaging protocols, uniform follow-up intervals for imaging, and a larger group of reviewers would be preferable. Alternatively, a register-based study collecting longitudinal data could offer the advantages of larger sample sizes and extended follow-up periods.

As is often the case in retrospective studies, we had challenges in controlling for confounding factors. For instance, in Study II, we had limited information concerning the fasting procedure prior to the AITT, which may have impacted the interpretation of refractoriness. Similarly, in Study III, it was difficult to obtain reliable information from medical records on the use of inhaled corticosteroids and hormonal contraceptives, both factors that may affect cortisol levels.

In Study II, the duration of the SSNP and the timing of blood sample collection varied to some extent, which may have impacted the interpretation of the results. Additionally, due to the retrospective study design, we could not study the duration of the refractory period in children with refractoriness or the length of hypoglycaemia across all subgroups. A prospective design with standardised protocols for fasting, test timing, and glucose monitoring would enhance the reliability and interpretability of the findings.

Study III had similar limitations. Testing was conducted in a clinical setting by different nurses, which may have led to procedural variability. The study population consisted of children primarily investigated for short stature, resulting in a heterogenous cohort with a potentially increased risk of pituitary abnormalities. As the study population was not selected for research purposes, the findings may not

be representative of the general paediatric population. Furthermore, the use of sex steroid priming, performed in accordance with the clinical protocol for evaluation of short stature, may have influenced the cortisol response. A prospectively recruited research cohort with clearly defined inclusion criteria and documentation of both previous and ongoing medications would represent an improved approach for Study III. Concerning the evaluation of the GST as a tool to assess the HPA axis, a prospective study with repeated testing in healthy children, comparing cortisol levels in the GST with those from another established stimulation test, would improve assessment of the GST's reliability and enable the development of reference values for the paediatric population.

Study IV, in contrast, was prospective and had more standardised testing procedures than Studies I–III. However, as the study was conducted in a clinical setting, it was still subject to limitations such as the patients' physical conditions and the influence of weekends and holidays, which affected the possible timepoints for testing. The study population consisted of children with ALL, which may limit the generalisability of the findings to other paediatric populations. An alternative methodological approach for Study IV would be a multicentre study with extended testing hours, including weekends, to minimise the impact of scheduling limitations and the patients' physical conditions. Additionally, expanding the study population to include children with a wider range of diagnoses treated with glucocorticoids would improve the generalisability of the results.

Although the findings of Studies I and IV were limited by small study populations, the larger cohorts of Studies II and III strengthened the results. Another strength of Studies II–IV was the consistent use of the same biochemical method for analyses related to the main results (GH and cortisol). Additionally, the blinded re-evaluation of all MRI examinations in Study I strengthened the result of that study and reduced the risk of several biases associated with repeated evaluation with MRI.

Conclusions

Based on the findings presented in this thesis, the following conclusions were drawn:

- **Pituitary lesions**:

  Non-functioning pituitary microadenoma and cystic lesions exhibited only minor size variations, frequently within the range of MRI slice thickness. MRI size measurements are subject to methodological limitations and should be interpreted with caution.

- **Pituitary refractoriness**:

  Approximately one fifth of the children with short stature demonstrated pituitary refractoriness to GH secretion during the AITT. Only a few clinical characteristics were identified to distinguish this group. When interpreting GH levels during stimulation tests, refractoriness should be carefully considered.

- **HPA axis assessment**:

  The GST is a reliable method for assessing the HPA axis function in children. Higher peak cortisol levels were observed in girls and younger children, which may support the need for sex- and age-specific reference ranges for cortisol.

  Following high-dose dexamethasone treatment, peak cortisol levels during the low-dose ACTH test correlated with the cortisol/cortisone ratio, suggesting that 11β-HSD-mediated interconversion plays an important role in the regulation of active cortisol levels in children at risk of secondary adrenal insufficiency. Plasma dexamethasone was not predictive of adrenal suppression and did not appear to be a useful biomarker for identifying patients at risk of secondary adrenal insufficiency.

In conclusion, I hope the findings of this project will support clinicians to reduce the overuse of MRI for incidentally discovered small NFPAs and cysts, raise awareness of pituitary refractoriness as a potential diagnostic pitfall in GHD, and provide evidence to support a more accurate assessment of cortisol levels in children.

Future perspectives

During the course of this research project, new questions and potential areas for further research have emerged. Below, I have summarised some of the reflections and ideas that arose along the way:

- **Pituitary lesions**: Considering the findings of Study I, it would be valuable to conduct a larger-scale evaluation of the natural course of small, non-functioning pituitary lesions in children. Longitudinal data, for instance by the Swedish Pituitary Registry, could enable an evaluation of whether our observed lack of size progression can be confirmed in a larger population.
- **Pituitary refractoriness**: Concerning pituitary refractoriness, it would be interesting to assess whether it is a clinically relevant problem in our current GH testing protocols, particularly when using sex steroid priming procedures. Moreover, a prospective study examining the duration of the refractory period under standardised conditions could be valuable. However, ethical concerns regarding extended fasting in children would lead to challenges in such a study design.
- **HPA axis assessment**: As for cortisol levels, a prospective comparative study of cortisol responses to both the GST and the low-dose ACTH test would be valuable to assess test reliability and support the development of evidence-based cortisol cut-off recommendations. Additionally, analysing both total and free cortisol concentrations could help clarify the role of CBG in sex-specific variations in cortisol levels.
- **Cortisol metabolism**: Studies of both children and adults have suggested the involvement of 11β-HSD activity in the pathophysiology of several diseases associated with altered cortisol levels, including cardiovascular, metabolic, and endocrine disorders [33, 34, 160, 171-173]. In Study IV, we found a correlation between peak cortisol and cortisol/cortisone ratio, suggesting a potential role of 11β-HSD in modulating adrenal cortisol responses. Although the sample size was limited, I find this result interesting and further research is needed to better understand the role of 11β-HSD in secondary adrenal insufficiency.
- **Personal priorities in future research**: Even given the interesting research questions reflected on above, the most important perspective for me in the nearest future is to complete the main study related to Study IV. The aim of this study is to investigate the prevalence and duration of secondary adrenal insufficiency in children with ALL and suggest strategies for clinical follow-up.

Ethical considerations

When conducting retrospective reviews of medical records, as in Studies I–III, general ethical concerns include the potential to encounter sensitive personal information or to discover an unintended personal relationship with the individual whose records are being reviewed. In such cases, it is crucial to carefully consider whether the potential benefits of the information collected outweigh the risks of compromising the patient privacy. In our studies, I believe this balance was appropriately maintained.

This research project did not involve collection of any sensitive patient data, and only a few designated individuals had access to the data. All personal information were, of course, covered by confidentiality. The data that have been published or will be published have been anonymised, and all the studies were approved by the relevant ethics committee (see Ethical approvals in the Methods section).

Moreover, as a part of this project, we conducted a re-evaluation of pituitary MRI scans. This procedure involved the potential risk of identifying previously unreported pathological findings. Fortunately, this was never the case, but in such a situation, the only responsible and ethically appropriate action would have been to contact the individual involved. However, this would have been an ethical challenge as the study had the appropriate ethical permit but was conducted without obtaining informed consent for participation.

Additionally, Studies II–III were conducted without parental informed consent to review medical records. For Study III, we proposed an opt-out consent process in our application for ethical permit, whereby patients and their parents would be informed via a secure electronic platform (1177) and could decline participation through a standardised procedure. However, the Swedish Ethical Review Authority expressed concern that the opt-out procedure could potentially 'cause harm to an already vulnerable research population'. Consequently, we were instructed to replace the opt-out procedure with not informing the research subjects about their participation at all. As a result, our attempt to implement consent in this retrospective study was overridden.

As for Study IV, which was a prospective study, written informed consent was obtained from all parents of the participating children. Two versions of research participant information were provided: a simplified version intended for individuals <15 years of age, and a more detailed version aimed at parents and older children.

An ethical consideration in this context is the timing of the consent process. Families generally receive the study information when they are still in the crisis of being informed that their child has a serious illness. In this situation, there is a risk that parents may not be able to fully process the implications of study participation. Their decision to consent may be influenced by the hope that involvement in the study could benefit their child and increase the chances of wellbeing. For those delivering the study information, it is important to be attentive to this risk and, if needed, to repeat the information at a later stage.

Moreover, during the planning phase of Study IV, we carefully considered how to limit the burden and potential discomfort for the participating children. Our aim was to minimise the volume of blood drawn and to limit the additional time required at the hospital for study-related procedures.

In the clinical practice at the Childhood Cancer Centres involved in Study IV, evaluation of secondary adrenal insufficiency is not routinely conducted outside of the study protocol, and it is generally unknown which children may be affected. At the outset of the study, we carefully considered how to manage situations involving the identification of children with subnormal cortisol responses. Although the study was primarily observational, we found it unethical not to offer treatment if subnormal cortisol values were identified. Consequently, children with suboptimal test results were considered for glucocorticoid replacement or stress-dose therapy.

Overall, I am confident that the benefits of this research project outweighed the potential risks related to both patient privacy and any associated potential discomfort.

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