

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/33195> holds various files of this Leiden University dissertation.

Author: Appelman-Dijkstra, Natasha Mireille

Title: Long-term consequences of growth hormone replacement and cranial radiation on pituitary function

Issue Date: 2015-06-04



Long-term consequences of growth hormone replacement and cranial radiation on pituitary function

Natasha Appelman-Dijkstra

Long-term consequences of Growth Hormone replacement and cranial radiation on pituitary function

Natasha Mireille Appelman-Dijkstra

COLOFON

ISBN 978-94-6295-147-1

Author Natasha M. Appelman-Dijkstra

Layout and printed by Proefschriftmaken.nl || 's-Hertogenbosch

Cover Proefschriftmaken.nl || 's-Hertogenbosch

Photo Paul Abspoel, www.paulabspoel.nl
View from the 'Burcht' on the 'Hooglandse Kerk'

The institute for Human rights and Medical research (instituut voor Mensenrechten en Medisch Onderzoek, iMMO) uses gates and doors as a metaphore for the passage of the assylum seekers' story on torture and ill treatment.

Copyright 2015, Natasha Mireille Appelman-Dijkstra, Zandvoort the Netherlands

All rights reserved. No part of this thesis may be reproduced, stored in a retrieval system of any nature, or transmitted in any form or by any means without prior permission of the author.

All studies were performed at the Center for Endocrine Tumors Leiden.

For publication of this thesis support from the department of Internal Medicine, division of Endocrinology of the Leiden University Medical Center is gratefully acknowledged

Long-term consequences of Growth Hormone replacement and cranial radiation on pituitary function

Proefschrift

Ter verkrijging van de graad van Doctor aan de Universiteit Leiden
op gezag van Rector Magnificus prof. mr.C.J.J.M.Stolker,
volgens besluit van het College voor Promoties te verdedigen
op donderdag 4 juni 2015 klokke 15:00 uur

Door

Natasha Mireille Appelman-Dijkstra

geboren te Haarlem 13 maart in 1980

PROMOTIECOMMISSIE

Promotor: Prof.dr. A.M.Pereira

Co-promotores: dr. N.R.Biermasz

dr. N.A.T.Hamdy

Overige leden: Prof. dr.M.L.Drent (Vumc, Amsterdam)

Prof. dr.H.Pijl

Prof. dr.C.A.M.Marijnen

Prof. dr.A.J.Rabelink

CONTENTS

Chapter 1	General introduction and outline of this thesis	7
Chapter 2	Pituitary failure after cranial irradiation for non-pituitary tumors: a systematic review and metaanalysis: <i>J Clin Endocrinol Metab.</i> 2011 Aug;96(8):2330-40	27
Chapter 3	Pituitary dysfunction in adult patients after cranial irradiation for head and nasopharyngeal tumors <i>Radiother Oncol.</i> 2014 Oct;113(1):102-7	49
Chapter 4	Long-term effects of recombinant human Growth Hormone (rGH) replacement in adults with Growth Hormone Deficiency (GHD):a systematic review <i>Eur J Endocrinol.</i> 2013 May 28;169(1):R1-14.	65
Chapter 5	Metabolic profile in growth hormone deficient (GHD) adults after long-term recombinant human growth hormone (rGH) therapy <i>J Clin Endocrinol Metab.</i> 2013 Jan;98(1):352-61	103
Chapter 6	Abnormal metabolic phenotype in middle-aged GH-deficient adults despite long-term recombinant human GH replacement <i>Eur J Endocrinol.</i> 2013 Dec 21 ;170(2):265-74	121

Chapter 7	The effects of chronic recombinant human GH (rGH) treatment on the bone metabolism in adult patients with Growth Hormone Deficiency(GHD) <i>Clin Endocrinol (Oxf).</i> 2014 Nov;81(5):727-35.	141
Chapter 8	Discontinuing recombinant human Growth Hormone therapy in adults: an observational study and systematic review of literature <i>submitted</i>	159
Chapter 9	General discussion and summary	177
Chapter 10	Nederlandse samenvatting	187
Appendix	Hypofyseuitval na bestraling: <i>Ned Tijdschr Geneeskd.</i> 2012;156:A4340	195
Curriculum vitae		216
Publications		217
Dankwoord		220

Chapter 1

General introduction and outline of this thesis

THE PITUITARY GLAND

The pituitary is a small gland that lies within the sella turcica, a recess in the sphenoid bone. Despite its small size, the pituitary gland is an important regulator of hormone homeostasis. Under the control of hypothalamic peptides, the anterior lobe, or adenohypophysis, synthesizes and secretes growth hormone (GH), prolactin, adreno-corticotroph hormone (ACTH), thyroid stimulating hormone (TSH), and the gonadotrophs LH and FSH. These hormones, in turn, stimulate their target glands, such as the adrenal cortex, thyroid, and ovaries/testicles to produce their respective end hormones: cortisol, thyroid hormone, estradiol/progesterone (in females) and testosterone (in males), respectively. The posterior lobe consists of axons of hypothalamic nerves expressing vasopressin and oxytocin. Vasopressin (also named anti-diuretic hormone ADH) is released in response to an increase in plasma osmolality resulting in increased renal water reabsorption. Oxytocin is released in large amounts during labour and causes contraction of the smooth muscle cells of the uterus. Furthermore it is responsible for the milk ejection reflex during lactation by contracting the smooth muscle cells of the mammary glands to release the milk into the duct system.

Disturbances in the secretion of pituitary hormones rapidly affect the levels of the hormones produced by their target glands. An increase in pituitary hormones leads to characteristic symptoms signs and phenotype, such as the Cushing phenotype in case of ACTH-induced cortisol excess or acromegaly in case of GH excess. Impairment in pituitary function is accompanied by non-specific complaints, such as tiredness, lack of concentration, and decreased physical tolerance and amenorrhoea. Furthermore, impaired bone mass will develop with a consequent increased fracture risk. (1) Disturbances in pituitary function have been shown to decrease overall survival, to cause metabolic dysregulation, as well as impaired quality of life. (2-4)

HYPOPITUITARISM

Hypopituitarism is the term used to describe insufficiency of pituitary hormone secretion and may affect anterior- or posterior pituitary function, or both. The reported incidence of hypopituitarism is around 12–42 new cases per million per year. The prevalence of hypopituitarism is 300–455 per million but this might be an underestimation as the disease is likely to be underreported since the clinical manifestations depend on the extent of hormone deficiency and are largely non specific, with complaints like fatigue, general unwell-being and cold intolerance. (5) The diagnosis of hypopituitarism relies on the measurement of basal and stimulated pituitary- and end-organ hormone secretion, and is more complicated than establishing the diagnosis of end organ deficiency.

INSUFFICIENCY OF THE GH-IGF-1 AXIS: GROWTH HORMONE DEFICIENCY

Growth Hormone (GH) plays an essential role in growth and maturation. Growth hormone is secreted in large amounts during childhood; and thereafter GH levels decline during aging.

In adults, Growth Hormone Deficiency (GHD) has been associated with hypercholesterolaemia and hypertriglyceridaemia.⁽⁶⁾ GHD is also associated with low muscle mass, a decrease in bone mass and diminished quality of life (QoL).^(1,7,8) It is generally accepted that 24-hour GH secretion poorly reflects GH status in adults. A number of tests evaluating GH secretory reserve may be used to diagnose GH deficiency (GHD).⁽⁹⁾ *Figure 1*

The standard for diagnosing GHD is the Insulin Tolerance Test, or alternatively when contra-indicated a Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) (with BMI-adjusted GH cut-offs).⁽¹⁰⁾ IGF-1 levels are not reliable to assess the GH/IGF-1 axis because normal levels do not exclude severe GHD. However, when IGF-1 levels are below -2 SD in the presence of 2 or more insufficient pituitary axes, severe GHD is present in almost all cases.⁽¹⁰⁾

Adult Growth Hormone Deficiency will be more extensively discussed in Section II.

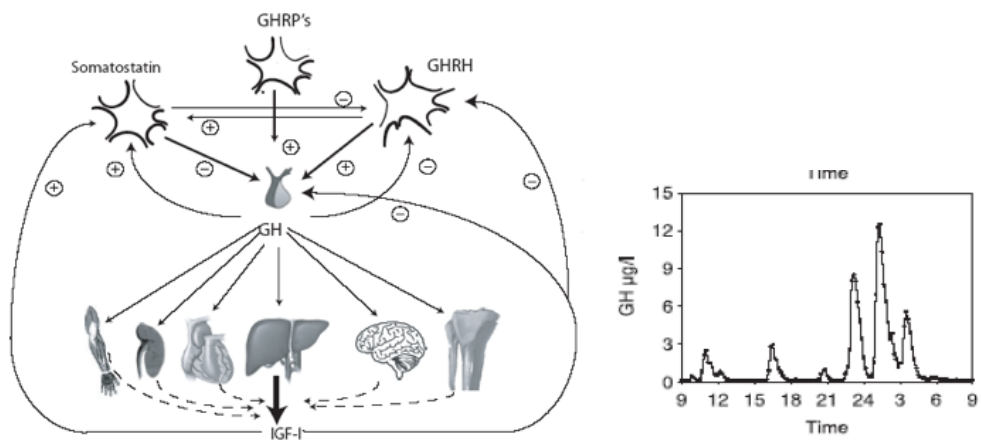


FIGURE 1.

GH/IGF-1 secretion secretion

GH; Growth Hormone, GHRH: Growth Hormone Releasing Hormone, IGF-1: Insulin-like Growth Factor 1, GHRP: Growth Hormone Releasing hexaPeptide

INSUFFICIENCY OF THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS (HPA-AXIS): SECONDARY ADRENAL INSUFFICIENCY.

The HPA-axis controls together with the sympathetic nervous system the stress response, and therefore, is of paramount importance for survival. Disruption or insufficient secretion of CRH and/or ACTH impairs adrenal glucocorticoid synthesis and secretion, a condition that is potentially lethal. In general, symptoms of secondary adrenal insufficiency are non-specific, but most patients with secondary adrenal insufficiency report fatigue and malaise (weight loss) characterized by abdominal discomfort and nausea. Blood pressure is usually normal or slightly decreased and since mineralocorticoid synthesis remains unaffected orthostatic hypotension will not occur until the maximal activation of the renin-angiotensin-aldosterone system (RAAS) cannot further compensate for electrolyte and fluid loss. In that case, an Addisonian crisis may develop.

The diagnosis of secondary adrenal insufficiency involves the evaluation of both basal (non-stimulated) cortisol concentrations as well as dynamic testing. Various dynamic tests have been developed to test the secretory capacity of the pituitary gland and are currently used in daily clinical practice. A single morning cortisol measurement after an overnight fast will already provide some information. In case of a fasting cortisol concentration > 500 - 550 nmol/l, in the absence of concurrent corticosteroid use or estrogen use, the chance of HPA-axis insufficiency is considered very low. Also, when cortisol concentrations are <80 nmol/l, the diagnosis of AI is very likely and dynamic testing is generally considered not necessary. (11) However, since basal cortisol concentrations are in the range between 80 - 500 in most cases, additional dynamic testing is crucial for the evaluation of corticotrope function. In the last decades several tests have been used to diagnose secondary hypocortisolism. To date, the insulin tolerance test (ITT) remains the golden standard for the evaluation of the HPA-axis. (11,12) In case of contraindications for the ITT, such as epilepsy or coronary artery disease, alternative dynamic tests such as the corticotrophin releasing hormone (CRH) test, the metyrapone test, or the ACTH stimulation test can be used to assess adrenal function. (13-15) The choice of test is made by the treating physician based on the patient's clinical condition and one's centers experience. Once diagnosed, hypocortisolism is treated with hydrocortisone replacement. Hydrocortisone therapy for hypopituitarism was associated with supraphysiological cortisol levels in the past, leading to adverse effects such as increased glucose intolerance, decline in bone mass and increased cardiovascular risk factors, such as hypertension and increased abdominal fat distribution. Decreasing the glucocorticoid dose from 20 - 30 mg/day to 15 mg/day was associated with beneficial effects in terms of body composition, lipid profile, and QoL. (16) Current replacement doses are 20 mg divided in three doses. Unfortunately, to date there are no adequate methods to monitor over-replacement of hydrocortisone in routine clinical practice.

INSUFFICIENCY OF THE HYPOTHALAMUS-PITUITARY-GONADAL AXIS: SECONDARY- OR HYPOGONADOTROPIC HYPOGONADISM.

Hypogonadotropic hypogonadism is defined as the presence of low or normal gonadotropin levels, (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) in the presence of low testosterone in males or estrogen levels/cyclic disturbances in pre-menopausal women. In pre-menopausal women, secondary hypogonadism manifests with (oligo-) amenorrhea, decreased libido as well as fertility and psychological disturbances. In post-menopausal women, hypogonadism might often be a-symptomatic and the absence of high serum LH/FSH values confirms the diagnosis. In males, hypogonadism presents with decreased libido, potency and fertility, and with increased prevalence of depressive like symptoms and other mood disturbances and osteoporosis. In males, testosterone replacement therapy is administered in the form of transdermal testosterone preparations allowing stable physiological serum testosterone levels whereas in pre-menopausal women, replacement therapy with oral estrogens alone or in combination with progestagens is administered.

INSUFFICIENCY OF THE HYPOTHALAMUS-PITUITARY-THYROID AXIS: SECONDARY HYPOTHYROIDISM

Symptoms of hypothyroidism are associated with complaints of tiredness, edema (especially periorbital), constipation, weight gain and depression. The symptomatology of secondary hypothyroidism does not differ from primary hypothyroidism, but may be masked by other concomitant pituitary insufficiencies. A diagnosis of secondary hypothyroidism is confirmed by the presence of a low serum FT4 levels and inappropriately normal or low TSH levels. TSH levels are non-diagnostic in secondary hypothyroidism. In case of multiple pituitary hormone deficiencies, secondary hypothyroidism should also be suspected when FT4 levels are within the lower tertile of the reference ranges. L-thyroxine is the treatment of choice but under-replacement is often the case resulting in a negative cardiovascular profile, although the awareness for the risks of chronic under-replacement is rising. (17)

Concomitant corticotropin deficiency should be excluded in order to avoid acute adrenal insufficiency before starting replacement therapy and retesting of the HPA-axis should be considered after re-institution of normal thyroid hormone values.

With the exception of congenital forms hypopituitarism is largely caused by pituitary tumours, or by the consequent treatment of those tumours with surgery or radiotherapy. Hypopituitarism may also arise after traumatic brain injury or vascular insult and after radiotherapy for non-pituitary tumours. In the course of treatment in various tumours like for instance nasopharyngeal carcinoma (NPC) the pituitary is within the radiation field of the primary tumour resulting in a high dose to the pituitary and the risk of

hypopituitarism. The aim of this thesis was, first of all, to critically evaluate the available literature on the long-term consequences of cranial irradiation, and subsequently to evaluate the prevalence of pituitary insufficiency in our tertiary referral center using state of the art endocrine evaluation (Section I). In addition, we aimed to investigate the metabolic phenotype of the adult GHD patient in more detail after long-term rGH replacement given the unknown contribution of growth hormone replacement therapy.

Section I: Radiation Induced Hypopituitarism

Irradiation of the hypothalamic-pituitary region is thought to result in a characteristic pattern of decline of pituitary function, explained by the cell content in the pituitary with growth hormone (GH) secretion declining first, followed by ACTH, gonadotrophins and TSH. (18)

Radiation-induced pituitary insufficiency is a dose-dependent late sequel of radiotherapy for pituitary tumours with prevalence rates for one or more pituitary insufficiencies of around 50% after 5 years, arising up to 75 % after 10 years, following a total dose of 40-45Gy *Figure 2*. (19-21) The prevalence of insufficiencies is correlated with the total radiation dose to the hypothalamic/pituitary region.

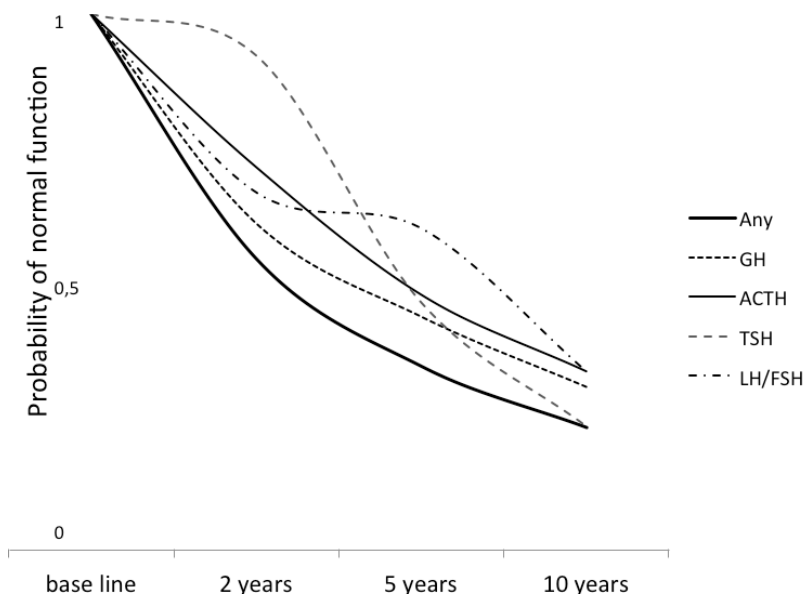


FIGURE 2.

Probability of normal pituitary function after irradiation for pituitary tumours with 40 Gy adapted from Little et al.¹⁸

Data from historical cohorts indicate that a total dose of 7- 24 Gy delivered to the pituitary/hypothalamus may cause growth hormone deficiency only whereas dosages as high as 50-60 Gy will most certainly cause multiple pituitary hormone deficiencies. (22) The incidence of multiple pituitary deficiencies thus increases both in time and with higher doses. (18, 23) The hypothalamic region is suggested to be more radiation sensitive causing elevated prolactin levels after irradiation in the presence of still intact pituitary function. This is indicative of a decreased dopaminergic inhibition by the hypothalamus and thus prolactin release, however hypothalamic dysfunction is difficult to observe since its hormones (GnRH, TRH, CRH, GHRH) are difficult to measure. The pathophysiological basis of the effect of irradiation is DNA damage, strand breaks, both in healthy- as well as in tumour tissue. This damage is generated by direct 3-dimensional dysfunctional changes in DNA as well as by the indirect negative effect of production of free radicals from water molecules in the cell. (24) These strand breaks may have direct lethal effects or will have accumulating sub-lethal effects that eventually will restrict potential cell replication. After radiation cell death occurs in the mitotic phase of the cell cycle (late G2-earleS phase) providing a partial explanation for the delayed onset of hormonal failure in slowly replicating tissue, such as that of the neuroendocrine tissue of the hypothalamus and pituitary. (25) *Figure 3*

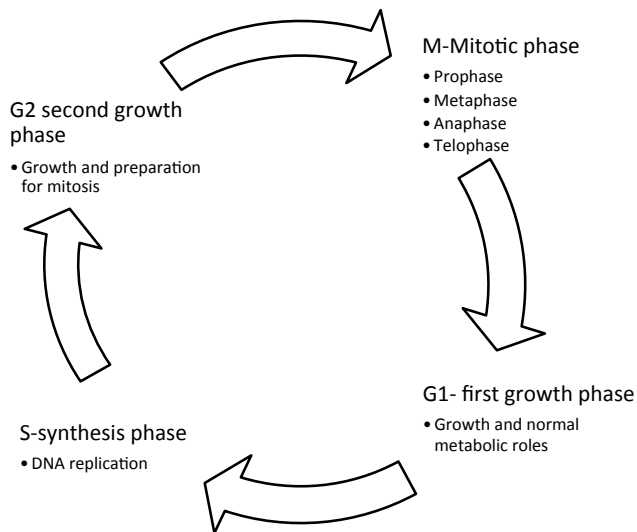


FIGURE 3.

Cell cycle

Ionising irradiation also induces degenerative changes in surrounding glia cells, causing decreased trophic support and demyelination resulting in chronic neuronal damage.

The vascular system is also compromised by the release of pro-inflammatory cytokines such as Necrosis Factor-kappa beta (NF- κ B). Due to radiation induced oxidative stress

NF- κ B causes endothelial proliferation by promoting the release of anti-inflammatory cytokines and adhesion molecules. Inflammatory cells are subsequently recruited, promoting foam cell formation which results in increased collagen synthesis, and thus of atherosclerosis. (26) Clinical studies performed in patients previously treated for e.g. lymphoma, breast cancer or head and neck cancer have demonstrated an increase in cardiovascular risk, also after adjustment for classic cardiovascular risk factors. Depending on the study, the relative risk of a clinical cardiovascular event (i.e., myocardial infarction, stroke) to be related to irradiation ranged from 1.5- to 4.0-fold, and this risk was even higher in the presence of traditional cardiovascular risk factors. (27,28)

Other reported late effects of cranial radiotherapy include a decline in neurocognitive function resulting in a decreased quality of life (29-31). In larger studies however the contributory role of cranial radiotherapy to the risk of cerebrovascular incidents was not higher than that of surgical trauma or glucocorticoid over-replacement or of undiagnosed hypopituitarism. (32)

Pituitary insufficiency is a well known late onset complication of cranial irradiation in children treated for cerebral tumours or after total body radiotherapy.(33-41) In the Childhood Cancer Survivor Study (CCSS) 43% of children treated for cerebral tumours had one or more endocrinopathies. Consequently, virtually all follow-up protocols for childhood cancer survivors include endocrine assessments as the development of hypopituitarism can still occur years after the initial treatment, especially in the doses < 45 Gy. Over the past decades, survival rates of patients treated with cranial radiotherapy for malignant or benign tumours have substantially improved by the introduction of new surgical, radiotherapy, and chemotherapy options. In contrast to the long-term survivors of cranial radiotherapy in childhood, endocrine surveillance programs have not been routinely incorporated in adults treated with cranial radiotherapy. The prevalence of hypopituitarism after cranial radiotherapy is influenced by a number of factors. First, the time interval between radiotherapy and the assessment of pituitary function is important, since the development of pituitary failure is likely to increase in time after radiotherapy. (42-44) Second, hypothalamic and pituitary insufficiencies are more likely to develop with increasing radiation exposure.

Furthermore, studies in survivors of childhood acute lymphoblastic leukaemia as well as in childhood brain cancer demonstrated an increased risk of metabolic and cardiovascular late effects after cranial irradiation, including effects on body composition, insulin resistance and hyperleptinaemia. Patients treated with radiotherapy for pituitary tumours had higher visceral to subcutaneous fat ratios when compared with controls who did not receive radiotherapy.(47) Further studies are needed to determine the clinical significance of these findings and, where clinically relevant, whether cardiovascular outcomes may be modulated by additional therapy.

A number of non-pituitary tumours, such as nasopharyngeal carcinomas, meningiomas or primary cerebral tumours are treated with high dose radiotherapy. Most often treatment schedules comprise multiple fractions of 2Gy, with a boost sometimes additionally delivered to the primary tumour. Depending on the tumour localisation, the pituitary/hypothalamic region will receive some dose despite improved radiotherapeutic techniques. *Figure 4.* In nasopharyngeal carcinoma, the total dose on the primary tumour area will be at least 6-70 Gy in fractions of 2 Gy. Depending on the location of the primary tumour this might result in an estimated dose of > 40 Gy to the pituitary gland, predicting a very high probability of pituitary insufficiency, as depicted in Figure 2. Structured follow up for pituitary function is not routinely incorporated in the long term monitoring of these patients. Since the overall survival of stage I and II NPC patients has improved throughout the years to a total of $> 80\%$ 5 year survival rate, structured monitoring of pituitary function is advisable. (48,49)

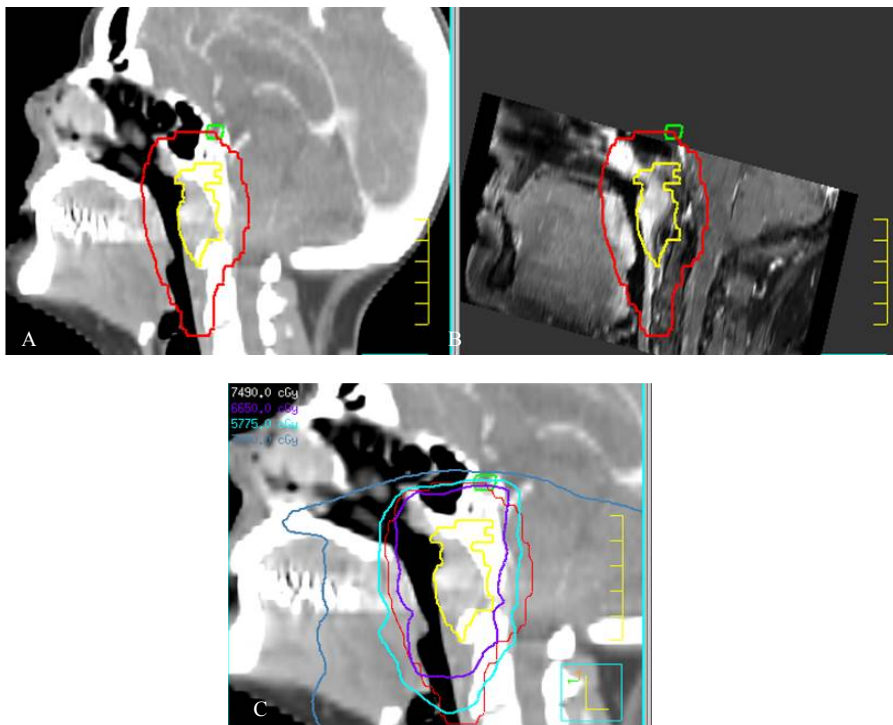


FIGURE 4.

Figure 4A and 4B show the position of the primary tumour (yellow line), the radiation field (red line) and the pituitary region (green line). Figure 4C shows the isodose lines of 66.5 Gy (purple), 57.75 Gy (Cyan) and 30 Gy (blue) in the same patient, notice the position of the pituitary region (green) within the radiation field.

When hypopituitarism develops in the course of treatment for an underlying disease, such as nasopharyngeal carcinoma, the symptoms of hypopituitarism may mimic those of

residual symptoms after chemotherapy, surgery or cranial irradiation and may therefore be easily overlooked. Cranial radiotherapy may also induce cognitive impairments, which may mask symptoms of hypopituitarism. Diagnosing hypopituitarism can be very difficult especially for physicians not familiar with endocrine pathology such as radiation oncologists and Ear-Nose-and Throat specialists. Even a trained endocrinologist cannot diagnose pituitary failure only on the basis of clinical signs and symptoms. Since hypopituitarism will develop several years after radiotherapy most patients will not consult an internist, and therefore the diagnosis may be easily overlooked. Despite the fact that there are many ways to screen for pituitary dysfunction, the evaluation of the effects of radiotherapy on pituitary or hypothalamic function in adult patients who were treated with cranial irradiation for different causes has not been incorporated into standard patient care. In addition, there are no protocols for standardizing the endocrinological evaluation or follow up for these patients.

In [Chapter 2](#) and appendix 1 of this thesis a review and meta-analysis of the published English literature is presented, whereas [Chapter 3](#) and the appendix 1 address the prevalence and time frame of onset of pituitary insufficiency after irradiation for head and nasopharyngeal tumours, including a proposed algorithm for standardized follow up.

Section II: Growth hormone secretion and growth hormone deficiency

The regulation of GH secretion is controlled by the net balance between stimulatory hypothalamic peptides such as GHRH and GHrelin, and on the other hand the inhibitory tone of somatostatin. The net result is a pulsatile secretion of GH, which is important because most of the secretion occurs in bursts. The magnitude of GH bursts correlates with somatic growth and with the hepatic actions of GH. Activation of the GH-receptor in the liver induces key intracellular signaling pathways such as PI3Kinase, MAP Kinase, and STAT5b, the latter resulting in the transcriptional activation of the IGF-1 gene. Together with IGF-1, GH exerts its anabolic effects on target tissues. (52) GH, as well as sex hormones, are thus extremely important for skeletal growth and maturation throughout puberty.

The largest amount of growth hormone is secreted during adolescence; with a further progressive decline in GH secretion throughout life.

Growth Hormone Deficiency (GHD) has been associated with an adverse metabolic profile and with an increased cardiovascular mortality due to abdominal obesity, hypercholesterolaemia and hypertriglyceridaemia in adults. (1-3) Untreated GHD is also characterized by a decrease in muscle bone mass, decreased bone turnover and increased fracture risk. (7,8) GHD is also associated with an overall decrease in quality of life. (51-53)

In 1990, Rosen *et al* reported increased mortality in patients with hypopituitarism, due to cardiovascular disease (CVD), particularly cerebrovascular disease, which was attributed, at least in part, to GHD. (2,54) Over the following decades a number of studies from 4 different cohorts compounding approximately 2000 patients with hypopituitarism, have reported increased mortality in these patients treated historically with conventional hormone replacement therapy, none of these patients were treated with recombinant GH (rGH) replacement. These studies only included patients with surgically resected non-secreting pituitary adenomas and craniopharyngiomas and excluded patients with Acromegaly and Cushing's disease, since it was assumed already that these specific diseases would increase mortality risk, as demonstrated by Dekkers *et al* in 2006 and 2008. (3,55-58) A significant number of these patients also received additional cranial irradiation, and the majority of pre-menopausal women were not treated for gonadotropin deficiency.

The classical clinical features of the adult patient with GHD is that of a patient suffering from overweight, with predominantly abdominal obesity, decreased muscle strength, reduced exercise intolerance and depressed mood. Additional investigations would reveal elevated serum lipids, particularly LDL cholesterol, reduced lean body/ increased fat mass and reduced bone mineral density. In the mid 1990's, human recombinant growth hormone (rGH) became available for the treatment of adults with severe GHD. Since then, consistent beneficial effects of replacement therapy with rGH have been reported on body composition and lipid profile, resulting in reduction of fat mass combined with an increase in lean body mass, and a reduction in total cholesterol (TC) levels, as meta-analysed and summarized by Maison *et al*. (6) Favourable effects were also reported on bone turnover, bone mineral density (BMD), muscle strength, cognitive function and QoL. (6, 59, 60)

In cross-sectional studies, beneficial effects of rGH replacement have been reported to sustain for at least 5 years of treatment; data with a longer follow-up duration are scarce. With respect to cardiovascular risk profile, a direct improvement of several cardiovascular risk factors was noted within the first year of treatment, which persisted during long-term rGH treatment. (61,62) A sustained improvement of lipid spectrum and diastolic blood pressure (DBP) was reported in a small study over 7 and 10 years of treatment, suggesting on-going beneficial effects of rGH even beyond 5 years. However whether rGH replacement has favourable effects on the incidence of cardiovascular events, including cardiovascular death is yet to be established.

GROWTH HORMONE AND THE SKELETON

The skeleton consists of cortical bone and trabecular (cancellous) bone. Cortical bone, which comprises 80% of the skeleton, is designed to provide rigidity and strength and

is predominantly found in the long bones, e.g. at the femur. Cancellous bone has a more flexible design with interconnecting trabecles, and is metabolically more active than cortical bone. There are three types of bone cells: osteoclasts, responsible for bone resorption, osteoblasts, responsible for bone formation and osteocytes that are the mechano-sensors of the skeleton. The three types of bone cells form the main components of the so-called basic multicellular unit (BMU). In a healthy adult there are more than 1 million BMU's, which are mostly active in cancellous bone. In a BMU bone resorption takes 3 weeks to be completed whereas bone formation may last up to 9 months. The balance between formation and resorption is essential for maintaining a normal bone mass and skeletal integrity. Disruptions in the ratio of bone formation and resorption in favour of resorption will ultimately lead to progressive bone loss and increased bone fragility.

GH and IGF-1 play an important role in bone growth and metabolism throughout the life span of an individual. (63) IGF-1 mediates skeletal growth by promoting endochondrial ossification in cartilage and by enhancing osteoblast formation and differentiation of both osteoblasts as osteoclasts, GH also exerts direct effects on bone. (64, 65) Both GH and IGF-1 act as anabolic hormones on bone by stimulating proliferation, and to some extent, differentiation of osteoblasts. IGF-1 promotes osteoblast differentiation through the mTOR pathway. This has been demonstrated in an IGF-1 knock-out mouse model in which decreased bone formation and subsequent decreased bone mass has been observed. (66) Up-regulation of IGF-1 mediated pathways in osteoblasts results in widening of the cortex and in an increase in the length of the long bones, whereas trabecular bone is hardly affected by these pathways. In osteocytes, knocking out IGF-1 results in a decrease in calvarial bone growth rate. (67) IGF-1 is also a co-regulator in osteoclast differentiation by induction of Receptor Activator of Nuclear factor κ B Ligand (RANK-L) expression. RANK-L is a stimulator of osteoclast differentiation, proliferation and activation and thus prolongs the lifespan of the osteoclast resulting in an overall increase in bone remodelling. (68) *Figure 5*

In contrast GH promotes the production of osteoprotegerin (OPG) which binds to RANK-L and inhibits osteoclast formation and differentiation. (69) The ratio between RANK-L and OPG is critical for optimal balance between bone formation and resorption. After the menopause for example, OPG levels drop tremendously due to estrogen deficiency, the most important stimulator of OPG formation, leading to a marked increase in bone loss particularly within the first year after the menopause.

IGF-1 knockout models have also shown that IGF-1 is required for adequate function of parathyroid hormone (PTH). In the absence of IGF-1, PTH does not longer have a stimulating effect on bone formation rates. IGF-1/GH plays a key role in bone cell growth and differentiation.

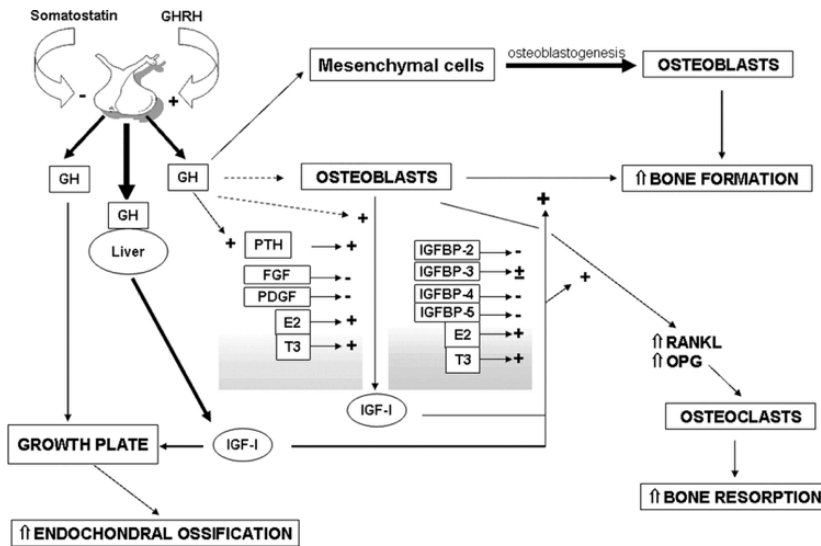


FIGURE 5.

Growth Hormone/IGF-1 and bone metabolism

GH: Growth Hormone, PTH: Parathyroid Hormone, FGF: Fibroblast Growth Factor, PDGF: Platelet Derived Growth Factor, E2: Estrogen, T3:thyroid hormone, IGFBP2-5: Insulin Growth Factor-like Binding Peptide 2-5, RANKL: Receptor activator of nuclear factor kappa-B ligand, OPG: Osteoprotegerin

In humans with growth hormone insensitivity due to inactivating mutations in the gene encoding the GH receptor (Laron syndrome) GH levels are very high and IGF-1 levels are reduced by more than 80%. These individuals are extremely short of stature due to short long-bones. In patients with Laron syndrome the cortex is of normal thickness and there is no increased incidence of osteoporosis or fractures. (70) In contrast, untreated GHD is characterized by low bone turnover, decreased bone mineral density (BMD) and increased fracture risk (1,7). Recombinant human GH (rGH) replacement therapy has been shown to increase bone turnover as reflected by an increase in bone formation and bone resorption markers, resulting in an initial decline in (or unchanged) BMD, followed by a small increase of about 1-2% in the first 2 years of rGH replacement due to a positive balance in favor of bone formation. (71,72)

Data on the effects of long-term rGH replacement on BMD are, however, scarce. Previous studies reported that rGH replacement induces a progressive increase in BMD and BMC up to 5-7 years of treatment, followed thereafter by a maintenance of BMD up to 7 years of treatment.(72-74) Götherström *et al.* found an increase in total body and lumbar spine BMD and BMC up to 10 years of rGH replacement, whereas femoral neck BMD and BMC reached a peak value after 5-7 years. Only one study has reported the effects of 15 years of rGH supplementation. This study reported a sustained increase in total body and lumbar BMD and BMC over 15 years, whereas femoral neck BMD and BMC returned towards baseline values after 7 years. (59)

Another important issue regarding long-term rGH replacement therapy in adults is the potential association between growth hormone/IGF-1 and cancer risk, which is an on going topic of debate. A number of animal studies as well as epidemiological studies in humans examined the relationship between low IGF-1 as well as reduced cancer risk and cardiovascular risk. The activity of the GH/IGF-1 axis decreases with ageing, yet smaller individuals within a species usually live longer (including ponies and small dogs). (75) Life span is also longer in mice lacking GHR, resulting in low IGF-1 levels. Although some of these mutant mice were also deficient in other hormones, such as TSH, their longevity is thought to be primarily due to their Growth Hormone Deficiency (GHD), since restoration of GH levels reverted their longevity to match that of non-mutants.(76,77)

Reduced IGF-1 levels as well as reduced IGF-1 signalling has been associated with reduced cancer risk in humans. For instance, cancer was virtually absent in a Ecuadorian cohort of patients with severe GHD due to GHR gene mutations). (78) Another cohort of patients with Laron syndrome showed a general identical clinical picture. (79) To date, only one case of (non-lethal) cancer has been reported in both Laron cohorts.

Whether IGF-1 *per se* alters longevity in humans remains questionable. Among others, the Leiden Longevity study showed that the relation between longevity and IGF-1 may be through insulin sensitivity. Rozing *et al.* showed a lower prevalence of diabetes, and non-fasted serum glucose levels in the offspring of familial nonagenarians compared to their partners although IGF-1 and IGFBP3 did not differ between both groups (80). It was already shown in the 90's that sporadic long-lived centenarians were very sensitive to insulin compared to young adults and that insulin sensitivity decreases with age. (81) A recent meta-analyses of IGF-1 in the general population showed an increase in mortality in both high as well as in the population with low IGF-1 levels, hazard ratio (HR) 1.18, (95%CI 1.04-1.34). (82) This U-shape relationship was present for both cancer and cardiovascular mortality. In addition, several other epidemiological reports showed an association of both low and high IGF-1 levels with DM2, sarcopenia, osteoporosis and cardiovascular disease. (75,83) However, this by no means proves that low and high IGF-1 levels are related to increased specific morbidity or mortality.

Taken together, these data do suggest that optimizing the activity of the GH/IGF-1 axis to promote healthy aging in humans is more complex than originally appreciated, and requires a greater understanding of its array of interactions and tissue specificity.

Chapter 4 describes the results of an extended literature search on long-term growth hormone replacement in adults beyond 5 years of treatment. In Chapter 5 the changes in metabolic profile after initiation of growth hormone replacement within a group of long-term recombinant GH therapy users are presented and discussed. In Chapter 6, the difference in metabolic profile of GHD patients on stable, defined as > 5 years, rGH replacement therapy to the metabolic profile of the general population will be

addressed. Chapter 7 discusses whether long-term rGH replacement has any beneficial effect on bone metabolism. Finally, in Chapter 8, a systematic literature search and cohort study is performed investigating the metabolic effects of discontinuing rGH replacement.

REFERENCES

1. Rosen T, Hansson T, Granhed H, Szucs J, Bengtsson BA. Reduced bone mineral content in adult patients with growth hormone deficiency. *Acta Endocrinol (Copenh)* 1993; 129(3):201-206
2. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990;336:285-288.
3. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet* 2001;357:425-431.
4. Erfurth EM. Update in mortality in GH-treated patients. *J Clin Endocrinol Metab.* 2013 Nov;98(11):4219-26.)
5. Ascoli P, Cavagnini FR. Hypopituitarism. *Pituitary*, December 2006, Volume 9, Issue 4, pp 335-342
6. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004; 89(5):2192-2199}
7. Bouillon R, Koledova E, Bezlepina O et al. Bone status and fracture prevalence in Russian adults with childhood-onset growth hormone deficiency. *J Clin Endocrinol Metab* 2004; 89(10):4993-4998.
8. Verhelst J, Abs R. Long-term growth hormone replacement therapy in hypopituitary adults. *Drugs* 2002; 62(16):2399-2412.
9. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008, Feb;18(1):1-16.
10. Aimaretti G, Corneli G, Rovere S, Granata R, Baldelli R, Grottoli S, Ghigo E, Insulin-Like Growth Factor I Levels and the Diagnosis of Adult Growth Hormone Deficiency. *Horm Res Paediatr* 2004;62(suppl 1):26-33
11. Grinspoon SK, Biller BM. Clinical review 62: laboratory assessment of adrenal insufficiency. *J Clin Endocrinol Metab.* 1994;79:923–931.
12. Fish HR, Chernow B, O'Brian JT. Review Endocrine and neurophysiologic responses of the pituitary to insulin-induced hypoglycemia: a review. *Metabolism.* 1986 Aug; 35(8):763-80.
13. Courtney CH, McAllister AS, McCance, Hadden, Leslie H, Sheridan B, Atkinson AB. The insulin hypoglycaemia and overnight metyrapone tests in the assessment of the hypothalamic-pituitary-adrenal axis following pituitary surgery. *Clin Endocrinol (Oxf)* 2000;53:309–312.
14. Maghnie M, Uga E, Temporini F, Di IN, Secco A, Tinelli C, Papalia A, Casini MR, Loche S. Evaluation of adrenal function in patients with growth hormone deficiency and hypothalamic-pituitary disorders: comparison between insulin-induced hypoglycemia, low-dose ACTH standard ACTH and CRH stimulation tests. *Eur J Endocrinol.* 2005;152:735–741.
15. Schmidt IL, Lahner H, Mann K, Petersenn S. Diagnosis of adrenal insufficiency: evaluation of the corticotropin-releasing hormone test and Basal serum cortisol in comparison to the insulin tolerance test in patients with hypothalamic-pituitary-adrenal disease. *J Clin Endocrinol Metab.* 2003;88:4193–4198
16. Danilowicz K, Bruno OD, Manavela M, Gomez RM, Barkan A. Correction of cortisol overreplacement ameliorates morbidities in patients with hypopituitarism: a pilot study. *Pituitary* 2008; 11(3):279-285
17. Klose M, Marina D, Hartoft-Nielsen ML, Klefter O, Gavan V, Hilsted L, Rasmussen AK, Feldt-Rasmussen U. Central hypothyroidism and its replacement have a significant influence on cardiovascular risk factors in adult hypopituitary patients. *J Clin Endocrinol Metab.* 2013 Sep;98(9):3802-10.
18. Littlely MD, Shalet SM, Beardwell CG, Robinson EL, Sutton ML. Radiation-induced hypopituitarism is dose-dependent. *Clin Endocrinol (Oxf)* 1989, Sep;31(3):363-73.
19. Darzy KH, Shalet SM. Hypopituitarism following radiotherapy. *Pituitary* 2009;12(1):40-50.

20. Brada M, Jankowska P. Radiotherapy for pituitary adenomas. *Endocrinol Metab Clin North Am* 2008, Mar;37(1):263-75, xi.
21. Biermasz NR, van Dulken H, Roelfsema F. Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. *J Clin Endocrinol Metab* 2000, Jul;85(7):2476-82.
22. Lam KS, Tse VK, Wang C, Yeung RT, Ho JH. Effects of cranial irradiation on hypothalamic-pituitary function--a 5-year longitudinal study in patients with nasopharyngeal carcinoma. *Q J Med* 1991, Feb;78(286):165-76.
23. Fernandez A, Brada M, Zabulienė L, Karavitaki N, Wass JA. Radiation-induced hypopituitarism. *Endocr Relat Cancer* 2009, Sep;16(3):733-72.
24. Belka C, Budach W, Kortmann RD, Bamberg M. Radiation induced CNS toxicity--molecular and cellular mechanisms. *Br J Cancer* 2001, Nov 2;85(9):1233-9.
25. Kunkler I 2003 Effects of radiation on normal tissues. In Walter and Miller's Textbook of Radiotherapy, pp 296–306 Eds Bomford CK & Kunkler IH. London: Churchill Livingstone.
26. Weintraub NL, Jones KJ, Manka D, Understanding Radiation-Induced Vascular Disease, *Journal of the American College of Cardiology*, Volume 55, Issue 12, 23 March 2010, 1237-1239
27. Little MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Q J Med* 70:145–160
28. Darzy KH, Shalet SM 2009 Hypopituitarism following radiotherapy. *Pituitary* 12:40–50
29. Erfurth EM, Hagmar L. Cerebrovascular disease in patients with pituitary tumors. *Trends Endocrinol Metab.* 2005;16(7):334–342.
30. Sherlock M, Reulen RC, Alonso AA, et al. ACTH deficiency, higher doses of hydrocortisone replacement, and radiotherapy are independent predictors of mortality in patients with acromegaly. *J Clin Endocrinol Metab.* 2009;94(11):4216–4223.
31. Taphoorn MJ, Schiphorst AK, Snoek FJ, Lindeboom J, Wolbers JG, Karim AB, Huijgens PC, Heimans JJ. Cognitive functions and quality of life in patients with low-grade gliomas: the impact of radiotherapy. *Ann Neurol.* 1994 Jul;36(1):48-54.
32. Erfurth EM, Hagmar L. Cerebrovascular disease in patients with pituitary tumors. *Trends Endocrinol Metab.* 2005;16(7):334–342
33. Alvarez JA, Scully RE, Miller TL, Armstrong FD, Constone LS, Friedman DL, Lipshultz SE 2007 Long-term effects of treatments for childhood cancers. *Curr Opin Pediatr* 19:23–31
34. Constone LS, Woolf PD, Cann D, Mick G, McCormick K, Raubertas RF, Rubin P 1993 Hypothalamic-pituitary dysfunction after radiation for brain tumors. *N Engl J Med* 328:87–94
35. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, Friedman DL, Marina N, Hobbie W, Kadan-Lottick NS, Schwartz CL, Leisenring W, Robison LL 2006 Chronic health conditions in adult survivors of childhood cancer. *N Engl J Med* 355:1572–1582
36. Schmiegelow M, Lassen S, Poulsen HS, Feldt-Rasmussen U, Schmiegelow K, Hertz H, Müller J 2000 Cranial radiotherapy of childhood brain tumours: growth hormone deficiency and its relation to the biological effective dose of irradiation in a large population based study. *Clin Endocrinol (Oxf)* 53:191–197
37. Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK, Poulsen HS, Müller J 2003 A population-based study of thyroid function after radiotherapy and chemotherapy for a childhood brain tumor. *J Clin Endocrinol Metab* 88:136–140
38. Shalet SM, Beardwell CG, Jones PH, Pearson D 1976 Growth hormone deficiency after treatment of acute leukaemia in children. *Arch Dis Child* 51:489–493
39. Shalet SM, Beardwell CG, Morris-Jones P, Bamford FN, Ribeiro GG, Pearson D 1976 Growth hormone deficiency in children with brain tumors. *Cancer* 37:1144–1148

40. Shalet SM, Beardwell CG, MacFarlane IA, Jones PH, Pearson D 1977 Endocrine morbidity in adults treated with cerebral irradiation for brain tumours during childhood. *Acta Endocrinol (Copenh)* 84: 673–680
41. Gurney JG, Kadan-Lottick NS, Packer RJ, Neglia JP, Sklar CA, Punyko JA, Stovall M, Yasui Y, Nicholson HS, Wolden S, McNeil DE, Mertens AC, Robison LL 2003 Endocrine and cardiovascular late effects among adult survivors of childhood brain tumors. *Childhood Cancer Survivor Study. Cancer* 97:663–673
42. Toogood AA 2004 Endocrine consequences of brain irradiation. *Growth Horm IGF Res* 14(Suppl A):S118–S124
43. Littlely MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Q J Med* 70:145–160
44. Darzy KH, Shalet SM 2009 Hypopituitarism following radiotherapy. *Pituitary* 12:40–50
45. Russell NS, Hoving S, Heeneman S, et al. Novel insights into pathological changes in muscular arteries of radiotherapy patients. *Radiother Oncol.* 2009;92:477– 83
46. Halle M, Gabrielsen A, Paulsson-Berne G, et al. Sustained inflammation due to nuclear factor-kappa B activation in irradiated human arteries. *J Am Coll Cardiol.* 2010;55:1227–36
47. Borgers AJ, Alkemade A, Venema HW, Fliers E, Bisschop PH. A history of cranial radiotherapy is associated with a higher visceral to subcutaneous fat ratio in men with pituitary insufficiency. *Eur J Endocrinol* 166 619–624
48. Lee AW, Ng WT, Chan YH, Sze H, Chan C, Lam TH. The battle against nasopharyngeal cancer. *Radiother Oncol* 2012, Sep;104(3):272-8.
49. Chan AT, Leung SF, Ngan RK, Teo PM, Lau WH, Kwan WH, et al. Overall survival after concurrent cisplatin-radiotherapy compared with radiotherapy alone in locoregionally advanced nasopharyngeal carcinoma. *J Natl Cancer Inst* 2005, Apr 6;97(7):
50. Greenspan FS Strewler GJ Basic and Clinical Endocrinology 8th edition
51. De Boer H, Block GJ, Van Der Veen EA. Clinical aspects of growth hormone deficiency in adults. *Endocr Rev.* 1995;16:63–86
52. Brabant G, Poll EM, Jönsson P, Polydorou D, Kreitschmann-Andermahr I. Etiology, baseline characteristics, and biochemical diagnosis of GH deficiency in the adult: are there regional variations? *Eur J Endocrinol.* 2009 Nov;161 Suppl 1:S25-31
53. Carroll PV, Christ ER, Bengtsson BA, Carlsson L, Christiansen JS, Clemmons D, Hintz R, Ho K, Laron Z, Sizonenko P, Sönksen PH, Tanaka T, Thorne M. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. *Growth Hormone Research Society Scientific Committee, J Clin Endocrinol Metab.* 1998 Feb;83(2):382-95.
54. Shahi M, Beshyah SA, Hackett D, Sharp PS, Johnston DG, Foale RA. Myocardial dysfunction in treated adult hypopituitarism: a possible explanation for increased cardiovascular mortality. 1992 *Br Heart J* 67:92–96
55. Bülow B, Hagmar L, Mikoczy Z, Nordström CH, Erfurth EM. Increased cerebrovascular mortality in patients with hypopituitarism. *Clin Endocrinol (Oxf).* 1997;46:75–81.
56. Dekkers OM, Horváth-Puhó E, Jørgensen JO, Cannegieter SC, Ehrenstein V, Vandenbroucke JP, Pereira AM, Sørensen HT. Multisystem morbidity and mortality in Cushing's syndrome: a cohort study. *J Clin Endocrinol Metab.* 2013 Jun;98(6):2277-84.
57. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. *J Clin Endocrinol Metab.* 2008 Jan;93(1):61-7
58. Dekkers OM, Biermasz NR, Pereira AM, Roelfsema F, van Aken MO, Voormolen JH, Romijn JA. Mortality in patients treated for Cushing's disease is increased, compared with patients treated for nonfunctioning pituitary macroadenoma. *J Clin Endocrinol Metab.* 2007 Mar;92(3):976-81

59. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. . Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012; 166(5):787-795.
60. Koltowska-Häggström M, Mattsson AF, Monson JP, Kind P, Badia X, Casanueva FF, Busschbach J, Koppeschaar HP, Johannsson G. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? *Eur J Endocrinol* 2006; 155(1):109-119.
61. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. *J Clin Endocrinol Metab* 2007; 92(4):1442-1445.
62. van der Klaauw AA1, Romijn JA, Biermasz NR, Smit JW, van Doorn J, Dekkers OM, Roelfsema F, Pereira AM. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. *Eur J Endocrinol* 2006; 155(5):701-708.
63. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev*. 2008 Aug;29(5):535-59.
64. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. *N Engl J Med*. 2007 Aug 30;357(9):905-16.
65. Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Słotweg MC. Growth hormone and bone. *Endocr Rev*. 1998 Feb;19(1):55-79.
66. Govoni KE, Wergedal JE, Florin L, Angel P, Baylink DJ, Mohan S. Conditional deletion of insulin-like growth factor-I in collagen type 1 α 2-expressing cells results in postnatal lethality
67. Cooper KL, Oh S, Sung Y, Dasari RR, Kirschner MW, Tabin CJ. Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions. *Nature* 2013;495: 375–378.
68. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003 May 15;423(6937):337-42.
69. Rubin J, Ackert-Bicknell CL, Zhu L, Fan X, Murphy TC, Nanes MS, Marcus R, Holloway L, Beamer WG, Rosen CJ. IGF-I regulates osteoprotegerin (OPG) and receptor activator of nuclear factor- κ B ligand in vitro and OPG in vivo. *J Clin Endocrinol Metab*. 2002 Sep;87(9):4273-9
70. Kornreich L, Konen O, Schwarz M, Siegel Y, Horev G, HersHKovitz I, Laron Z. Abnormalities of the axial and proximal appendicular skeleton in adults with Laron syndrome (growth hormone insensitivity). *Skeletal Radiol*. 2008 Feb;37(2):153-60.
71. Janssen YJH, Hamdy NA, Frolich M, Roelfsema F. Skeletal effects of two-years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. *J Clin Endocrinol Metab* 1998; 83(6):2143-2148.
72. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001; 86(10):4657-4665.
73. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rGH) alone and rGH combined with alendronate in GH-deficient adults: a seven-year follow-up study. *Clin Endocrinol (Oxf)* 2004; 60(5):568-575.
74. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *Eur J Endocrinol* 2007; 156(1):55-64.
75. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012; 61(6):1315-1322.
76. Russell SJ, Kahn CR. Endocrine regulation of ageing. *Nat Rev Mol Cell Biol* 2007; 8(9):681-691.
77. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; 299(5611):1346-1351.

78. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M et al. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med* 2011; 3(70):70ra13
79. Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958-2003. *J Clin Endocrinol Metab*. 2004 Mar;89(3):1031-44.
80. Rozing MP1, Westendorp RG, Frölich M, de Craen AJ, Beekman M, Heijmans BT, Mooijaart SP, Blauw GJ, Slagboom PE, van Heemst D; Leiden Longevity Study (LLS) Group. Human insulin/IGF-1 and familial longevity at middle age. *Aging (Albany NY)*. 2009 Jul 24;1(8):714-22.
81. Paolisso G, Gambardella A, Ammendola S, D'Amore A, Balbi V, Varricchio M, D'Onofrio F. Glucose tolerance and insulin action in healthy centenarians. *Am J Physiol*. 1996; 270
82. Burgers AM, Biermasz NR, Schoones JW et al. Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. *J Clin Endocrinol Metab* 2011; 96(9):2912-2920.
83. Tackema DG, Ling CH, Blauw GJ, Meskers CG, Westendorp RG, de Craen AJ, Maier AB. Circulating levels of IGF1 are associated with muscle strength in middle-aged- and oldest-old women. *Eur J Endocrinol*. 2011 Feb;164(2):189-96.

Chapter 2

Pituitary Dysfunction in Adult Patients after Cranial Radiotherapy: Systematic Review and Meta-Analysis

Natasha M. Appelman-Dijkstra, Nieke E. Kokshoorn, Olaf M. Dekkers,
Karen J. Neelis, Nienke R. Biermasz, Johannes A. Romijn,
Johannes W. A. Smit and Alberto M. Pereira

ABSTRACT

Cranial radiotherapy is an important cause of hypopituitarism. The prevalence of hypopituitarism varies considerably between studies. We conducted a systematic review and meta-analysis of reported prevalences of hypopituitarism in adults radiated for nonpituitary tumours.

We searched PubMed, EMBASE, Web of Science, and the Cochrane Library to identify potentially relevant studies. Studies were eligible for inclusion with the following criteria: 1) cranial radiotherapy for nonpituitary tumours and/or total body irradiation for hematological malignancies; 2) adult population (>18 yr old); and 3) report on endocrine evaluation.

Data review was done by two independent reviewers. Besides extraction of baseline and treatment characteristics, also endocrine tests, definitions, and cutoff values used to define pituitary insufficiency were extracted.

Eighteen studies with a total of 813 patients were included. These included 608 patients treated for nasopharyngeal cancer (75%) and 205 for intracerebral tumours. The total radiation dose ranged from 14 to 83 and 40 to 97 Gy for nasopharyngeal and intracerebral tumours, respectively.

The point prevalence of any degree of hypopituitarism was 0.66 [95% confidence interval (CI), 0.55–0.76]. The prevalence of GH deficiency was 0.45 (95% CI, 0.33–0.57); of LH and FSH, 0.3 (95% CI, 0.23–0.37); of TSH, 0.25 (95% CI, 0.16–0.37); and of ACTH, 0.22 (95% CI, 0.15–0.3), respectively. The prevalence of hyperprolactinemia was 0.34 (95% CI, 0.15–0.6). There were no differences between the effects of radiotherapy for nasopharyngeal vs. for intracerebral tumours.

Hypopituitarism is prevalent in adult patients after cranial radiotherapy for nonpituitary tumours. Therefore, all patients treated by cranial radiotherapy should have structured periodical assessment of pituitary functions.

INTRODUCTION

Pituitary insufficiency is a late onset sequel of cranial irradiation for intracerebral and nasopharyngeal tumours or total body radiotherapy for haematological malignancies in children (1-9). In the Childhood Cancer Survivor Study (CCSS) 43% of children treated for cerebral tumours had one or more endocrinopathies (10). Consequently, structured follow-up programs for childhood cancer survivors include endocrine assessments. In the last decades, survival rates of patients treated with cranial radiotherapy for various malignancies as well as for benign tumours have improved substantially by introduction of new surgical, radiotherapeutical, and chemotherapeutical options. In contrast to the long-term survivors of cranial radiotherapy in childhood, endocrine surveillance programs have not been routinely incorporated in adults treated with cranial radiotherapy. The prevalence of hypopituitarism after cranial radiotherapy is affected by several factors. First, the time interval between radiotherapy and the assessment of pituitary function is important, since the development of pituitary failure is likely to increase in time after radiotherapy (11-13). Second, hypothalamic and pituitary insufficiencies are more likely to develop with increasing radiation exposure (10;14). Finally, methodological differences between the studies with respect to endocrine evaluation, like the use of different endocrine tests with different criteria for pituitary insufficiency, will also affect the prevalence of hypopituitarism.

The aim of this study was to systematically assess the reported prevalence of pituitary insufficiency after cranial or total body radiotherapy for intracerebral tumours, nasopharyngeal tumours or haematological malignancies at the adult age.

DESIGN

Search strategy and eligibility criteria

We searched the following databases for studies on cranial radiotherapy and pituitary failure: PubMed, Cochrane Library, Web of Science, EMBASE, CINAHL database, Academic Search Premier, and Science Direct. The search was performed on August 14th, 2010.

In collaboration with a trained clinical librarian, we composed a search strategy for the above mentioned databases, focussing on radiotherapy, pituitary function, cerebral tumours and nasopharyngeal tumours. We used all relevant keyword variations, including free text words. The complete strategy is provided in appendix 1 (see supplemental data). Furthermore, the references of relevant articles were checked for additional articles.

Only original articles in English were included. Studies were eligible for inclusion in this review if they fulfilled the following criteria: 1) cranial radiotherapy for non-pituitary

tumours and/or total body irradiation for haematological malignancies 2) > 18 years at the time of radiotherapy 3) report on endocrine evaluation.

In case of mixed cohorts (i.e. including both paediatric and adult patients), patients <18 years were filtered from the results. In case of duplication of reports involving the same patient cohort the results on the different axes were combined in the analyses and tables, and only the paper with the longest duration of follow-up was included.

Data review and analysis

Initial selection of studies by title and abstract was performed by two reviewers (N.A en N.K.). These studies were retrieved for full assessment. All studies were evaluated by the two reviewers independently. Disagreements were resolved by consensus. Data extraction was based on data from each study provided at the population level. The definition of hypopituitarism had to be stated in the paper, including the endocrine tests used for the evaluation, definitions and cut-off values used to define pituitary insufficiency for each axis, hormone assays and reference values provided by the authors.

Statistical analysis

The main outcome of the present meta-analysis was the pooled proportion of patients with pituitary insufficiency after cranial radiotherapy. For all studies, the proportion of patients with hypopituitarism was calculated as the number of patients with the pituitary insufficiency divided by the total number of tested patients.

Meta-analysis was performed using an exact likelihood approach. The method used was a logistic regression with a random effect at the study level (15). Given the expected clinical heterogeneity a random effects model was performed by default and no fixed effects analyses were performed. For meta-analysis of proportions the exact likelihood approach based on a binomial distribution has advantages compared to a standard (DerSimonian and Laird) random effects model that is based on a normal distribution. Firstly, estimates from a binomial model are less biased than estimates from models based on a normal approximation (15;16). This is especially the case for proportions that are close to 0 or 1. Secondly, no assumptions are needed for the exact approximation when dealing with zero-cells, whereas the standard approach needs to add an arbitrary value (often 0.5) when dealing with zero-cells. Adding values to zero-cells is known to contribute to the biased estimate of the model (17;18).

Meta-regression analyses were also performed with an exact likelihood approach. A random effects meta-regression was performed to address the question whether the tumor site (nasopharyngeal *vs* intracerebral) influences the prevalence of pituitary insufficiency. All analyses were performed with STATA 10.0 (Stata Corp, Texas, USA).

Risk of Bias assessment

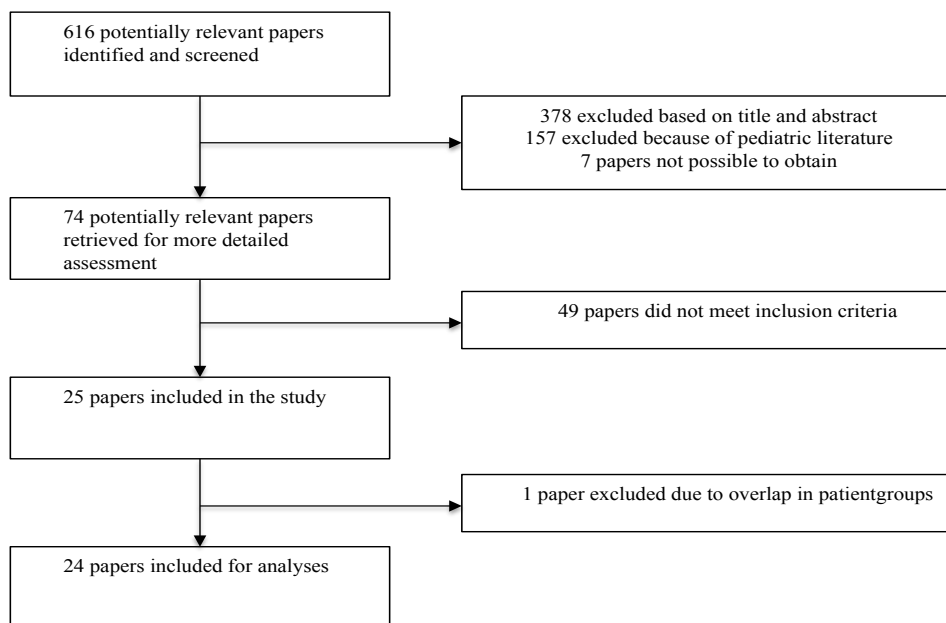
An additional evaluation of the risk of bias was performed in order to identify components that could potentially bias an association between cranial radiotherapy and hypopituitarism. The following study characteristics were evaluated: 1) adequacy of exposure determination, 2) adequacy of inclusion and follow-up, and 3) adequacy of outcome determination. For exposure determination 1 point was given if it was stated clearly that the pituitary was involved in the radiation field and 1 point if the estimated dose at the pituitary was reported. For the evaluation of inclusion of patients, 1 point was given for each study that included (consecutive) non-selected patients. For outcome determination, 1 point was given when the hormonal evaluation also included dynamic tests and 1 point if all patients in the study were tested. Consequently, each study could attain a maximum score of 5 points. Studies that scored 0-2 points were considered to have a high risk of bias, studies with 2-3 points as intermediate risk, and studies with 4-5 points as studies with a low risk of bias.

RESULTS

Systematic literature search (Figure 1)

The initial search resulted in a total of 849 articles (301 in PubMed, 4 in Cochrane Library, 131 in Web of Science, 353 in EMBASE, 16 in CINAHL database, 27 in Academic Search Premier, and 17 in Science Direct). Of these articles, 616 were unique without duplications. We excluded 378 papers based on title and abstract or language, 157 studies which evaluated patients aged <16 years and 7 papers which were not available for evaluation. Consequently, a total of 74 potentially relevant papers were retrieved for full assessment. Of these 74 publications, 51 papers were excluded from further analysis because the studies did not fulfil one or more of the eligibility criteria.

Therefore, ultimately, our search strategy resulted in 23 manuscripts meeting the inclusion criteria. However, some reports described data from the same patient cohort (19-26). In these cases the results were combined as one study in the analysis or the study with the longest follow-up was included. Consequently, a total of 18 studies were included in the present review, comprising 813 patients. The number of included patients per study ranged from only 6 (27) to 312 (20). Five studies included patients < 18 yrs (26-30). However, in only three of these five studies (27;28;30) it was possible to obtain the numbers of patients treated after the age of 18 yrs. The remaining two were included because of the low number of patients <18 years or sub-analysis on the patient group <18 years was performed with no different outcome than the older group (Table 1).

**FIGURE 1.**

Flowchart of study assessment and exclusion stages.

Study characteristics (Table 1&2)

Details of the 18 included studies are summarized in Table 1 and 2. The studies were published between 1975 and 2009. Seventy-five percent of the patients (608/813) were treated for nasopharyngeal cancer (Table 1). The remaining 25% were treated for intra-cerebral tumours (table 2). The majority of studies were cross-sectional studies with time after irradiation ranging from 4 months to 30 years. However the time after radiation varied considerable between individuals, even within one single study. Two studies did not report the time interval between irradiation and endocrine evaluation (28;31). None of the included papers evaluated patients treated with prophylactic body irradiation in the course of stem cell transplantation for haematological malignancies.

In three reports patient selection criteria were not stated (20;28;31) whereas four studies selected symptomatic patients by recruiting from a radiotherapy complication outpatient clinic or by inclusion of only patients suspected for any degree of hypopituitarism (21;30;32;33). For example, two studies used questionnaires on fatigue or diminished libido in combination with basal hormone samples to select patients for further endocrinological evaluation (22;28).

TABLE 1. Head and neck tumors

Author	Number of patients, age and gender	Tumor characteristics	Cranial irradiation (Gy)	Time since Rx (months)	Endocrine evaluation	Bias assessment	Pituitary insufficiency per axis (%)					
							Any	GH	HPA	TSH	LH/FSH	PRL
Snyers A et al; 2009 ⁽³⁸⁾	Total n=32 ^a	Sinonasal cancer	44-66	107 (11-253)	Basal morning serum samples, if abnormal : ITT, ACTH stim test or GHRH-arg	4	62% (13/21)	24% (5/21)	19% (4/21)	14% (3/21)	19% (4/21)	10% (2/21)
	n=21 endocrine evaluation 56 (28-74) yr		Mean dose pituitary = 51-56 Mean dose hypothalamus = 44-52									
Bhandare MS et al; 2008 ^(20b)	Total n=312 200M/112F	Nasal cavity n=56 Nasopharynx n=119 Frontal sinus n=2	Chemo n= 23 Broad range: < 40 - >70	63 (6-365)	Basal serum samples: TSH, FT4, PRL, GH, Cortisol, LH, FSH, T	2	60% (67/112)	36% (16/44)	32% (14/44)	70% (31/44)	27% (12/44)	15% (10/68)
	n= 112 endocrine evaluation	Etmoid sinus n=5 Sphenoid sinus n=34 Maxillary sinus n=59 Other n=37	Chemo n= 40		If abnormal: ACTH-test, metyrapone, CRH-test, ITT + vasopressin, GST, GHRH-test, Arginine-test, TRH-test							
	No age mentioned											
Lam KS et al; 1991 ^{(23)c}	n=20 22M/9F	Nasopharyngeal	Total dose: 60 Estimated dose pituitary: 62	60	Basal serum samples: LH, FSH, PRL, TSH, T4, T, E2	5	75% (15/20)	55% (11/20)	25% (5/20)	15% (3/20)	35% (7/20)	30% (6/20)
	M: 43.7±8.4 yr F: 36.8±9.0 yr				ITT, LHRH 100 µg, TRH 200 µg							
Woo E et al; 1988 ⁽³²⁾	n=11 8M/3F Age: 48(33-64) yr	Nasopharyngeal	Estimated dose pituitary: 62-67	72-240	Basal serum samples: LH, FSH, TSH, T4, cortisol, PRL, T, E2 ITT, LHRH 100 µg, TRH 200 µg,	4	82% (9/11)	90% (9/10)	18% (2/11)	45% (5/11)	55% (6/11)	27% (3/11)

TABLE 1. Head and neck tumors

Samaan NA et al; 1987 ^{(16)d}	Total n=166 98M/68F Age: 47(6-80) yr	Nasopharyngeal n=114 Paranasal sinus tumors n=29 Optic nerve/ eye tumors n=23	Estimated dose anterior pituitary: 57 (4-75) Estimated dose hypothalamus : 50 (11-75)	12-312	ITT, TRH 500 µg, LHRH 100 µg; Total T4, T3 resin uptake	5	75% (124/166)	75% (124/166)	18% (30/166)	20% (33/166)	35%/20% (68/166 FSH) (33/166 LH)	36% (60/166)
Lam KS et al; 1987 ^{(12)e}	n=32 21M/11F n=14 dynamic testing Age: 27-50 yr	Nasopharyngeal	46-60	60-204	n=32 Questionnaires on sexual impairment, galactorrhea Basal serum samples: PRL, T4, FTI, TSH, T If abnormal: E2, T, ITT, LHRH-test, TRH-test	3	25% (8/32)	19% (6/32)	6% (2/32)	13% (4/32)	16% (5/32)	19% (6/32F)
Lam KS et al; 1986 ^{(23)f}	n=8 1M/7F Age: 27-52 yr	Nasopharyngeal	46-61 Estimated dose to pituitary: 55-67	>60	Basal serum samples: LH, FSH, TSH, PRL, fT4, E2, T ITT, LHRH-test	5	100% (8/8)	50% (4/8)	50% (4/8)	50% (4/8)	25% (2/8)	88% (7/8)
Huang KE; 1979 ⁽³⁴⁾	n=11 All females Age: 19-40 yr	Nasopharyngeal	Nasopharyngeal area: 70 Estimated dose HP area: 60-65	12-156	ITT (n=2), LHRH test, TRH test, GHRH-test (n=6), CRH test(n=4) LHRH 25 µg	3	82% (9/11)	NR	NR	NR	82%/64% (9/11 LH) (7/11 FSH)	100% (8/8)
Rosenthal MB et al; 1976 ^(15g)	n=6 All Male Age: 35-66 yr	Nasopharyngeal	55-65 Chemo n=2	12-96	Basal serum samples: T4, TSH, FTI, T3, cortisol, LH, FSH, GH TRH test, ITT (n=2)	3	67% (4/6)	100% (2/2)	50% (1/2)	67% (4/6)	NR	NR

TABLE 1. Head and neck tumors

Samaan SA et al: 1975 ⁽³⁷⁾	n=10 7M/3F Age: 26-55 Age Rtx: 41 (8-58 yr) ^g	Nasopharyngeal	Estimated dose HP area: 50-83 50-65 to thyroid lobes (n=12)	60-240	ITT, Chlorpromazin: 25 mg, TRH-test 500 µg, LHRH 100 µg, T4, T3 resin uptake, 24 hour 131-I thyroid uptake	4	100% (6/10)	60% (5/10)	40% (4/10)	30% (3/10)	50% (5/10)
---	--	----------------	--	--------	---	---	--------------------	-------------------	-------------------	-------------------	-------------------

ACTH adrenocorticotrope hormone; E2, estrogens; F, female; FSH, follicle stimulation hormone; GH, growth hormone; GHRH, growth hormone releasing hormone; GST, glucagon stimulation test; Gy, Gray; HPA, hypothalamus pituitary axis; ITT, insulin tolerance test; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; N, number; NR, not reported; M, male; PRL, prolactin; Rtx, radiotherapy; T, testosterone; T4, thyroxine; T3, triiodothyronine; TRI, thyrotropin releasing hormone, TSH, thyroid stimulating hormone; a Total population n=168, however only n=32 long term follow-up in late morbidity clinic and n=21 endocrine evaluation

b Same patient group as (19): Clinical hypopituitarism: 14.1% (44/312), n=68 had dynamic testing and n=23 (33.8%) had subclinical hypopituitarism

c Combination of results with (45) which described follow up after 2 yrs of same cohort

d Part of the results were reported previously in (25). Patients were divided into 2 groups >15 yrs and <15. There was no difference between both groups in pituitary failure after 4 yrs.

e All patients received questionnaires; only those with suggested hypopituitarism had detailed endocrine assessment (n=14)

f All patients referred because of symptoms of hypopituitarism

g n=5 < 16 years during radiotherapy

Risk of bias assessment

One study was classified as high risk (received 1 point) (28), eight studies as intermediate risk (20;22;29-31;33-35), and 9 studies as low risk for selection bias (21;23;26;27;32;36-39).

Radiotherapy

The radiation dose was reported in all, but one, study (31), and ranged from 14-83 Gy, and 40-97 Gy in patients treated for nasopharyngeal carcinomas and intra-cerebral tumours, respectively. A total of nine studies (6 involving patients treated for nasopharyngeal tumours and 3 for intracerebral tumours) also calculated the estimated dose delivered to the pituitary, ranging from 46-83 Gy (in patients treated for nasopharyngeal tumours) and 25-97 Gy (in patients with intra-cerebral tumours) (21;23;26;29;30;36-39).

Endocrine assessment (Table 1 &2)

The overall prevalence of any degree of hypopituitarism differed considerably between the studies, ranging from 25% (22) to 100% (21;37) in studies involving patients treated for nasopharyngeal tumours, and from 37% (31) to 77% (39) in patients treated for intra-cerebral tumours.

Growth hormone (GH)-IGF-I axis (n=724):

Fourteen studies evaluated the GH-IGF-I axis. However, in only 61% (440/724) of the patients the axis was evaluated using either basal serum IGF-I and/or GH levels and/or a stimulation test (glucagon stimulation test, insulin tolerance test (ITT), arginine test, and combined growth hormone releasing hormone plus arginine test) (20-23;26;27;30-32;35-40). The prevalence of GH deficiency (GHD) varied between 24-100%. The prevalence of 100% was assessed in one study of 6 patients that evaluated only 2 patients using ITT (35).

Hypothalamic-pituitary-adrenal (HPA)-axis (n=751):

The HPA-axis was evaluated in 14 studies. In only 61% (460/751) of the patients the axis was tested by either basal serum cortisol levels and/or a stimulation test (corticotropin releasing hormone test, ITT, Glucagon- or ACTH-test). Adrenal insufficiency was diagnosed in 0-50% of patients with nasopharyngeal tumours and in 3-62% of the patients with intra-cerebral tumours.

TABLE 2. Primary intracerebral tumors

Author	Number of patients, age and gender	Tumor characteristics	Cranial irradiation (Gy)	Time since RTx (years)	Endocrine evaluation	Bias assessment	Pituitary insufficiency per axis (%)					
							Any	GH	HPA	TSH	LH/FSH	PRL
Schneider HJ et al; 2006 ⁽³¹⁾	n= 44 28 M/40 F Age: 45(20-79) yr	Glioma n= 40 Neuroblastoma n= 1 Meningeoma n= 18 Schwannoma n= 5 Dysgerminoma n= 2 Neuroblastoma n= 1 Chondrosarcoma n= 1 Hemangiopericytoma n= 1	NR Chemo n=27	NR	Basal serum samples: cortisol, 24-h urinary cortisol, T3, T4, TSH, PRL, FSH, LH, test, E2 GHRH+Arg	2	37% (17/44)	27% (12/44)	18% (8/44)	16% (7/44)	29.5% (13/44)	7% (3/44)
Agha A et al; 2005 ⁽³⁶⁾	n= 56 28 M/28 F Age: 39.3±11.9 yr	Osteosarcoma n= 1 Glioma n= 43 Meningeoma n= 5 Pinealoma n= 3 Medulloblastoma n= 2 Other n= 3	Estimated dose pituitary: 54(4-97)	6	Basal serum samples: FSH, LH, TSH, T4, PRL, IGF-I, T, E2 ITT (n=25), Arginine stim test, ACTH stim test	5	41% (23/56)	32% (18/56)	21% (12/56)	9% (5/56)	27% (15/56)	32% (18/56)
Johannese n TB et al; 2003 ⁽²⁹⁾	n= 33 18M/15F n= 25 endocrine evaluation Age Rbx: 38 (14-68) yr	Low grade glioma n= 27 High grade glioma n= 5 Anaplastic glioma n= 1	54 (45-59)	13.1 (6-25.6)	Basal serum levels If abnormal: TRH-test, CRH-test	3	64% (16/25)	NR	4% (1/25)	56% (14/25)	16% (4/25)	0
Popovic V et al; 2002 ⁽³⁰⁾	Total n= 22 n= 6 >18 yr	Medulloblastoma n= 4 Pinealoma n= 2	56 Estimated dose pituitary: 25-30	7.6 ± 0.7 (2-13)	ITT, GHRH-GHRP6	3	67% (4/6)	ITT: 67% (4/6)	NR	NR	NR	NR
GHRH-GHRP6: 33% (2/6)												

TABLE 2. Primary intracerebral tumors

Taphoorn MJ et al ; 1995 ⁽³⁹⁾	n=13 9M/4F Age:24-66 yr	Low grade astrocytoma n=7 Low grade oligodendroma n=6	45-61 Mean calculated dose pituitary 36(0-50)	3 (1-11.5)	Basal serum levels TRH-test 200 µg GnRH- test 100 µg hCRF –test 100 µg GHRH 50 µg	5	77% (10/13)	31% (4/13)	62% (8/13)	0%	15% (2/13)	23% (3/13)
Constine LS et al; 1987 ⁽²⁸⁾ a	Total n=65 n=30 (>18 yr) Age: 36.5(19-65) yr	Glioma n= 43 Medulloblas n= 7 Meningioma n= 5 Ependymoma n= 4 Others n= 5 No biopsy n= 2	Various amounts n = 47 cranial Rtx n= 18 craniospinal Rtx Chemo n=8	NR	T4 and TSH n= 65 PRL n= 47 No dynamic tests n= 35 cranial Rtx	1	NR	NR	NR	25% (7/28)	Only measure d in 9 people	29% (7/24)
Mechanick JJ et al; 1986 ⁽³³⁾	n=15 5M/10F Age: 22-59 yr	Astrocytomas n= 14 Medulloblast n= 1	40-50 to whole brain 1-22 local boost Chemo n=13	2-9	PRL n=9,T4, T3, T3- resin uptake TRH 200 µg	2	60% (9/15)	NR	NR	14.3% (1/7)	NR	100% (9/9)
Harrop JS et al; 1976 ⁽²⁷⁾	Total n=17 n= 8 > 18 yr Group 1 : tumors remote from the HP-area n= 5 3M/2F Group 2: tumors in hypothalamic area n= 3 1M/2F	Astrocytoma: n= 2 Meningeoma: n= 3 Angioma: n= 1 No biopsy: n= 2	40-52	6 (1-15)	ITT (n=6), GST (n=6), TRH (n= 8) 200 µg 250 µg Synacthen, Clomiphene stim test (n= 5), LHRH 100 µg	4	62.5% (5/8)	50% (4/8)	12.5% (1/8)	12.5% (1/8)	37.5% (3/8)	NR

ACTH adrenocorticotrope hormone; E2, estrogens; F, female; FSH, follicle stimulating hormone; GH, growth hormone; GHRH, growth hormone releasing hormone; GST, glucagon stimulation test; Gy, Gray; HPA, hypothalamus pituitary axis; ITT, insulin tolerance test; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; N, number; NR, not reported; M, male; PRL, prolactin; Rtx, radiotherapy; T, testosterone; T4, thyroxine; T3, triiodothyronine ;TRH, thyrotropin releasing hormone, TSH, thyroid stimulating hormone; a Evaluation galactorrhea, libido, menstrual function, texture of skin and hair, weight change, constipation, leg cramping, heat/cold intolerance. When abnormal quotationaire: basal hormone levels, 2 patients did fill out questionnaire but did not proceed to testing.

Hypothalamic-pituitary-thyroidal-axis (n=488):

Sixteen studies evaluated thyroid function using either basal serum hormone levels or a TRH-stimulation test. Thyrotropin deficiency was diagnosed in 26% of the patients (126/488). The prevalence rates ranged between 0-67% and 13-25% in patients with nasopharyngeal cancers and intracerebral tumours, respectively.

Hypothalamic-pituitary-gonadal axis (n=469)

The pituitary-gonadal axis was assessed in 14 studies. Hypogonadotropic hypogonadism was present in 30% (143/469): in 30-82% of cases treated for nasopharyngeal cancer and in 38-61% of cases treated for intracerebral tumours.

Hyperprolactinemia (n=515):

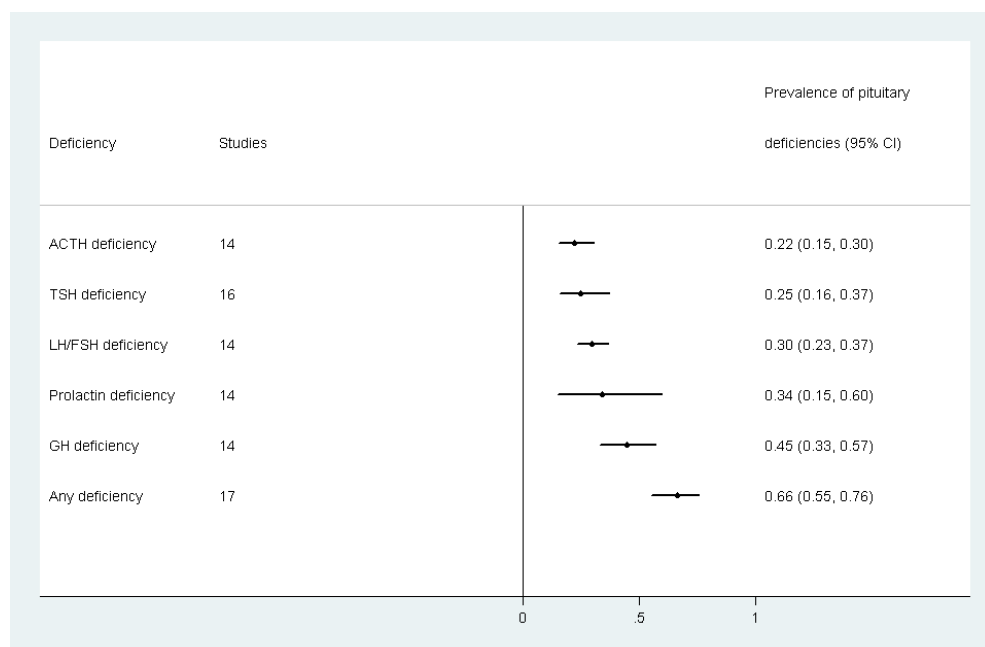
Prolactin levels were measured in 15 studies, documenting hyperprolactinemia in 147 of 515 patients (29%, 2-100% in patients treated for nasopharyngeal cancers, and 7-100% in patients treated for intra-cerebral tumours).

Meta-analysis

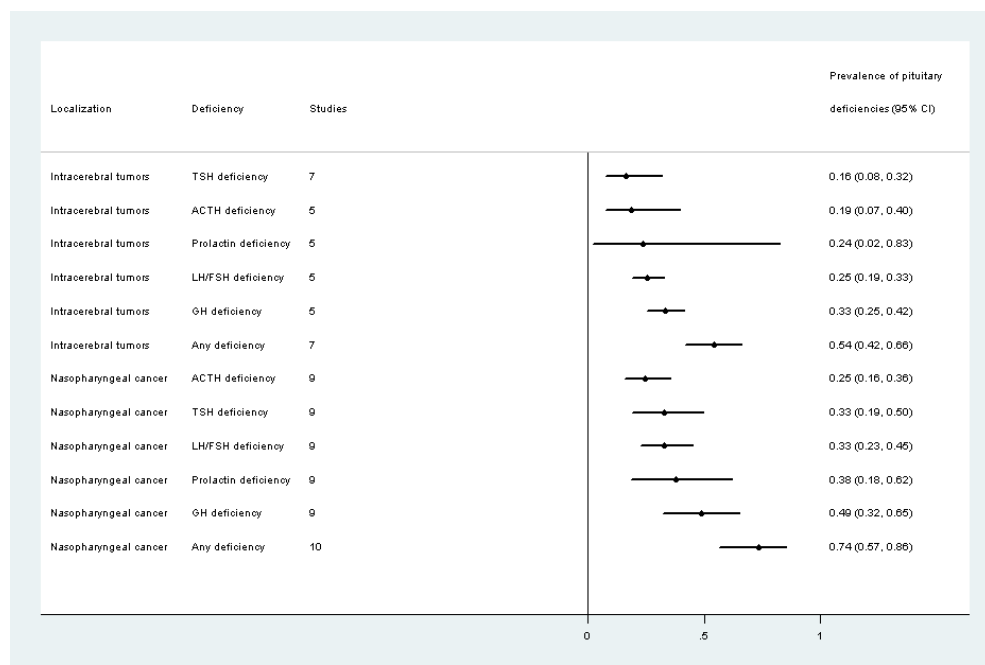
The pooled prevalence of any degree of hypopituitarism was 0.66 (95% CI 0.55-0.76). Growth hormone deficiency was the most prevalent pituitary deficiency, with a prevalence of 0.45 (95% CI 0.33-0.57), followed by LH/FSH deficiency 0.3 (95% CI 0.23-0.37) and TSH deficiency 0.25 (95% CI 0.16-0.37), respectively. The prevalence of hyperprolactinemia was 0.33 (95% CI 0.16-0.56) and ACTH deficiency had the lowest prevalence 0.22 (95% CI 0.15-0.3).

In a random effects meta-regression, the effect of tumor localization (nasopharyngeal vs cerebral) on the prevalence of deficiencies was assessed. No statistically significant association between the probability of any pituitary deficiency ($p=0.14$), GH deficiency ($p=0.36$), ACTH deficiency ($p=0.75$), TSH deficiency ($p=0.11$) LH/FSH deficiency ($p=0.21$) as well as hyperprolactinemia ($p=0.44$) and the indication for radiotherapy (nasopharyngeal cancer *vs.* intra-cerebral tumours).

A sensitivity analysis with four studies explicitly mentioning the inclusion of consecutive unselected patients was performed (23;29;36). The pooled prevalence of any pituitary deficiency was 0.62 (95% CI 0.45, 0.77), which is similar to the pooled prevalence of 0.66 when combining all the studies.

**FIGURE 2.**

Random effects meta-analysis of prevalence of pituitary insufficiency after cranial radiotherapy.

**FIGURE 3.**

Random effects meta-analysis of prevalence of pituitary insufficiency according to tumor site.

Pituitary insufficiency related to duration of follow-up after radiotherapy

Two studies reported on the occurrence in time of hypopituitarism (23;26). The prevalence of pituitary failure in patients treated for nasopharyngeal tumours was 6% after 1 year, 35% after 2 years, 56% after 3 years and 62% after 4 and 5 years (23). Samaan, and colleagues (26), reported on the classical sequential order of failure of individual pituitary functions in time. GH deficiency occurred after a mean of 2.6 years, followed by failure of the pituitary-gonadal axis and hyperprolactinemia after approximately 3.8 years, ACTH insufficiency after 6 years, and finally TSH insufficiency after a mean of 11 years.

DISCUSSION

This systematic review and meta-analysis demonstrated that pituitary insufficiency is a highly prevalent condition in adult patients after cranial radiotherapy for nasopharyngeal and intra-cerebral tumours. The prevalence of any form of hypopituitarism was 0.66 (CI 0.55-0.76). There were considerable variations in the reported prevalence rates of hypopituitarism after cranial radiotherapy, ranging from hardly any effect on pituitary function to almost 100% of the patients being affected. These variations were associated with differences in the number of patients included in the study and manner of endocrine evaluation.

The risk of development of hypopituitarism after cranial radiotherapy is a well recognized phenomenon in children. The likelihood to develop hypothalamic-pituitary insufficiency increases with increasing radiation exposure and with prolonged duration of follow-up up after radiotherapy (11;12). In the Childhood Cancer Survivor study (CCSS) 43% of paediatric patients treated for cerebral tumours had one or more endocrinopathies (10). Our meta-analysis showed that hypopituitarism is present in approximately two third of all adult patients previously treated with cranial irradiation. The prevalence of hypopituitarism after cranial radiotherapy is affected by several factors including radiation dose and techniques. Furthermore, the time interval between radiotherapy and the assessment of pituitary functions is important, since the development of pituitary failure is likely to increase during prolongation of follow-up after radiotherapy (11;12).

A random effects meta-regression revealed no significant different effects of underlying disease on pituitary function between the two groups of adult patients reported in literature: those treated for nasopharyngeal cancer *vs* intra-cerebral tumours. In addition, the overall difference in radiation dosage did not differ between groups: 40-83 Gy for the patients treated for nasopharyngeal carcinoma and 40-97 Gy for the patients with cerebral tumours. The use of different radiotherapeutical techniques however will most likely affect the rate of subsequent hypopituitarism, because higher cumulative radiation doses are associated with increasing incidence rates of pituitary failure (11;12).

Patients treated for nasopharyngeal tumours are usually treated with a higher average dosage and additive high dose single tumour boost. Therefore, a separate analysis of the studies concerning nasopharyngeal tumours vs. the other studies was performed (Fig. 3). However, there were no significant differences in the prevalence of any pituitary insufficiency between both groups. There are various possible explanations for this lack of significant differences between the two patient groups despite differences in irradiation dose. In nine studies from which the estimated doses delivered to the pituitary could be extracted (six involving patients treated for nasopharyngeal tumours and three for intracerebral tumours), the dose ranges were wide in both patient groups and showed a considerable overlap. In patients treated for nasopharyngeal tumours, the dose ranged from 46–83 Gy, and in patients with intracerebral tumours the dose ranged from 25–97 Gy. Duration of follow-up since radiotherapy varied from 11–253 months in the patient group treated for nasopharyngeal tumours and from 12–156 months in the group treated for intracerebral tumours. Finally, the overall prevalence of pituitary insufficiency is already high in both groups, with 0.54 (95% CI, 0.42–0.66) for intracerebral tumours vs. 0.74 (95% CI, 0.57–0.86) for nasopharyngeal tumours. This 0.2 difference in prevalence, however, did not result in significant differences between groups ($P = 0.14$). According to our risk of bias stratification, nine studies were considered as being at low risk of bias. The majority of studies used cross-sectional study designs with large differences in the time of evaluation in relation to previous radiotherapy. The selection of patient groups differed largely between the studies. Patient selection criteria were not stated in some of the reports (19, 27, 30), whereas the description of the selection procedures in other studies suggests preselection of patients, like recruitment from a radiotherapy complication outpatient clinic or by including only symptomatic patients suspected for any degree of hypopituitarism (20, 29, 31, 32). Two studies ($n=186$) used a prospective design; the reasons for loss to follow-up were not mentioned in any of the studies, precluding definite answers after 5 years of follow-up (22, 25). We additionally performed a sensitivity analysis of studies that qualified as a potential low–intermediate risk of bias (22, 28, 35, 37). This analysis revealed a pooled proportion of any pituitary deficiency of 0.62 (95% CI, 0.45–0.77), which is remarkably close to the overall found prevalence of 0.66 (95% CI, 0.55–0.76) calculated for all studies. This outcome illustrates that signs and symptoms of hypopituitarism after cranial irradiation apparently are not predictive of hypopituitarism in individual patients with their condition. Differences in endocrine evaluation may also affect the reported prevalence. The majority of patients were not evaluated by proper stimulation tests. Therefore, it is likely that the estimates of hypopituitarism after cranial radiotherapy represent a rather conservative estimation of the true prevalence of hypopituitarism. In addition, studies that did perform stimulation tests used different tests and cut-off values, and only 10 of 18 studies assessed all pituitary axes (but even then, not all patients were tested for each axis). Moreover, the hypothalamic-pituitary-

thyroidal axis and the hypothalamic-pituitary-gonadal axis can also be influenced by the use of alkylating chemotherapeutics and exposure of the thyroid gland and gonads to irradiation (18, 39–42). Primary hypothyroidism in patients treated for nasopharyngeal carcinoma occurs up to 20–30% within the first year after treatment (42). In the random effects regression model, the prevalence of TSH deficiency was 0.33 (95% CI, 0.19–0.5) and 0.16 (95% CI, 0.08–0.32) for the patients treated for intracerebral tumours. These differences were not significant, with $p = 0.11$. Hypogonadism after cranial radiotherapy was difficult to quantify because some studies reported testosterone or estrogen levels, whereas others used delayed or insufficient LH/FSH responses to GnRH as a criterion for hypogonadism. In addition, primary gonadal failure is highly prevalent in patients treated with chemotherapy, especially when treated with alkylating agents that could overestimate the results (39). Patients treated for nasopharyngeal tumours were more likely to receive chemotherapy, but again, no significant differences between groups were found. Prolactin might be another possible tool to estimate the likelihood of radiation-induced damage of the hypothalamic area. Because hyperprolactinemia might be a consequence of decreased hypothalamic dopamine secretion, hyperprolactinemia is variable in severity and often subclinical; it diminishes and might even normalize in time due to slowly evolving radiation-induced damage of lactotrophs. If hyperprolactinemia is to be considered as an indicator of disturbed hypothalamic dopamine secretion, one could expect the prolactin level to be high in the first years after radiation and normal after several years when lactotroph function has declined. Unfortunately, none of the studies provided information on prolactin levels in relation to the time interval since radiotherapy. Considering the high prevalence of hypopituitarism found, 0.66 (95% CI, 0.55–0.76), and that there was no significant difference between groups, assessment of pituitary function should be included in the long-term follow-up of all cranially irradiated patients. Current literature does not provide a timeline or a sequence of axis failure. Hypopituitarism can occur as soon as the first year after treatment, but it can also occur 11 years after treatment (22, 25). Taking into consideration the improved survival of patients, duration of follow-up for at least 15 years should be advisable. This follow-up period should include a basal morning hormone sample and dynamic testing of the HPA axis in every patient not on corticosteroids. Dynamic testing of the somatotrope axis should be defined per patient because GH failure will not have therapeutic consequences, although it might be an indicator for radiation induced pituitary damage. This systematic review underscores the need for structured periodical endocrine assessments of all patients who survive after cranial radiotherapy for all kinds of diagnoses. Therefore, an increasing number of patients will require a structured, tailored, periodic evaluation of pituitary functions after cranial radiotherapy.

ACKNOWLEDGMENTS

We thank Johannes W.Schoones, trained librarian, for his advice on the structured literature search.

REFERENCES

1. Alvarez JA, Scully RE, Miller TL, Armstrong FD, Constine LS, Friedman DL, Lipshultz SE 2007 Long-term effects of treatments for childhood cancers. *Curr Opin Pediatr* 19:23–31
2. Constine LS, Woolf PD, Cann D, Mick G, McCormick K, Raubertas RF, Rubin P 1993 Hypothalamic-pituitary dysfunction after radiation for brain tumours. *N Engl J Med* 328:87–94
3. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, Friedman DL, Marina N, Hobbie W, Kadan-Lottick NS, Schwartz CL, Leisenring W, Robison LL 2006 Chronic health conditions in adult survivors of childhood cancer. *N Engl J Med* 355:1572–1582
4. Schmiegelow M, Lassen S, Poulsen HS, Feldt-Rasmussen U, Schmiegelow K, Hertz H, Müller J 2000 Cranial radiotherapy of childhood brain tumours: growth hormone deficiency and its relation to the biological effective dose of irradiation in a large population based study. *Clin Endocrinol (Oxf)* 53:191–197
5. Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK, Poulsen HS, Müller J 2003 A population-based study of thyroid function after radiotherapy and chemotherapy for a childhood brain tumor. *J Clin Endocrinol Metab* 88:136–140
6. Shalet SM, Beardwell CG, Jones PH, Pearson D 1976 Growth hormone deficiency after treatment of acute leukaemia in children. *Arch Dis Child* 51:489–493
7. Shalet SM, Beardwell CG, Morris-Jones P, Bamford FN, Ribeiro GG, Pearson D 1976 Growth hormone deficiency in children with brain tumours. *Cancer* 37:1144–1148
8. Shalet SM, Beardwell CG, MacFarlane IA, Jones PH, Pearson D 1977 Endocrine morbidity in adults treated with cerebral irradiation for brain tumours during childhood. *Acta Endocrinol (Copenh)* 84: 673–680
9. Gurney JG, Kadan-Lottick NS, Packer RJ, Neglia JP, Sklar CA, Punyko JA, Stovall M, Yasui Y, Nicholson HS, Wolden S, McNeil DE, Mertens AC, Robison LL 2003 Endocrine and cardiovascular late effects among adult survivors of childhood brain tumours. *Childhood Cancer Survivor Study. Cancer* 97:663–673
10. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. *J Clin Endocrinol Metab* 85:2476–2482
11. Toogood AA 2004 Endocrine consequences of brain irradiation. *Growth Horm IGF Res* 14(Suppl A):S118–S124
12. Little MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Q J Med* 70:145–160
13. Darzy KH, Shalet SM 2009 Hypopituitarism following radiotherapy. *Pituitary* 12:40–50
14. Hamza TH, van Houwelingen HC, Stijnen T 2008 The binomial distribution of meta-analysis was preferred to model within-study variability. *J Clin Epidemiol* 61:41–51
15. Chang BH, Waternaux C, Lipsitz S 2001 Meta-analysis of binary data: which within study variance estimate to use? *Stat Med* 20: 1947–1956
16. Platt RW, Leroux BG, Breslow N 1999 Generalized linear mixed models for meta-analysis. *Stat Med* 18:643–654
17. Sweeting MJ, Sutton AJ, Lambert PC 2004 What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. *Stat Med* 23:1351–1375
18. Bhandare N, Kennedy L, Malyapa RS, Morris CG, Mendenhall WM 2007 Primary and central hypothyroidism after radiotherapy for head-and-neck tumours. *Int J Radiat Oncol Biol Phys* 68:1131–1139
19. Bhandare N, Kennedy L, Malyapa RS, Morris CG, Mendenhall WM 2008 Hypopituitarism after radiotherapy for extracranial head and neck cancers. *Head Neck* 30:1182–1192

19. Lam KS, Wang C, Yeung RT, Ma JT, Ho JH, Tse VK, Ling N 1986 Hypothalamic hypopituitarism following cranial irradiation for nasopharyngeal carcinoma. *Clin Endocrinol (Oxf)* 24:643–651
20. Lam KS, Ho JH, Lee AW, Tse VK, Chan PK, Wang C, Ma JT, Yeung RT 1987 Symptomatic hypothalamic-pituitary dysfunction in nasopharyngeal carcinoma patients following radiation therapy: a retrospective study. *Int J Radiat Oncol Biol Phys* 13:1343–1350
21. Lam KS, Tse VK, Wang C, Yeung RT, Ho JH 1991 Effects of cranial irradiation on hypothalamic-pituitary function—a 5-year longitudinal study in patients with nasopharyngeal carcinoma. *Q J Med* 78:165–176
22. Samaan NA, Bakdash MM, Caderao JB, Cangir A, Jesse Jr RH, Ballantyne AJ 1975 Hypopituitarism after external irradiation. *Ann Intern Med* 83:771–777
23. Samaan NA, Vieto R, Schultz PN, Maor M, Meoz RT, Sampiere VA, Cangir A, Ried HL, Jesse Jr RH 1982 Hypothalamic, pituitary and thyroid dysfunction after radiotherapy to the head and neck. *Int J Radiat Oncol Biol Phys* 8:1857–1867
24. Samaan NA, Schultz PN, Yang KP, Vassilopoulou-Sellin R, Maor MH, Cangir A, Goepfert H 1987 Endocrine complications after radiotherapy for tumours of the head and neck. *J Lab Clin Med* 109:364–372
25. Harrop JS, Davies TJ, Capra LG, Marks V 1976 Hypothalamic-pituitary function following successful treatment of intracranial tumours. *Clin Endocrinol (Oxf)* 5:313–321
26. Constine LS, Rubin P, Woolf PD, Doane K, Lush CM 1987 Hyperprolactinemia and hypothyroidism following cytotoxic therapy for central nervous system malignancies. *J Clin Oncol* 5:1841–1851
27. Johannesen TB, Lien HH, Hole KH, Lote K 2003 Radiological and clinical assessment of long-term brain tumour survivors after radiotherapy. *Radiother Oncol* 69:169–176
28. Popovic V, Pekic S, Golubicic I, Doknic M, Dieguez C, Casanueva FF 2002 The impact of cranial irradiation on GH responsiveness to GHRH plus GH-releasing peptide-6. *J Clin Endocrinol Metab* 87:2095–2099
29. Schneider HJ, Rovere S, Corneli G, Croce CG, Gasco V, Ruda` R, Grottoli S, Stalla GK, Soffietti R, Ghigo E, Aimaretti G 2006 Endocrine dysfunction in patients operated on for non-pituitary intracranial tumours. *Eur J Endocrinol* 155:559–566
30. Woo E, Lam K, Yu YL, Ma J, Wang C, Yeung RT 1988 Temporal lobe and hypothalamic-pituitary dysfunctions after radiotherapy for nasopharyngeal carcinoma: a distinct clinical syndrome. *J Neurol Neurosurg Psychiatry* 51:1302–1307
31. Mechanick JI, Hochberg FH, LaRocque A 1986 Hypothalamic dysfunction following whole-brain irradiation. *J Neurosurg* 65:490–494
32. Huang KE 1979 Assessment of hypothalamic-pituitary function in women after external head irradiation. *J Clin Endocrinol Metab* 49:623–627
33. Rosenthal MB, Goldfine ID 1976 Primary and secondary hypothyroidism in nasopharyngeal carcinoma. *JAMA* 236:1591–1593
34. Agha A, Sherlock M, Brennan S, O'Connor SA, O'Sullivan E, Rogers B, Faul C, Rawluk D, Tormey W, Thompson CJ 2005 Hypothalamic-pituitary dysfunction after irradiation of nonpituitary brain tumours in adults. *J Clin Endocrinol Metab* 90:6355–6360
35. Snyers A, Janssens GO, Twickler MB, Hermus AR, Takes RP, Kappelle AC, Merks MA, Dirix P, Kaanders JH 2009 Malignant tumours of the nasal cavity and paranasal sinuses: long-term outcome and morbidity with emphasis on hypothalamic-pituitary deficiency. *Int J Radiat Oncol Biol Phys* 73:1343–1351
36. Taphoorn MJ, Heimans JJ, van der Veen EA, Karim AB 1995 Endocrine functions in long-term survivors of low-grade supratentorial glioma treated with radiation therapy. *J Neurooncol* 25:97–102

37. Chen MS, Lin FJ, Huang MJ, Wang PW, Tang S, Leung WM, Leung W1989 Prospective hormone study of hypothalamic-pituitary function in patients with nasopharyngeal carcinoma after high dose irradiation. *Jpn J Clin Oncol* 19:265–270
38. Levine J, Canada A, Stern CJ 2010 Fertility preservation in adolescents and young adults with cancer. *J Clin Oncol* 28:4831–4841
39. Jereczek-Fossa BA, Alterio D, Jassem J, Gibelli B, Tradati N, Orecchia R 2004 Radiotherapy-induced thyroid disorders. *Cancer Treat Rev* 30:369–384
40. Mercado G, Adelstein DJ, Saxton JP, Secic M, Larto MA, Lavertu P 2001 Hypothyroidism: a frequent event after radiotherapy and after radiotherapy with chemotherapy for patients with head and neck carcinoma. *Cancer* 92:2892–2897
41. Lin Z, Wu VW, Lin J, Feng H, Chen L 2011 A longitudinal study on the radiation-induced thyroid gland changes after external beam radiotherapy of nasopharyngeal carcinoma. *Thyroid* 21:19–23
42. Lam KS, Tse VK, Wang C, Yeung RT, Ma JT, Ho JH 1987 Early effects of cranial irradiation on hypothalamic-pituitary function. *J Clin Endocrinol Metab* 64:418–424

Chapter 3

Pituitary dysfunction in adult patients after cranial irradiation for head and nasopharyngeal tumours

Natasha M. Appelman-Dijkstra, Frank Malgo, Karen J. Neelis, Ida Coremans,
Nienke R. Biermasz and Alberto M. Pereira

ABSTRACT

Background: Pituitary insufficiency after radiotherapy in the hypothalamic pituitary region is a well-known complication. However, endocrine assessments are not incorporated in the follow-up after cranial irradiation for head and neck tumours.

Aim of the study: to evaluate pituitary function in patients cranially irradiated for non-pituitary tumours.

Patients and Methods: Evaluation of pituitary function in all available patients treated at our center with cranial radiotherapy for head and neck tumours.

Results: We included 80 patients. Forty patients were treated for cerebral tumours, 15 for nasopharyngeal tumours, and 25 for different tumours like meningioma or cerebral metastasis. Mean age was 47.5 (18.6-89.7) years. Mean radiation dose delivered at the pituitary region was 56.27 Gy (40.0-70.0). Pituitary insufficiency was present in 16 patients within 2 years after irradiation within 2 years after irradiation. 23/49 patients (47%) after 5 years and 27/45 (60%) after 10 years and 31/35 patients (89%) after 15 years, respectively.

Conclusion: Pituitary insufficiency is highly prevalent in adult patients treated with cranial radiotherapy for head and nasopharyngeal tumours. These prevalence rates are comparable to those observed after radiotherapy for pituitary tumours. Because hormone replacement of endocrine deficits improves quality of life and prevents potential severe complications, such as Addisonian crises, periodical evaluation of pituitary function is advocated.

INTRODUCTION

Radiation-induced pituitary insufficiency is a well-known dose-dependent late sequel of pituitary irradiation for pituitary tumours with prevalence rates of any pituitary insufficiency of approximately 50% after 5 years, and up to 75 % after 10 years following a total dose of 40-45Gy (1-3). In accordance, when the hypothalamic-pituitary region is within the radiation field, cranial irradiation for non-pituitary tumours may also induce pituitary insufficiency (4). In children, the high incidence of pituitary insufficiency after cranial irradiation for cerebral or nasopharyngeal tumours, including total body irradiation (TBI) for hematological malignancies, is well established (5-9). The Childhood Cancer Survivor Study (CCSS) reported one or more endocrinopathies to be present in 43% of children treated for cerebral tumours. Consequently, these children are subjected to structural endocrine surveillance also when reaching adulthood (10).

Currently, guidelines on endocrine surveillance in adult patients are not available. Survival rates for e.g. cerebral and nasopharyngeal tumours have improved substantially by new treatment modalities (11,12). Therefore, it is likely that an increasing number of adult patients will also be affected by endocrine complications of cranial irradiation. Since complaints of hypopituitarism are generally non-specific, endocrine tests are required for an accurate diagnose of pituitary failure. Endocrine function tests are necessary for diagnosing hypocortisolism or growth hormone deficiency. Growth hormone deficiency however might not be clinically relevant for a patient treated for a malignant tumour since cancer patients generally will not be substituted with growth hormone, although in childhood cancer survivors no there was no statistically significant increased overall risk of the occurrence of neoplasms associated with rh-GH exposure (13). But diagnosing growth hormone deficiency will still be useful since it may serve as an indicator of radiation induced hypothalamic/pituitary damage. In a recent meta-analysis, we have concluded that the low number of adult patient cohorts followed after cranial radiotherapy for non-pituitary tumours with sufficient long-term data and proper dynamic endocrine evaluation precludes strong conclusions on the incidence and prevalence rates of pituitary insufficiency. However, the meta-analysis suggested a high prevalence of any form of pituitary insufficiency, like in children, of 0.66 (95% CI, 0.55–0.76) after cranial radiotherapy for both nasopharyngeal and cerebral tumours (14). In addition, the time of onset of pituitary failure was documented in only 3 studies (15-17). The aim of this cohort study was to evaluate the prevalence, and the time of onset, of pituitary insufficiency in adult patients after cranial irradiation for non-pituitary tumours in our center.

SUBJECTS AND METHODS

The patient registry of the department of Radiotherapy was checked for patients who underwent cranial radiotherapy for non-pituitary tumours from 1990-2010 with the hypothalamus and/or the pituitary within the radiation field and whom received >20Gy to the hypothalamus-pituitary region (n=1504). We selected a threshold of 20 Gy based on data from historical cohorts indicating that a total dose of 7-24 Gy delivered to the pituitary/hypothalamus region, may cause growth hormone deficiency only, whereas dosages as high as 50-60 Gy will most certainly cause multiple pituitary hormone deficiencies (18). When alive and not already known to the outpatient clinic of the department of Endocrinology, patients were invited for endocrine testing. Patients were excluded if: 1) radiotherapy was given for pituitary or parasellar tumours 2) radiotherapy was applied as TBI, 3) pituitary insufficiency was already present before radiotherapy 4) was a life expectancy of less than 12 months at time of screening. Pituitary functions were checked at first outpatient clinic visit with basal hormone samples and endocrine function tests, afterwards patients were monitored by a standardized protocol (vide infra). In addition we used clinical and amnesic information on their condition prior to radiation. In case of pituitary insufficiency medication was started at the discretion of the treating physician. This study represents a combined analysis of the patients already known at the department of endocrinology and patients from the screening of records of radiotherapy.

Mean applied radiation dose:

All files were screened for a calculated/estimated dose to the pituitary/hypothalamic region. For patients irradiated before 2004, the isodose lines plotted on 2D images and on plain x-rays of the skull were used to estimate the pituitary dose. After the introduction of CT based treatment planning in our department CT images were used to read the plotted dose. Since 2007 the planning software gives a calculated dose to the hypothalamic/pituitary region, and mean doses to the pituitary were collected.

Parameters

At baseline disease specific information was extracted from the medical files.

The following parameters were assessed:

Anthropometric parameters: body weight and height were measured. Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. Additional information on medication use, Karnofsky score, IADL score, and co-morbidity was gathered.

Endocrine assessment

Biochemical parameters: IGF-1, GH, TSH, fT₄, ACTH, cortisol, LH, FSH, estradiol, testosterone, Sex Hormone Binding Globulin (SHBG), and prolactin concentrations were measured, furthermore general laboratory parameters including renal and liver function and cardiovascular parameters were obtained. Blood samples were taken for laboratory measurements after an overnight fast.

For diagnosing cortisol deficiency we used the following stepwise approach. First a basal cortisol level was performed. When basal cortisol concentrations were <80 nmol/L, in the absence of glucocorticoid use, the diagnosis of hypocortisolism was considered evident and no additional tests were performed (19). To date, the insulin tolerance test (ITT) remains the golden standard for the evaluation of the HPA-axis (19). In case of contraindications for the ITT, such as epilepsy or coronary artery disease, alternative dynamic tests such as the corticotrophin releasing hormone (CRH) test ($n=10$), the metyrapone test ($n=4$), or the ACTH stimulation test were used as to assess adrenal function (19-23). The choice for a specific test was made by the treating physician on an individual basis depending on the patient's clinical condition. Cortisol deficiency was defined by a peak cortisol below 0.55 $\mu\text{mol/L}$, either after insulin-induced hypoglycemia (glucose nadir <2.2 mmol/L) or after stimulation with 100 mcg CRH. A limited number of patients were tested using metyrapone ($n=4$) since in the past CRH was not available for some time, two of them were retested with the insulin tolerance test when possible. Metyrapone (30 mg/kg, Metopiron, Novartis Pharma B.V., Arnhem, the Netherlands) was administered orally at midnight. The next morning post absorptive blood samples were obtained for measurement of 11-deoxycortisol, cortisol and ACTH levels. A cut-off value for 11-deoxycortisol of 200 nmol/L was used to define normal adrenal function (20-22). Growth hormone deficiency was defined by a GH peak response to the insulin induced hypoglycemia of <3 $\mu\text{g/L}$ or by a GH peak after combined Growth Hormone Releasing Hormone + Arginine-test (GHRH/Arg test) using BMI-adjusted GH cut-offs. (24,25). When secondary amenorrhea was present for >1 year in the presence of a low serum estradiol (estradiol $0-200$ pmol/L) with normal or low serum levels of FSH and LH, premenopausal women were classified as gonadotropin-deficient. In men, gonadotropin deficiency was defined as a testosterone level <8.0 /l with and normal or low serum levels of FSH and LH (FSH $1.5-12.5$ U/L; LH $2.0-9.0$ U/L). Thyroid-stimulating hormone (TSH) deficiency was defined as free T₄ level below the reference range (<12 pmol/L). Hyperprolactinemia was defined by a basal serum prolactin level above the reference range (15.0 and 23 $\mu\text{g/L}$, for men and women, respectively).

Assays

In patients, serum IGF-1 concentrations were measured. From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5 nmol/l and an interassay CV less than 11%. IGF-1 was expressed as SD score for age- and gender-related normal levels determined in the same laboratory. Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (26,27).

FT4, TSH, LH, FSH, and PRL concentrations were measured by electrochemiluminescence immunoassay (ECLIA), using a Modular E170 (Roche Diagnostics). The maximal inter-assay CV was 5.0%. ACTH was determined by immunoluminometric assay using an Immulite 2500 (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The maximal inter-assay CV was between 5.0 and 10.0%. For the measurement of estradiol, a RIA (Orion Diagnostica, Espoo, Finland) was used (CV was 6% at 70 pmol/l) with a detection limit of 20 pmol/l. Testosterone was measured using an RIA (Siemens Healthcare Diagnostics; CV was 20% at 1.0 nmol/l and 12% at 14 nmol/l). The detection limit was 0.2 nmol/l.

Statistical analysis

SPSS for Windows, Version 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Results are presented as mean, age and follow up are stated as median, unless stated otherwise.

RESULTS

We identified 1504 patients from the records of the radiotherapy department. We excluded 426 patients treated with TBI for hematological malignancies. Of the 1078 remaining patients, 243 patients were still alive at screening (23%). Another 130 patients did not fulfill inclusion criteria: 102 patients (9%) had progressive disease at the time of the study, 28 had other exclusion criteria. Finally, 8 patients were lost to follow up. 105 patients were enrolled in the current study.

Of this total of 105 patients, 59 patients had been assessed at the endocrine department since the beginning of their follow up period. The remaining 45 patients were invited for endocrine evaluation, of whom 24 declined, and therefore 21 new patients underwent a complete endocrine work-up (Figure 1).

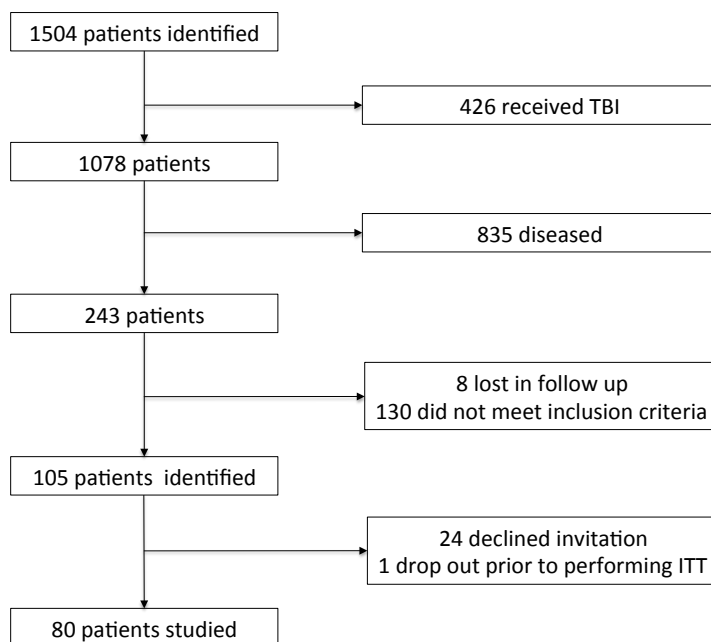


FIGURE 1.

Flowchart patient selection

TBI: total body irradiation, ITT : Insulin Tolerance Test

Patient characteristics

A total of 80 patients were enrolled in the study. Forty were treated for cerebral tumours, 15 for nasopharyngeal tumours, 25 for different tumours like meningioma, or metastasis. Median age was 47.5 years and median time of follow-up was 6 years (range 0.5-35) (Table 1.)

General assessment of Karnofsky score and ADL in general showed a normal score, in agreement with the inclusion criteria and outpatient clinic setting of this study.

For 39 patients, treated after 2007, the planning software calculated a estimated dose to the pituitary, from all other patients, if possible, retrospective dose calculations were made from the the isodose lines plotted on 2D images and on plain x-rays of the skull or from 2004 onwards CT images were used. Mean radiation dose was 56.27 Gy (range 40-70).

For 39 patients, treated after 2007, the planning software calculated an estimated dose to the pituitary, mean 31.7 Gy. Stereotactic radiotherapy was fractionated and performed elsewhere, it was not possible to retrieve the radiation plans.

TABLE 1 Patient characteristics

Baseline characteristics	Number of patients (n=80)
Diagnosis (n):	
Cerebral tumor	40
Nasopharyngeal tumor	15
Metastasis	2
Meningioma	14
Other	9
Radiotherapy (n):	
Conventional	77
Stereotactic	3
Mean Dose Rtx (Gy)	56.3 (40.0-70.0)
Median Age (years)	47.5 (18.6-89.7)
Follow up	6.0 (0.5-35.0)

Other tumours: Chondrosarcoma n=2, Ethmoidcarcinoma n=1, Arachnoidsarcoma n=1, Osteosarcoma n=1, Parachordoma n=1, Haemangiopericytoma n=1, Non-Hodgkin Lymphoma n=1. Gy; Grey, Rtx; Radiotherapy

Endocrine assessment

No adverse events occurred during the insulin tolerance test.

Of the total of 80 patients, fifty patients (62%) were diagnosed with any form of hypothalamic-pituitary insufficiency. When patients with isolated hyperprolactinemia (n=9) were excluded, the prevalence of any pituitary insufficiency was 51%. Pituitary insufficiency was present in 16 patients within 2 years after irradiation. This number increased to 23/49 patients (47%) after 5 years and 27/45 (60%) after 10 years. Pituitary insufficiency was present in 31/35 patients (89%) with a follow-up duration of at least 15 years.

Of the 21 patients first tested during this cross-sectional study, 9 (43%) were found to have any form of pituitary insufficiency; including previously undiagnosed symptomatic pituitary insufficiency.

Growth hormone- IGF-1 axis:

Severe GH deficiency (GHD) was documented in 27 patients (33%).

In 70% IIT was performed, 18 patients did not undergo dynamic testing and were diagnosed with GHD using serum IGF-1 followed by calculating the standard deviation

of the value in the age- and sex-specific reference range $<-2SD$ in addition of 2 other failing pituitary hormones. Duration of follow up before diagnosing GHD was 4,5 years after irradiation (0,5-35 years) in the 59 patients known on the outpatient clinic of the endocrinology department

Hypothalamic-Pituitary-Adrenal-axis:

Secondary adrenal insufficiency was diagnosed in 25 patients (31%). In 73% (n=59) of the patients tested for HPA-axis IIT was performed, 7 (12%) were diagnosed with secondary hypocortisolism with IIT. Secondary adrenal insufficiency was diagnosed in the other patients with the ACTH test (n=4), the CRH test (2), the Metyrapon test (n=4). Duration of follow up before diagnosing cortisol deficiency was after 6 years (range 0.5- 24 yrs) in the 59 patients known on the outpatient clinic of the endocrinology department.

Gonadal axis

Hypogonadotropic hypogonadism was present in 20 patients (25%) of the 59 patients known and the duration of follow up before diagnosis was 7 years (range 0,5-16). Hypogonadotropic hypogonadism in the presence of hyperprolactinemia was transient and resolved after initiation of anti-dopaminergic treatment and these patients were not classified as having gonadal deficiency.

Pituitary-Thyroid axis

Central hypothyroidism was observed in 11 patients (14%), as defined by a low Ft4 and and inappropriate TSH. TSH deficiency was seen after a duration of follow up of 5.1 (1.5-10.3) years after irradiation in the 59 patients known on the outpatient clinic of the endocrinology department.

Prolactin

Hyperprolactinemia was seen in 17 patients (21%) of the patients known to the outpatient clinic after a follow up of 2,5 (0,5-21) years, but was symptomatic in only 2 patients (females).

Disease specific pituitary insufficiency.

Cerebral tumours

40 patients (22 males) were treated for primary cerebral tumours with a mean dose of 55.8 Gy $\pm$ 4.4 Gy, range 46-61 Gy. Eleven patients (28%) were GHD, 9 (27%) suffered

from hypocortisolism, 13% were TSH deficient, and 13% had hypogonadotropic hypogonadism. Hyperprolactinemia was seen in 27% of patients, none were symptomatic.

Nasopharyngeal carcinoma

Fifteen patients (11 males) were treated for nasopharyngeal carcinoma. Mean radiation dose was 63 Gy $\pm$ 9.8 range 40-70 Gy. 2 patients from the group never tested prior to this study turned out to have symptomatic pituitary insufficiency. In the total of 15 patients treated for nasopharyngeal carcinoma 3 (21%) were GHD, 5 (33%) were HPA deficient. TSH deficiency was seen in 20%, whereas 40% of patients were hypogonadal and 40% suffered from hyperprolactinemia. Two patients (females) were symptomatic and were subsequently treated with cabergoline with reinstatement of the gonadotroph function.

Other tumours

Twenty-five patients were treated for various other tumours with a mean dose of 53 Gy $\pm$ 7 with a range of 40-69 Gy. In patients treated for cerebral metastases or other tumours 52% were GHD, 40% HPA deficient, 28% TSH deficient, 36% were hypogonadal and 28% of patients had hyperprolactinemia.

DISCUSSION

This study, using optimal endocrine testing to evaluate pituitary function in a group of adult patients after cranial radiotherapy for head and neck tumours with the pituitary within the radiation field, showed that pituitary insufficiency was highly prevalent, since 62% of the patients were diagnosed with any form of hormone deficiency. These findings add up to the presently available reports (14,17,28). The additional value of the present study is the standardized approach of stringent endocrine testing and long-term follow up in the majority of patients. In addition, the long-term follow-up enabled to produce an overall timeframe showing that pituitary insufficiency of any form was present in 55% of the cases within 5 years after radiotherapy, and amounted to 75% and 90% of the patients after 10 and 15 years, respectively. Another study following the same testing protocol reported a slightly lower rate of pituitary deficiencies in patients irradiated for non-pituitary brain tumours in adulthood (28). The lower rate of deficiencies was mainly driven by the approximately 20% of patients with diagnosed as adrenal insufficient, which can be explained, at least in part, by the use of different cut of values for the ITT (500 nmol/L *vs* 550 nmol/L in our hospital). A similar pattern of

radiation-induced pituitary insufficiency with a prevalence of 38% within 32 months of cranial irradiation was reported by Madaschi and colleagues (17).

This retrospective study, as others, suffers from several limitations. We fully acknowledge the fact that the patients from the Endocrinology department (n=59) studied in the present study reflect a very selected cohort since they were actively referred. A large number of patients from our registry were deceased, due to their malignancies or had progressive disease and were not tested at time of the study, which largely influences the population at risk to develop pituitary insufficiency. In addition, concurrent medication use like corticosteroids for the treatment of cerebral edema or already diagnosed and treated primary thyroidal or gonadal failure precludes complete testing of pituitary function in a significant number of cases (29). We acknowledge the fact that the patients studied in the long-term in the present study reflect a selected cohort since they were actively referred. Although we screened the files of all eligible patients treated with cranial radiation (Figure 1) and invited all patients alive with reasonable life expectancy, we were only able to study a minority of all irradiated patients (n=80). Of note, 59 patients treated with cranial radiotherapy at our center was actively referred for endocrine testing. However these patients did not differ from the patients actively recruited in terms of pituitary deficiencies, since pituitary insufficiency was present in 43% of cases after a mean follow up of 4.8 ± 3.2 years after radiotherapy (range 1-10 years). However, despite the high level of heterogeneity in this study, as in the other available studies on this subject, and the methodological flaws, the prevalence of pituitary insufficiencies is significant and clinically relevant. Despite the lack of prospective data, this condition merits a much greater awareness of all health care providers involved, including the development of recommendations or guidelines.

Lastly, the use of different radiation schemes and patient cohorts, creating a very diverse cohort. The degree of pituitary deficiencies depends on the radiation dose delivered to the hypothalamic/pituitary region. Previous studies have shown that 7-24 Gy as used for the treatment of haematological malignancies result in growth hormone deficiency only (18,30). The incidence of multiple deficiencies increases in time and with higher dosages due to the radiation induced changes to the DNA as well as indirectly by production of free radicals from intracellular water molecules (31,32). These radiation-induced changes can cause direct lethal effects or will accumulate to sublethal effects that will restrict potential cell replication. Cell death after radiation occurs in the mitotic phase of the cell cycle providing the explanation for the delayed onset of hormonal failure in slowly replicating tissue, like neuroendocrine tissues of the hypothalamic and pituitary (33). Furthermore, radiation may also induce tissue damage and dysfunction through chronic inflammation and release of pro-inflammatory cytokines, compromising endothelial function by increasing endothelial proliferation and collagen synthesis (31-34). We selected a threshold of 20 Gy based on data from historical cohorts indicating that a total dose of 7-24 Gy delivered to the pituitary/hypothalamus region, may

cause growth hormone deficiency only, whereas dosages as high as 50-60 Gy will most certainly cause multiple pituitary hormone deficiencies (30). On the basis of the current data and other studies we consider the chance of developing hypopituitarism with pituitary doses > 20 Gy high enough to justify endocrine follow-up.

The presence of pituitary insufficiency *per se* is associated with a decrease in quality of life as well as an increase in morbidity and mortality (35,36). Although available studies have some limitations, because studies are small sized, retrospective nature and use different diagnostic criteria to study pituitary function, it is clear that patients treated with cranial irradiation are at increased risk to develop pituitary insufficiency and that this easily treatable side effect can not be neglected. Therefore, although we advocate routine endocrine testing in every patient after cranial radiotherapy, the decision to proceed to pituitary testing with stimulation tests should be individually based. Diagnosing hypopituitarism can be challenging since it might be difficult to distinguish between symptoms of hypopituitarism or radiation/chemotherapy induced changes. Furthermore biochemical findings might not be that clear e.g the finding of low FT4 combined with an abnormally low TSH makes a diagnosis of central hypothyroidism obvious. However milder defects, characterized by FT4 levels still within the normal range, but decreasing following radiotherapy may be also clinically relevant and remain undiagnosed (37). Rose et al. reported on the fact that in childhood cancer survivors many patients with mild thyrotrope insufficiency were not diagnosed on the basis of basal thyroid function screening (38). Patients treated for malignancies often complain of irritability, anxiety and depression as well as fatigue (39-41). In patients with hypopituitarism, for example with growth hormone deficiency, the psychological symptoms include decreased energy levels, social isolation, and lack of positive well being, depressed mood and increased anxiety (42). Whereas physical complaints like fatigue and decreased libido can be seen due to hormonal dysfunction as well. Even for endocrinologists, it remains very difficult to distinguish between hypopituitarism and general complaints after radiation or chemotherapy. Nevertheless, endocrine replacement is able to improve complaints caused by hypopituitarism. For example 2 of the 21 patients who were invited for endocrinological screening were already under long-term treatment for depression and their decrease libido was considered secondary to depression instead of hypogonadism and 2 patients cured from nasopharyngeal carcinoma even presented in the past with unexplained hypotension before the diagnosis of panhypopituitarism was made. This exemplifies the need for endocrine testing in any of these patients since detection purely based on anamnesis will not be sufficient.

We propose a follow up schedule according to both the total radiation dosage delivered to the pituitary and the expected life expectancy that is currently incorporated in our standard protocols in the follow up postcranial radiation (Figure 2). For all patients treated by cranial radiotherapy an estimated dose to the pituitary should be calculated. If this estimated dose to the pituitary is > 20 Gy, depending on the physical condition

of the patient endocrine follow up is mandatory. How to proceed within the first years after radiation has to be tailored for each patient personally, since this is a very complex and fragile patient cohort. A follow up protocol as proposed needs to be validated prospectively, but this matter should be addressed since pituitary insufficiency is highly prevalent in adult patients treated with cranial radiotherapy with the pituitary within the radiation field. Replacement of endocrine deficits is associated with improvement of quality of life and prevents severe complications such as Addisonian crises. Therefore, we advocate that all patients with a life expectancy of more than 1 year should be referred and followed for the development of pituitary insufficiencies.

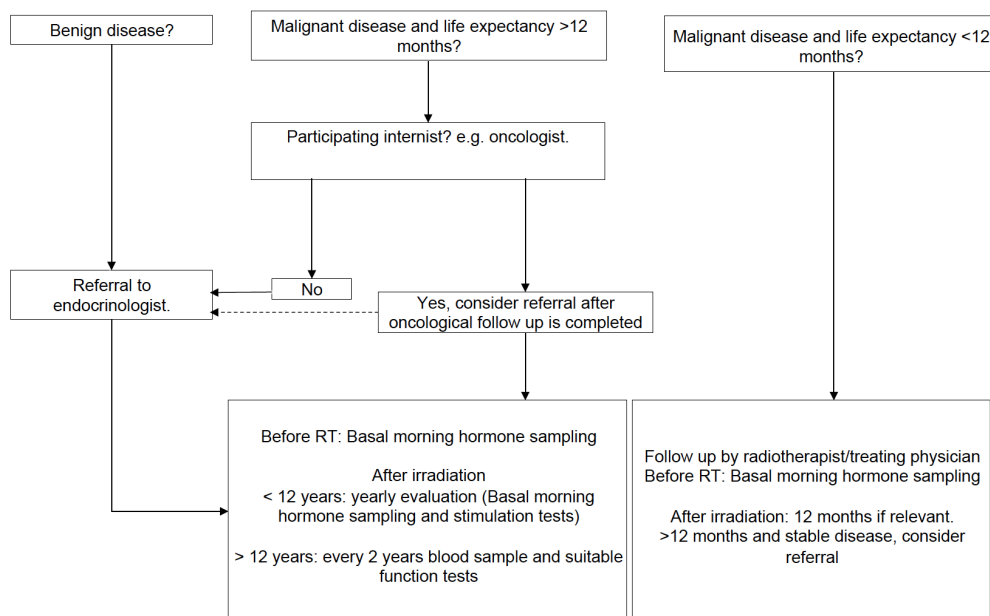


FIGURE 2.
Proposed follow up

ACKNOWLEDGEMENTS

Nieke E. Kokshoorn for contributing to the database.

REFERENCES

1. Darzy KH, Shalet SM. Hypopituitarism following radiotherapy. *Pituitary* 2009;12(1):40-50.
2. Brada M, Jankowska P. Radiotherapy for pituitary adenomas. *Endocrinol Metab Clin North Am* 2008, Mar;37(1):263-75, xi.
3. Biermasz NR, van Dulken H, Roelfsema F. Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. *J Clin Endocrinol Metab* 2000, Jul;85(7):2476-82.
4. Fernandez A, Brada M, Zabulienė L, Karavitaki N, Wass JA. Radiation-induced hypopituitarism. *Endocr Relat Cancer* 2009, Sep;16(3):733-72.
5. Alvarez JA, Scully RE, Miller TL, Armstrong FD, Constine LS, Friedman DL, Lipshultz SE. Long-term effects of treatments for childhood cancers. *Curr Opin Pediatr* 2007, Feb;19(1):23-31.
6. Constine LS, Woolf PD, Cann D, Mick G, McCormick K, Raubertas RF, Rubin P. Hypothalamic-pituitary dysfunction after radiation for brain tumors. *N Engl J Med* 1993, Jan 14;328(2):87-94.
7. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health conditions in adult survivors of childhood cancer. *N Engl J Med* 2006, Oct 12;355(15):1572-82.
8. Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK, Lange M, Poulsen HS, Müller J. Assessment of the hypothalamo-pituitary-adrenal axis in patients treated with radiotherapy and chemotherapy for childhood brain tumor. *J Clin Endocrinol Metab* 2003, Jul;88(7):3149-54.
9. Shalet SM, Beardwell CG, Jones PH, Pearson D. Growth hormone deficiency after treatment of acute leukaemia in children. *Arch Dis Child* 1976, Jul;51(7):489-93.
10. Gurney JG, Kadan-Lottick NS, Packer RJ, Neglia JP, Sklar CA, Punyko JA, et al. Endocrine and cardiovascular late effects among adult survivors of childhood brain tumors: Childhood cancer survivor study. *Cancer* 2003, Feb 1;97(3):663-73.
11. Chan AT, Leung SF, Ngan RK, Teo PM, Lau WH, Kwan WH, et al. Overall survival after concurrent cisplatin-radiotherapy compared with radiotherapy alone in locoregionally advanced nasopharyngeal carcinoma. *J Natl Cancer Inst* 2005, Apr 6;97(7):536-9.
12. Lee AW, Ng WT, Chan YH, Sze H, Chan C, Lam TH. The battle against nasopharyngeal cancer. *Radiother Oncol* 2012, Sep;104(3):272-8.
13. Patterson BC, Chen Y, Sklar CA, Neglia J, Yasui Y, Mertens A, Armstrong GT, et al. Growth hormone exposure as a risk factor for the development of subsequent neoplasms of the central nervous system: a report from the childhood cancer survivor study. *J Clin Endocrinol Metab*. 2014 Jun;99(6):2030-7.
14. Appelman-Dijkstra NM, Kokshoorn NE, Dekkers OM, Neelis KJ, Biermasz NR, Romijn JA, et al. Pituitary dysfunction in adult patients after cranial radiotherapy: Systematic review and meta-analysis. *J Clin Endocrinol Metab* 2011, Aug;96(8):2330-40.
15. Lam KS, Tse VK, Wang C, Yeung RT, Ho JH. Effects of cranial irradiation on hypothalamic-pituitary function--a 5-year longitudinal study in patients with nasopharyngeal carcinoma. *Q J Med* 1991, Feb;78(286):165-76.
16. Samaan NA, Schultz PN, Yang KP, Vassilopoulou-Sellin R, Maor MH, Cangir A, Goepfert H. Endocrine complications after radiotherapy for tumors of the head and neck. *J Lab Clin Med* 1987, Mar;109(3):364-72.
17. Madaschi S, Fiorino C, Losa M et al. Time course of hypothalamic-pituitary deficiency in adults receiving cranial radiotherapy for primary extrasellar brain tumors. *Radiother Oncol* 2011, Apr;99(1):23-8.
18. Darzy KH. Radiation-induced hypopituitarism. *Curr Opin Endocrinol Diabetes Obes* 2013, Aug;20(4):342-53.
19. Grinspoon SK, Biller BM. Clinical review 62: laboratory assessment of adrenal insufficiency. *J Clin Endocrinol Metab*. 1994;79:923-931.

20. Courtney CH, McAllister AS, McCance, Hadden, Leslie H, Sheridan B, Atkinson AB (2000) The insulin hypoglycaemia and overnight metyrapone tests in the assessment of the hypothalamic-pituitary-adrenal axis following pituitary surgery. *Clin Endocrinol (Oxf)* 53:309–312
21. Fiad TM, Kirby JM, Cunningham SK, McKenna TJ (1994) The overnight single-dose metyrapone test is a simple and reliable index of the hypothalamic-pituitary-adrenal axis. *Clin Endocrinol (Oxf)* 40:603–609
22. Staub JJ, Noelpp B, Girard J, Baumann JB, Graf S, Ratcliffe JG (1979) The short metyrapone test: comparison of the plasma ACTH response to metyrapone and insulin-induced hypoglycaemia. *Clin Endocrinol (Oxf)* 10:595–601
23. Kokshoorn NE, Romijn JA, Roelfsema F, Rambach AH, Smit JW, Biermasz NR, Pereira AM. The use of an early postoperative CRH test to assess adrenal function after transsphenoidal surgery for pituitary adenomas. *Pituitary*. 2012 Sep;15(3):436–44.
24. Corneli G, Di Somma C, Baldelli R, Rovere S, Gasco V, Croce CG, et al. The cut-off limits of the GH response to gh-releasing hormone-arginine test related to body mass index. *Eur J Endocrinol* 2005, Aug;153(2):257–64.
25. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008, Feb;18(1):1–16.
26. Cole TJ The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990, Jan;44(1):45–60.
27. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and igf-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 1998, Sep;50(3):166–76.
28. Agha A, Sherlock M, Brennan S, O'Connor SA, O'Sullivan E, Rogers B, et al. Hypothalamic-pituitary dysfunction after irradiation of nonpituitary brain tumors in adults. *J Clin Endocrinol Metab* 2005, Dec;90(12):6355–60.
29. Lin Z, Wu VW, Lin J, Feng H, Chen L. A longitudinal study on the radiation-induced thyroid gland changes after external beam radiotherapy of nasopharyngeal carcinoma. *Thyroid* 2011, Jan;21(1):19–23.
30. Littlely MD, Shalet SM, Beardwell CG, Robinson EL, Sutton ML. Radiation-induced hypopituitarism is dose-dependent. *Clin Endocrinol (Oxf)* 1989, Sep;31(3):363–73.
31. Belka C, Budach W, Kortmann RD, Bamberg M. Radiation induced CNS toxicity--molecular and cellular mechanisms. *Br J Cancer* 2001, Nov 2;85(9):1233–9.
32. Chiang CS, Hong JH, Stalder A, Sun JR, Withers HR, McBride WH. Delayed molecular responses to brain irradiation. *Int J Radiat Biol* 1997, Jul;72(1):45–53.
33. Kunkler I 2003 Effects of radiation on normal tissues. In Walter and Miller's Textbook of Radiotherapy, pp 296–306 Eds Bomford CK & Kunkler IH. London: Churchill Livingstone.
34. Kyrkanides S, Olschowka JA, Williams JP, Hansen JT, O'Banion MK. TNF alpha and il-1beta mediate intercellular adhesion molecule-1 induction via microglia-astrocyte interaction in CNS radiation injury. *J Neuroimmunol* 1999, Mar 1;95(1-2):95–106.
35. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, et al. Association between premature mortality and hypopituitarism. West midlands prospective hypopituitary study group. *Lancet* 2001, Feb 10;357(9254):425–31.
36. Klose M, Marina D, Hartoft-Nielsen ML, Klefter O, Gavan V, Hilsted L, et al. Central hypothyroidism and its replacement have a significant influence on cardiovascular risk factors in adult hypopituitary patients. *J Clin Endocrinol Metab* 2013, Sep;98(9):3802–10.
37. Persani L, Clinical review: Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. *J Clin Endocrinol Metab*. 2012 Sep;97(9):3068–78.

38. Rose SR, Lustig RH, Pitukcheewanont P, Broome DC, BurGHen GA, Li H, Hudson et al, Diagnosis of hidden central hypothyroidism in survivors of childhood cancer. *J Clin Endocrinol Metab* 1999 84:4472–4479
39. Xiaohong Wu, Mofa Gu, Guijuan Zhou, Xue Xu, Mengmeng Wu and Haiwei Huang ; Cognitive and neuropsychiatric impairment in cerebral radionecrosis patients after radiotherapy of nasopharyngeal carcinoma; *BMC Neurol.* 2014; 14: 10.
40. Greene-Schloesser D, Robbins ME. Radiation-induced cognitive impairment--from bench to bedside. *Neuro Oncol.* 2012 Sep;14 Suppl 4:iv37-44
41. Dützmänn S, Schatlo B, Lobrinus A, Murek M, Wostrack M, Weiss C et al. A multi-center retrospective analysis of treatment effects and quality of life in adult patients with cranial ependymomas *J Neurooncol.* 2013 Sep;114(3):319-27.
42. Doga M., Bonadonna S., Gola M, Mazziotti G., Giustina A. Growth hormone deficiency in the adult; *Pituitary* (2006) 9:305–311

Chapter 4

Long-term effects of recombinant human Growth Hormone (rGH) replacement in adults with Growth Hormone Deficiency (GHD): a systematic review

Natasha M. Appelman-Dijkstra, Kim M.J.A. Claessen, Ferdinand Roelfsema,
Alberto M. Pereira, Nienke R. Biermasz

Eur J Endocrinol. 2013 May 28;169(1):R1-14.

ABSTRACT

Background: The beneficial effects of recombinant human growth hormone (rGH) therapy in growth hormone deficient (GHD) adults are well-established in the short-term. However, data documenting the effects during prolonged follow-up are relatively scarce.

Objective: To evaluate the reported effects of rGH replacement (≥ 5 years) in GHD adults on biochemical and anthropometric parameters, quality of life (QoL), bone metabolism, muscle strength, serious adverse events (SAEs), and mortality.

Methods: We conducted a systematic literature search. Quality assessment of retrieved papers was performed using a quality assessment based on the modified STROBE statement.

Results: We included 23 prospective studies with a rGH treatment duration ranging from 5 to 15 years. Overall, beneficial effects were reported on QoL, body composition, lipid profile, carotid IMT and BMD. In contrast, the prevalence of the metabolic syndrome, glucose levels, BMI and muscle strength were not, or negatively influenced. Most of the studies were uncontrolled, lacked the presence of a control group (of non-treated GHD patients), and reported no data on lipid-lowering and anti-diabetic medication. Overall mortality was not increased.

Conclusion: RGH treatment in adult GHD patients is well-tolerated and positively affects QoL in the long-term. However, the metabolic and cardiovascular effects during long-term treatment are variable. The low numbers of long-term studies and studied patients and lack of control data hamper definite statements on the efficacy of prolonged treatment. Therefore, continuous monitoring of the effects of rGH replacement to enable an adequate risk-benefit analysis that may justify prolonged, potentially life-long, treatment is advisable.

INTRODUCTION

Growth hormone deficiency (GHD) in adults has been associated with an adverse metabolic profile and increased cardiovascular mortality by inducing abdominal obesity, hypercholesterolaemia and hypertriglyceridaemia (1;2). Consistent beneficial effects of treatment with recombinant human growth hormone (rGH) were reported on body composition and lipid profile, resulting in reduction of fat mass combined with an increase in lean body mass and a reduction in total cholesterol (TC) levels (3). In addition, favorable effects were reported on bone turnover, bone mineral density (BMD), muscle strength, cognitive function and quality of life (QoL) (3-5). Most of these effects have been documented in short-term studies, sometimes placebo-controlled, with a follow-up duration ranging from 6 to 18 months.

The beneficial effects of rGH therapy have been reported to sustain for at least 5 years on treatment; however data with a longer follow-up duration are scarce. With respect to cardiovascular disease, a direct improvement of several cardiovascular risk factors was noted within the first treatment year, which was reported to sustain during prolonged rGH treatment (6;7). In a small study, sustained improvement of lipid spectrum and diastolic blood pressure (DBP) was reported over 7 and 10 years, suggesting ongoing beneficial effects even beyond 5 years (6;7). However, it is presently unknown whether rGH therapy has favorable effects on the incidence of cardiovascular events, including cardiovascular death. With respect to bone, rGH replacement therapy increases bone remodeling, indicated by an increase of biochemical markers of both bone formation and resorption. This results in an initial decline in BMD, followed by a significant increase in BMD, reaching a plateau phase after 5 to 7 years of treatment (8-10).

In general, rGH replacement therapy in adults is regarded as safe. Recently, Van Bunderen *et al.* reported no differences in overall or malignancy-related mortality rate between GHD treated patients and the background population (11). However, an increased risk in cardiovascular death was observed in rGH-treated women. This increased cardiovascular mortality, however, is not easily explained. These recent findings accentuate the necessity to critically evaluate long-term efficacy and safety data.

We performed a systematic review of all available papers on long-term rGH replacement in adult GHD patients. We aimed to evaluate the effects of long-term rGH replacement in GHD adults on cardiovascular parameters, bone metabolism, muscle strength, QoL, mortality and adverse events.

MATERIALS AND METHODS

Search strategy and eligibility criteria

To identify studies that investigated the long-term effects of rGH replacement in GHD adults, we searched the following databases, up to September 2012 (Date of initial search 9th March, 2012): Medline, EMBASE, Web of Science, and the Cochrane Library, in collaboration with a trained clinical librarian (J.S.). We composed a search strategy focusing on long-term rGH treatment, GHD adults, bone metabolism, QoL, metabolic effects, muscle strength, mortality, and adverse events. Chronic stable replacement was arbitrarily defined as replacement for ≥ 5 years, since such a period will reflect a stable metabolic situation without confounding by ongoing metabolic changes induced by the start and titration of rGH replacement (3;12). We used all relevant keyword variations, including free text words. Duplicated articles were excluded. The complete search strategy is provided in *Appendix 1*. References of relevant articles were checked for additional articles. Only original articles were included.

Studies were eligible when all of the following criteria were met: (1) they investigated effects on biochemical, metabolic/anthropometric parameters, bone metabolism, QoL, muscle strength, mortality, or adverse events, (2) in adult patients with either childhood-onset (CO) or adult-onset (AO) GHD and (3) with at least 5 years of rGH treatment and (4) were written in English. Only human studies were evaluated.

Data extraction and analysis

Data extraction and eligibility were assessed by two independent investigators (K.M.J.A.C. and N.M.A.-D.). Inconsistencies were resolved by consensus. The following data were extracted: (1) study population (patient characteristics, population size, GHD assessment, control population); (2) exposure to rGH (duration of rGH treatment, rGH dose), (3) outcome (biochemical and anthropometric parameters, QoL, bone metabolism, muscle strength, mortality, serious adverse events (SAEs)) (4) potential confounders (age, sex, CO/AO).

Methodological quality assessment

The quality of each included paper was assessed in consensus by two independent reviewers (K.M.J.A.C. and N.M.A.-D.), using a standardized set of criteria based on the STROBE statement (*Appendix 2*), which was modified to cover the topic of our review (12). A maximum score of 10 points could be obtained. According to the total quality score, articles were considered as having poor (0-2), intermediate (3-5), good (6-8), or excellent methodological quality (9-10).

RESULTS

Identification and selection of the literature

From initial 841 potentially relevant studies identified, 803 were excluded on the basis of title and abstract. Thirty-eight papers were retrieved for detailed assessment: 9 were excluded because of rGH treatment duration <5 years, 2 studies described pediatric patients only, 1 study did not report original data and 3 studies did not address the long-term effects of rGH suppletion. Consequently, 23 studies were eligible for inclusion, several describing part of the same patient cohort (Göteborg) (4;6;9;10;13-16) (KIMS database, (5;17)) (*Figure 1 & Table 1*). Therefore, we also mention the specific study centre.

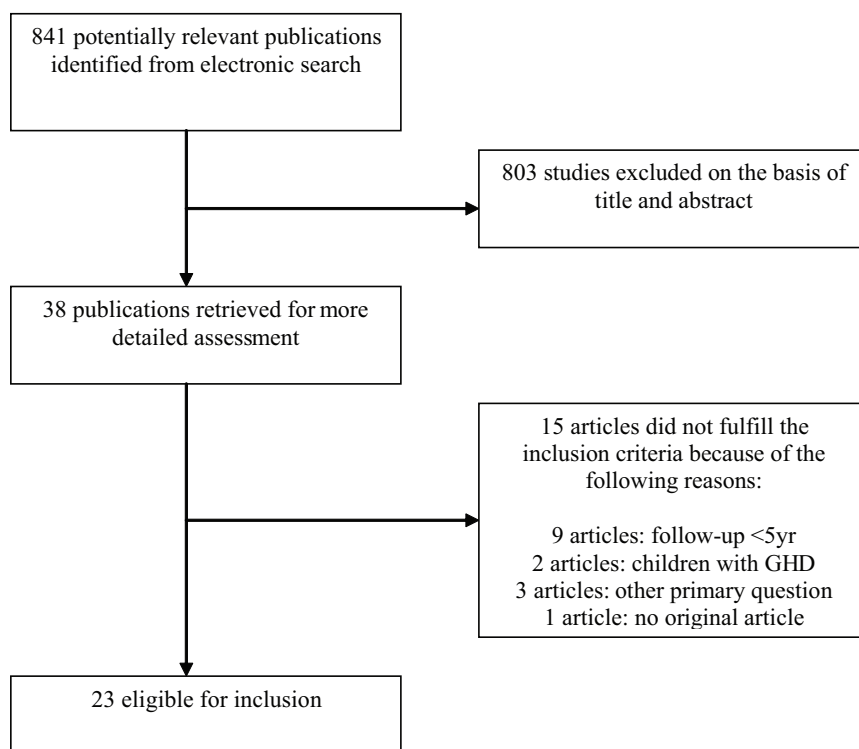


FIGURE 1.

Flow diagram of study selection and exclusion stages.

Quality assessment

According to the predefined quality criteria, no studies were classified as having poor quality. Two studies scored intermediate (18;19), 15 studies had a good quality (4-6;9;11;15;18;21-28) and 6 studies had an excellent methodological quality (7;10;13;15;16;20) (*Table 1*). Mean quality score was 7.3 (range 5.0-9.0), indicating that overall methodological quality was good. However, especially the items on potential bias, handling with missing data, reasons for non-participation at each study stage and the use of/handling with co-medication were not clearly described.

Methodology

All selected studies (n=23) were prospective follow-up studies, eleven of these had a controlled design. Healthy controls were included in six studies (5;13;19-22), of which two studies used National Registries. Four other studies included GHD patients with intermittent rGH treatment or untreated GHD patients as controls (11;22;24;25). Gibney *et al.* compared rGH-treated patients with both untreated GHD patients and healthy controls (23). Four reports involved multi-center studies (5;11;17;24). No randomized controlled trials were available.

Endocrine evaluation

Different established stimulation tests were used for the diagnosis of severe GHD. The insulin tolerance test (ITT) was used as standard test in 16 studies and the combined GHRH-arginine test, glucagon stimulation test or stimulation with clonidine alone, in case of contraindications for ITT. Eight studies did not specify which stimulation tests were used (5;11;14;17;22;24-26). Thirteen studies applied the generally used cut-off value for severe GHD of a peak GH <3µg/L (4;6;7;9;10;13;15;16;19;20;23;27;28), in seven studies no exact cut-off value was reported (5;11;14;17;22;24;26), and three studies used, respectively, <1.7µg/L (21), <5µg/L (18) and <10mU/L (25) as cut-off. In all studies, except for one (25), information on additional pituitary deficiencies was given. Most patients had multiple anterior deficiencies, especially gonadotrope deficiency, but thyreotrope and corticotrope deficiency was also frequently encountered.

Duration of rGH treatment

Mean treatment duration with rGH ranged from 5 years to 15 years: 8 studies reported 5-year effects, 2 studies had a 6-year follow-up (11;18), 3 studies investigated 7-year effects (7;21;28), 1 study investigated the 9-year effects (25), 8 studies reported 10-year effects (6;10;13-15;23;24;26), 1 study described 15 years of rGH supplementation (4). In one study, treatment duration varied among the different countries from 4-8 years (5).

TABLE 1. Included studies on the effects of long-term rhGH supplementation

Author (yr ^(ref))	Center	Total (M/F)	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Tested parameters					Quality score
							Glucose	Cardio vascular [#]	Anthropometric/ Body composition ^{##}	Muscle strength	Bone QoL	
Gibney (1999 ⁽²²⁾)		10 (7/3)	38 (21-48)	Untreated	0.025IU/kg/d	10	x	x	x	x	x	7.5
		NR		GHD (N=11)								
Chrisoulidou (2000 ⁽²⁰⁾)		12 (6/6)	52 ± 10	Healthy controls, age, sex & BMI matched (N=12)	NR	7	x	x	x			6.5
		10/2		rhGH (N=11)								
Clanget (2001 ⁽¹⁸⁾)		12 (8/4)	42.5 (24-61)	-	μ 2.4IU (0.80mg)	6				x		5.0
		9/3										
Götherström (2001 ⁽⁹⁾)**		118 (70/48)	49.3 (22-74)	-	0.3 (median)	5	x	x	x	x		7.0
Göteborg Svensson (2002 ⁽²⁶⁾)**	Göteborg	118/0										8.0
		11 (7/4)	48.0 (20-62)	Healthy controls, age & sex & BMI & WHR matched (N=11)	initial 1.10 end 0.61	10	x	x	x			
Göteborg		9/2										

TABLE 1. Included studies on the effects of long-term rhGH supplementation

Author (yr ^(a))	Center	Total (M/F)	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Tested parameters					Quality score	
							Glucose	Cardio vascular [#]	Anthropometric/ Body composition ^{##}	Muscle strength	Bone QoL		SAE/ Mortality
Gilchrist (2002 ^(2b))		61 (27/34)	37.9 ± 11.8	Continuous	0.25IU/kg/wk	9					x		6.0
		43/1		use, no use, intermittent.									
Svensson (2003 ^(7b))**	Göteborg	109 (61/48)	50.0 (22-74)	-	initial 0.88 end 0.46	5			x			x	9.0
		109/0											
Svensson (2004 ^(7b))**	Göteborg	289 (186/103)	47.6 ± 14.8	National Swedish Death Registry	N=80 11.9µg/ kg/d	5						x	7.5
		225/64			N=209 indiv dose titration based on IGF1								
Giavoli (2004 ^(9b))		20 (11/9)	44 ± 14	Healthy controls, age & sex & BMI & WHR matched (N=11)	initial 0.98 end 0.47	5		x		x			5.0
		20/0											
Götherström (2005 ^(10b))**	Göteborg	26 (12/14)	65.0 (61-74)	Reference population Göteborg	initial 0.73 end 0.36	5			x		x		9.0
		26/0											

TABLE 1. Included studies on the effects of long-term rhGH suppletion

Author (yr ^(ref))	Center	Total (M/F)	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Tested parameters					Quality score	
							Glucose	Cardio vascular [#]	Anthropometric/ Body composition ^{##}	Muscle strength	Bone		QoL
Koltowska- Hägström (2006 ⁽⁵⁾)	UK	UK 758	UK	General pop	NR	UK 7							
		(363/395)	48.5±12.6	UK N=892									
		704/54											
KIMS database													
	NL	247	NL	NL N=1038		NL 6							
		(123/124)	48.2±13.3										
		222/25											
	Spain	197	Spain	Spain N=868		Spain 4							
		(75/122)	45±11.1										
		176/21											
	Sweden	484	Sweden	Sweden		Sweden 8							
		(247/237)	51.4±13.0	N=1682									
		453/31											
Van der Klaauw (2006 ⁽⁷⁾)***		63 (30/33)	46.7 ± 14.3	-	initial 0.2	7	x	x	x				8.5
Leiden		52/11			end 0.5								

TABLE 1. Included studies on the effects of long-term rhGH suppletion

Author (yr ^(ref))	Center	Total (M/F)	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Tested parameters					Quality score
							Glucose	Cardio vascular [#]	Anthropometric/ Body composition ^{##}	Muscle strength	Bone QoL	
Van der Klaauw (2007 ⁽²⁹⁾)***	Leiden	50 (24/26)	45.2 ± 9.1	MORGEN cohort (N=1062)	initial 0.2 end 0.5	5	x	x	x			8.5
Götherström (2007 ⁽⁶⁰⁾)**		87 (52/35)	44.1 (22-74)	-	initial 0.98 end 0.47	7	x	x	x	x		8.5
Göteborg												
Cenci (2008 ⁽²⁸⁾)	Göteborg	14 (10/4)	(33-62)	-	initial 0.87 end 0.64	5	x	x				7.5
Götherström (2009 ⁽¹⁵⁾)**		109 (61/48)	50 (22-74)	-	initial 0.88 end 0.47	10			x			7.0
Göteborg												
Roemmler (2010 ⁽²⁴⁾)*	Göteborg	22 (14/8)	51.5 (33-75)	Untreated GHID (N=30)	0.3 (median)	10 (2-42)	x	x	x	x		5.5
Götherström (2010 ⁽⁶⁰⁾)**		24 (11/13)	65.2 ± 3.4	-	initial 0.72 end 0.37	10				x		8.5
Göteborg												

TABLE 1. Included studies on the effects of long-term rhGH suppletion

Author (yr ⁽ⁿ⁾) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Tested parameters					Quality score		
						Glucose	Cardio vascular [#]	Anthropometric/ Body composition ^{##}	Muscle strength	Bone		QoL	SAE/ Mortality
Spielhagen (2010 ⁽²⁵⁾)*****	440	F 36.7± 8.4 M 35.1±8.1	-	F initial 0.27 end 0.46 M	10	x					x		7.5
	(224/216)												
	301/139	(20-49)											
Van Bunderen (2011 ⁽¹⁰⁾)	2229	42.6 ±16.3	Untreated GHID (N=109)	μ 0.48 initial 0.31 end 0.4	5.7 (median)							x	7.0
	(1160/ 1069)		Partly treated GHID (N=356)										
	1718/511		General Dutch population Untreated GHID (N=1411)										
Elbornsson (2012 ⁽⁶⁾)**	126	49.4	-	initial 0.63 end 0.41	15			x			x		7.5
	(72/54)	(22-74)											
	126/0												
Göteborg													

TABLE 1. Included studies on the effects of long-term rhGH supplementation

Author (yr ^(e6))	Total (M/F)	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Tested parameters					Quality score			
						Glucose	Cardiovascular [#]	Anthropometric/ Body composition [#]	Muscle strength	Bone		QoL	SAE/ Mortality (points)	
Gaillard (2012 ²⁰)	13983	CO	General reference population	NR	4.9							x	6.5	
*****	(7174/6809)	AO												
KIMS database	(10767/3216)	48.8±13.0												
#	Cardiovascular: lipid metabolism, blood pressure, cardiac function, carotid IMT, or MS													
##	Anthropometric: BMI, weight, WHR													
*	3 patients treated <5yr													
**	Overlapping patient cohorts Götherström 2010, Götherström 2005, Götherström 2001, Götherström 2007, Götherström 2009, Elbornsson 2012, Svensson 2003, Svensson 2004 (4;6;9;10;14-17;24)													
***	Overlapping patient cohorts Van der Klaauw 2006 & Van der Klaauw 2007(7;27)													
****	Control group at baseline differed from controls at 10 year													
*****	Overlapping patient cohorts KIMS database Kolowska-Häggström 2006, Spielhagen 2010 Gaillard 2012 (5;22;26)													
rhGH, recombinant human growth hormone; GHD, growth hormone deficiency; AO, adult-onset GHD; CO, childhood-onset GHD; M, Male; F, Female; R, range; BF, body fat; LBM, lean body mass; ITT, insulin-tolerance test; GHRH, growth hormone releasing hormone; BP, blood pressure; MS, metabolic syndrome; WHR, waist-hip ratio; Carotid IMT, intima media thickness of carotid arteries; QoL, quality of life; UK, United Kingdom; NL, Netherlands; SAE, serious adverse event; NR, not reported.														

Dose of rGH treatment & IGF-1 levels

Fourteen studies (nine from Göteborg) calculated the GH starting dose based on body weight (4;6;9;10;13-16;19;21-23;25;27). Of the remaining nine studies, three studies used dose titration of rGH on an individual basis in the beginning of treatment, with the aim to reach IGF-1 levels within the normal age- and sex-related range, and adjusted the rGH dose accordingly (7;20;26). Six studies did not report their rGH dosage regimen (5;11;17;18;24;28). GH start dose ranged from 0.2mg/day (7;20) to 1.1mg/day (21). Twelve studies reported mean GH dose after dose titration (at 1 year) (6;9;10;13-17;19;21;24;27), ranging from 0.3mg/day (19) to 0.85mg/day (21) (median dose 0.54 mg/day).

Sixteen studies reported mean IGF-1 levels (4;7;9;10;13-16;18;19;21-24;26;28), and thirteen studies described IGF-1 SDS to evaluate treatment response (4;6;9;10;13-17;20-22;24) (*Appendix 3*). Based on available IGF-1 values, we can conclude that all patients were physiologically substituted at least after 5 to 7 years treatment with rGH, independently of the applied starting dosage regimen.

Outcome of long-term rGH replacement (Table 2)

Anthropometric parameters

Thirteen studies (N=1185) assessed the effects on body weight, body mass index (BMI) and/or waist-hip ratio (WHR) (4;6;7;9;10;13;14;20;21;24;26-28). rGH suppletion did not affect WHR or waist circumference (7;20;21;26-28), except for one study reporting an increased waist circumference (24). Seven studies reporting BMI found a significant increase during rGH suppletion ≥ 5 years, the other six studies reported no change (4;6;7;9;13;14;16;19-21;24;27;28). Two of the eight studies reporting body weight found an increase in weight during rGH suppletion; the other studies reported no change (4;10;13;14;16;21;27;28).

In conclusion, results on long-term effects on BMI appear to be inconclusive with seven studies reporting an increase, and six reporting no change. In addition, there is little evidence for an increase in body weight.

Body composition

Eleven studies (N=538) (6;9;13;14;16;19;21;23;26-28) assessed the effects of rGH suppletion on body composition using dual-energy X-ray absorptiometry (DEXA) scan (6;9;13;14;16;26;28), bioelectrical impedance analysis (BIA) (19;21) or a helical CT scan (27). Gibney *et al.* measured body composition with radioisotope potassium (23). The majority of studies (n=9) reported consistent effects with an increase in lean body mass (LBM) and a decrease in total body fat (BF) (6;9;13;14;16;19;21;23;28). One study

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Nr of studies						
Anthropometric parameters						
N=13	Chrisoulidou (2000(21)) Göteborg	12 (6/6) 10/2	52 ± 10	NR	7	↑ weight & BMI, = WHR
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	0.3 (median)	5	↑ BMI
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	10	= weight & WHR & BMI
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	= weight & BMI
	Giavoli (2004(28)) Göteborg	20 (11/9) 20/0	44 ± 14	initial 0.98 end 0.47	5	= BMI
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	= weight & BMI
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	= WC & WHR ↑ BMI
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= waist circ & WHR ↑ BMI
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	7	= weight, ↑ BMI
	Cenci (2008(20)) Göteborg	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	= weight & WC & WHR & BMI
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R(22-74)	initial 0.88 end 0.47	10	= weight & BMI
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	↑ BMI & WC & HC = WHR
	Elbornsson (2012(4))** Göteborg	126 (72/54) 126/0	49.4 (R 22-74)	initial 0.63 end 0.41	15	↑ weight & BMI

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Nr of studies Body composition	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	= BF ↑ LBM ↑ thigh muscle area ↓ BF ↑ LBM
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↑ LBM ↓ skinfold thickness (subscap)
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓ BF ↑ LBM
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	↓ BF ↑ LBM
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↓ BF ↑ LBM
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 3-8 µg/kg/d end 0.3	5	↓ BF ↑ LBM
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↓ BF ↑ LBM
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓ BF ↑ LBM
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ visceral fat
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R(22-74)	initial 0.88 end 0.47	10	= BF ↑ LBM
	Roemmeler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BF = LBM

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion			
Nr of studies Lipid metabolism	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	TC =	HDL ↑	LDL ↓	TG =
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓	=	↓	=
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓	↑	↓	↓
	Svensson (2002(26))***** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	=	↑	↓	=
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	↓	↑	↓	=
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	NR	↑	NR	=
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓	↑	↓	NR
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	=	=	=	=
	Roemmier (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	initial 1.10 end 0.61	10 (2-42)	=	=	=	=
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	=	NR	=	NR

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Nr of studies						
Glucose	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025 IU/kg/d	10	= glucose, = insulin
N=11						
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	initial 0.98 end 0.48	7	= NEFA, = glucose, = insulin
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	0.3 (median)	5	↑ glucose, = insulin, ↓ HbA1c
	Svensson (2002(26))**** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 3-8 µg/kg/d end 0.3	7	= glucose & insulin sensitivity
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 0.98 end 0.47	5	↑ glucose & insulin = HOMA-IR & QUICKI
	Van der Klaauw (2006(7))*** Leiden	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.2 end 0.5	7	= glucose
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= glucose
	Götherström (2007(6))** Göteborg	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	10	↑ glucose, ↓ HbA1c
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.6	5	= glucose & insulin & HOMA-IR
	Roemmier (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	initial 1.10 end 0.61	10 (2-42)	= all glucose parameters (including glucose, insulin, HbA1c, insulin sensitivity, insulin resistance, β-cell-function, HOMA)
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	= glucose

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Nr of studies Cardiovascular parameters Including Metabolic syndrome N=5	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025 IU/kg/d	10	= SBP & DBP = LVM & ESM & EDM ↓ carotid IMT
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	5	= SBP & DBP = IVRT, = LVM = HR, ↓ resting DBP, = SBP = exercise time = SBP & DBP
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	= SBP & DBP ↑ MS prevalence
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= SBP & DBP ↑ MS prevalence
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ carotid IMT
Neuromuscular function N=5	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/ kg/d	10	= muscle strength
N=5	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↑ knee flexor & extensor strength ↑ hand grip strength
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↑ knee flexor & extensor strength ↑ peak hand grip strength
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R(22-74)	initial 0.88 end 0.47	10	↑ knee flexor & extensor strength ↑ hand grip strength
	Götherström (2010(16))** Göteborg	24 (11/13) 24/0	65.2 ± 3.4	initial 0.72 end 0.37	10	↑ knee flexor strength = hand grip strength = muscle endurance

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Nr of studies Bone N=5	Clanget (2001(19))	12 (8/4) 9/3	42.5 (R 24-61)	μ 2.4IU (0.80mg)	6	↑ BMC & BMD (lumbar spine, trochanter, = femur neck)
	Götherström (2001(9))** Göteborg	118 (70/48) 118	49.3 (R 22-74)	initial 0.98 end 0.48	5	↑ BMC & BMD = osteocalcin & Ca2+ & PTH
	Götherström (2007(10))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↑ BMC & BMD (total body, lumbar spine, prox femur) ↑ osteocalcin & Ca2+ = BMD
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	
	Elbornsson (2012(4))** Göteborg	126 (72/54) 126/0	49.4 (R 22-74)	initial 0.63 end 0.41	15	↑ BMC & BMD (total body, lumbar spine, plateau) ↑ BMC, = BMD (prox femur, peak at 7yr, thereafter ↓) Fractures: 1 hip, 1 symp VF ↑ overall psychological well-being, energy, emotional reaction
Quality of life N=4	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	
	Gilchrist (2002(23))	61 (27/34) 43/1	37.9 ± 11.8	0.25IU/kg/wk	9	NHP: ↑ energy levels, = sleep & emotional reaction & social isolation & physical mobility & pain PGWB: ↑ vitality score

TABLE 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters	Author (yr(ref)) Center	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Nr of studies	Koltowska-Hägström (2006(5))***** KIMS database	UK 758 (363/395) 704/54	UK 48.5±12.6	NR	UK 7	QoL-AGHDA: ↑ in 1 st yr, after that regression to country- specific mean (normalization)
		NL 247 (123/124) 222/25	NL 48.2±13.3		NL 6	
		Spain 197 (75/122) 176/21	Spain 45±11.1		Spain 4	
		Sweden 484 (247/237) 453/31	Sweden 51.4±13.0		Sweden 8	
		Sweden 440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	
Mortality/SAE N=3	Spielhagen (2011(25))***** KIMS database					QoL-AGHDA: ↑ in 1 st yr, sustained during longer follow- up
	Svensson (2004(27))** Göteborg	289 (186/103) 225/64	47.6 ± 14.8	N=80 11.9µg/kg/d N=209 indiv dose titration based on IGF1 µ 0.48	5	= overall mortality & malignancy rate ↓ MI rate, ↑ CVA rate
	Van Bunderen (2011(11))	2229 (1160/1069) 1718/511	42.6 ± 16.3		5.7 (median)	= mortality compared to background pop (after exclusion high-risk) = malignancy mortality ↑ CVD mortality in ♀ ↑ overall mortality in ♀ (moderate)
	Gaillard(2012(18))***** KIMS database	13983 (7174/6809) 10767/3216	CO 26.9 ± 9.9 AO 48.8 ± 13.0	NR	4.9	= malignancy & CVD mortality ↑ CVA & infection mortality ↑ progression primary tumour & endocrine complications

- * 3 patients treated <5yr
- ** Overlapping patient cohorts Götherström 2010, Götherström 2005, Götherström 2001, Götherström 2007, Götherström 2009, Götherström 2012, Svensson 2003, Svensson 2004
- *** Overlapping patient cohorts Van der Klaauw 2006 & Van der Klaauw 2007
- **** Control group at baseline differed from controls at 10 year
- ***** Overlapping patient cohorts KIMS database Koltowska-Häggström 2006, Gaillard 2012, Spielhagen 2011
- rhGH, recombinant human growth hormone; GHD, growth hormone deficiency; ΔQ, adult-onset GHD; CO, childhood-onset GHD; R, range; BF, body fat; LBM, lean body mass; ITT, insulin-tolerance test; GHRH, growth hormone releasing hormone; BMD, bone mineral density; BMC, bone mineral content; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BP, blood pressure; MS, metabolic syndrome; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin check index, Carotid IMT, intima media thickness of carotid arteries; QoL, quality of life; NHP, Nottingham Health Profile; PGWB, psychological general well-being schedule; QoL-AGHDA, quality of life-assessment of GH deficiency in adults; LVM, left ventricular mass; ESM, end systolic diameter; EDM, end diastolic diameter; Ca2+, calcium; NEFA, nonesterified fatty acids; Lp(a), lipoprotein (a); APU, apparent phalangeal ultrasound transmission velocity; CVD, cardiovascular disease; IVRT, isovolumic relaxation time; LVM, left ventricular mass; VF, vertebral fracture; UK, United Kingdom; NL, Netherlands; MI, myocardial infarction; CVA, cerebrovascular attack; SAE, serious adverse event; NR, not reported.

could not detect any effect of rGH on body composition (26). Cenci *et al.* reported only a decrease in visceral fat (27).

In conclusion, rGH suppletion had favourable effects on body composition, with an increase in LBM and a decrease in total BF. Several methods are available to evaluate body composition, which may account for differences between studies. These differences might have influenced the results significantly. In literature, DEXA is reported to be the best currently available technique for measuring body composition (29); however, in this respect, it has to be noted that DEXA cannot distinguish between body cell mass (BCM) and water. Therefore, the LBM data may not be accurate, since rGH replacement is associated with an increase in the intracellular water component (30-33). However, also when using other modalities to evaluate body composition, positive long-term effects of rGH replacement were found.

Lipid metabolism

Ten studies (N=827) assessed the effects of rGH suppletion on fasting plasma lipid profiles (6;7;9;20;21;23;24;26-28). Three studies did not find any effect of rGH therapy on plasma lipids (24;26;27). The seven remaining studies reported generally similar results, *i.e.* reduction of total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol, an increase in high-density lipoprotein (HDL) cholesterol and no effect on triglycerides (6;7;9;20;21;23;28). In 6 patients, new-onset hyperlipidaemia was reported (Göteborg). However, only four studies reported the number of patients on lipid-lowering medication, especially statins, (6;7;20;26) but without excluding these patients from analysis.

In summary, there are ongoing beneficial effects of rGH suppletion on plasma lipids. However, a strong limitation is the lack of data concerning lipid-lowering treatment, which has a considerable influence on the outcome parameter. Due to the lack of information on lipid-lowering drugs and the overlap in patient groups, meta-analysis was not an option.

Glucose metabolism

Eleven studies (N=847) assessed the effects of rGH suppletion on glucose and insulin levels (6;7;9;19-21;23;24;26-28). Seven studies showed no effect on glucose (7;20;23;24;26-28), three an increase (6;9;19) and one study found a transient increase of glucose levels only during the first year of rGH therapy (21). Insulin levels increased only in one study (19). HbA1c was evaluated in three studies, two of which observed a decrease (6;9); the other found no change (26). Three studies investigated insulin resistance by the homeostasis model assessment (HOMA) (19;26;27), reporting no significant effects of rGH suppletion. Insulin sensitivity was not affected in two studies

(21;26), while another study reported a transient decrease, using the quantitative insulin check index (QUICKI), showing a trend to return towards basal values afterwards (19). In at least ten patients, new-onset diabetes mellitus type 2 (DM2) was diagnosed (4 Göteborg, 6 KIMS (24)). Five studies reported the number of patients starting anti-diabetic medication during follow-up (6;9;21;26;27). Only one study excluded diabetic patients from analysis (9), the other studies did not report the handling with anti-diabetic medication in their statistical analysis.

In conclusion, there is moderate evidence for an increase in mean glucose levels after chronic rGH treatment; however, insulin sensitivity appeared not to be affected in the long-term. Due to the lack of information on anti-diabetic medication and the overlap in patient cohorts, we were not able to perform a meta-analysis on this parameter.

Cardiovascular parameters

Four studies (N=135) investigated the effects of rGH suppletion on blood pressure (7;9;20;23;28). No effect was found on systolic blood pressure (SBP); one study reported a decrease in resting diastolic blood pressure (DBP) (28). Only two studies described the number of patients on antihypertensive medication (7;20), without excluding them from analysis. Prevalence of the metabolic syndrome (MS) was assessed in one study, reporting a strong increase when compared to healthy controls, despite 5 years of rGH treatment (38.0% *vs* 15.7%) (20).

Two studies (N=22) evaluated the long-term effects on cardiac function (23;28) using echocardiography; Chrisoulidou *et al.* also performed an exercise test (28). Left ventricular mass (LVM) was not affected. In addition, no effect on end diastolic or systolic diameter (23) nor on isovolumetric relaxation time (IVRT) or LVM, was demonstrated (23;28). Two studies (N=24) evaluated carotid intima media thickness (IMT), reporting a decrease in IMT up to 18% after long-term rGH therapy (23;27).

In conclusion, no effect on SBP or LVM was reported. In addition, conclusions on the prevalence of MS cannot be made, since this parameter was only evaluated in one study. Long-term rGH suppletion had positive effects on IMT.

Muscle strength

Five studies (N=278, all AO-GHD) investigated the effects of rGH suppletion on muscle strength (13-16;23). In general, 5 years of rGH improved knee flexor and extensor strength and hand-grip strength. However, these effects were not sustained after 10 years. One study also investigated muscle endurance; no significant effects of rGH suppletion were demonstrated (15). In addition, rGH suppletion did not affect muscle strength in the elbow, shoulder or hip (23). Compared with population-based

controls, rGH was found to be protective to the normal age-related decline in muscle performance and neuromuscular function (13;14).

In general, rGH supplementation improved muscle strength during the first 5 years of treatment; however, these effects were not sustained after prolonged follow-up. Since almost all studies described one patient group, we were not able to perform a meta-analysis on this parameter.

Bone parameters

Four studies (N=343) evaluated the long-term effects of rGH on bone (4;9;10;18). After an initial decline, all studies reported an increase in both BMD and BMC within the first 5 years of treatment. After 5 years, all studies reported a plateau phase, which lasted for the entire study period (*i.e.* sustained effects) (4;9;10;18). Only one study investigated (symptomatic) fracture prevalence, reporting 1 hip fracture and 1 symptomatic vertebral fracture (4). None of the studies described the use of calcium or Vitamin D supplementation, or the use of bisphosphonates, selective estrogen receptor modulator (SERM), teriparatide or strontium ranelate. Again, most patients were from the same study center, prohibiting the performance of a meta-analysis.

QoL

Two large cohort studies (both KIMS database, N=1686 and N=440, respectively) and two smaller studies (N=71) addressed the effects on QoL and neuropsychological well-being, by QoL-Assessment of GHD in Adults (QoL-AGHDA), Nottingham Health Profile (NHP), and Psychological General Well-Being (PGWB) questionnaires (5;23-25). Within the first year of treatment, a direct beneficial response on well-being was noticed, as assessed by QoL-AGHDA, with marginal changes after the dose-titrating phase (5;24). Improvement was related to the degree of QoL impairment at baseline (5). Scores on NHP and PGWB improved in the long-term, especially with respect to energy level and emotional reaction (23;25).

In conclusion, QoL seemed to improve during long-term rGH replacement, but positive effects on QoL were especially seen within the first year of treatment. Since studies are scarce and none of them had a placebo-controlled randomized controlled design, which is of paramount importance for the interpretation of QoL, no firm conclusions can be drawn on the effects of long-term rGH replacement on QoL.

Effects of rGH on mortality

Three cohort studies (N=16501) reported data on mortality after long-term rGH supplementation (11;17;22), of which one study reports KIMS data (N=13983). In Gaillard *et*

al., overall mortality rate was moderately increased, especially in females, young attained age, patients with aggressive tumours and lower IGF-1 SDS during therapy (17). The other studies did not report differences in overall mortality or malignancy-related death. In two studies (17;22), the rate of cerebrovascular accidents (CVAs) was increased, but the incidence of myocardial infarction was decreased (risk ratio (RR) 0.27, 95%CI 0.03-0.99) when compared to the background population (22). In addition, rates of death due to infections (standardized mortality ratio (SMR) 4.97, 95%CI 3.98-6.14), primary tumour progression (SMR=6.96, 95%CI 4.45-10.3) and endocrine complications (SMR=2.79, 95%CI 1.30-5.23) were increased (17). Van Bunderen *et al.* (N=2229; median rGH treatment of 5.7 yr) reported an increase in cardiovascular-related deaths in GHD-treated women (SMR 2.36) (11). This was not supported by the other long-term studies (17;22).

In summary, there is evidence for a moderate increased mortality rate despite long-term rGH replacement, especially in females. Differences in pituitary disease, hormone replacement- and radiation therapies may account for differences between studies. However, since long-term studies on mortality are scarce, at this moment, no firm conclusions on the safety of long-term rGH replacement can be drawn.

Serious adverse events (SAEs)

Twelve studies reported SAEs during long-term rGH treatment (n=9 from Göteborg) (4;6;7;9;13-16;21;24;28). Since several studies described the same patient cohort, the total number of SAEs could not be extracted.

Malignancies

A total of 6 malignancy cases were reported in the GHD cohort of Göteborg: colonic carcinoma (N=1), prostate carcinoma (N=1), chronic lymphatic leukaemia (N=1), renal carcinoma (N=1), bladder carcinoma (N=1), and pulmonary carcinoma (N=1). In addition, 12 cases of *de novo* malignancies, not further specified, were reported (7;24).

Cardiovascular events

Three cases of myocardial infarction (Göteborg, Van der Klaauw *et al.* (7)) were reported, of which two with fatal outcome. Five patients suffered from a CVA (Göteborg, Van der Klaauw *et al.* (7)), resulting in death in four cases, and one patient died from a subarachnoid hemorrhage (Göteborg). In addition, angina pectoris was reported (N=3) (Göteborg), and 1 patient died from a pulmonary embolism (Göteborg). In at least ten patients, new-onset DM2 was diagnosed (4 Göteborg, 6 KIMS (24)). In, respectively, 6

and 4 patients new-onset hyperlipidaemia and hypertension were reported (Göteborg, (28)).

Other SAEs

Two studies reported primary tumour growth (N=11), either recurrence of the pituitary or central nervous system tumour (7;24). In conclusion, half of the studies reported SAEs during rGH therapy. Due to the lack of a control group including non-treated GHD patients, no firm conclusions can be drawn.

DISCUSSION

The short-term effects of rGH treatment are well-established, showing favourable effects of rGH therapy within the first 3 years on body composition, lipid profile, bone turnover, BMD, muscle strength, cognitive function, and QoL. This is the first systematic review focusing on the long-term effects of rGH treatment (beyond ≥ 5 years of treatment). Overall, long-term rGH therapy beneficially affected QoL and BMD. In addition, sustained beneficial effects were reported on lipid spectrum and on carotid IMT after 10 years of treatment. Consistent effects on body composition were reported with an increased LBM and a decreased total BF. Muscle strength increased after 5 years of rGH suppletion, but these effects were not sustained during longer follow-up. Negative effects were reported on mean glucose levels; however, insulin sensitivity appeared not to be affected by long-term rGH treatment.

The effects of rGH replacement on bone turnover, and thus on BMD, are dynamic over time. Initially, BMD decreases due to increased bone resorption and formation, followed by an increase within the first years of treatment, finally resulting in a plateau phase. The five studies addressing this topic, showed a significant increase within the first 5 years of treatment. Thereafter, BMD was conserved but did not further increase (4;9;10). Only one study described the number of symptomatic fractures (4), however, without radiographic assessment of the vertebral column. As a consequence, the reported fracture rate is most probably underestimated, since 21% of the fractures in patients with osteopenia or osteoporosis are asymptomatic (34). Fracture incidence was also studied by Holmer *et al.* (35), but a considerable proportion of patients were treated < 5 years. In CO-GHD women, not in AO-GHD women or CO-GHD men, fracture risk was doubled, and in men with AO-GHD fracture incidence was even decreased (35). Further research has to establish the efficacy of rGH treatment with respect to fracture risk.

Presently, in clinical practice, QoL is the most frequently addressed outcome parameter, but in the long-term, QoL was studied only in four studies (5;23-25). The two largest

studies (KIMS database) showed improvement within the first year of treatment, with thereafter steady progress towards the country-specific mean (*i.e.* normalization) (5;24). The other two studies found ongoing beneficial effects on energy level and emotional reaction (23;25). Unfortunately, none of these studies had a placebo-controlled randomized controlled design. Thus, based on current literature, long-term effects on QoL are inconclusive, and therefore, in daily clinical practice, QoL should be individually evaluated in each patient.

With respect to plasma lipids, seven out of ten studies investigating the long-term effects of rGH replacement on the lipid profile report ongoing beneficial effects, with a reduction of TC and LDL-C levels, an increase in HDL-C and no effect on triglycerides. However, in this respect, a strong limitation is the lack of data concerning lipid-lowering treatment, prohibiting to draw firm conclusions on this outcome parameter. When comparing these long-term results to short-term studies on plasma lipids, it has been noted that there were also several (randomized controlled) short-term studies reporting no change or minimal effects on lipid levels, and that even these effects were limited to men (36;37). Overall, however, as shown in a meta-analysis of Maison *et al.* investigating 37 placebo-controlled randomized controlled studies (follow-up up to 18 months), rGH replacement was shown to improve LDL-C and TC levels in the short-term (3).

Concerning the safety of rGH suppletion, in children, long-term safety data have shown conflicting data, raising the question whether long-term use of rGH is safe. Several large studies did not find an increased mortality in pediatric GHD patients after rGH replacement, especially not due to new-onset malignancies or recurrence of central nervous system tumors. Cause of death was assessed as unrelated to rGH replacement in the majority of patients (38-42). In contrast, a recent study of Carel *et al.* showed an increased incidence in bone tumor-related mortality and mortality due to cardiovascular diseases in a large cohort of French children, especially in those with highest GH doses ($>50\mu\text{g/kg/day}$) (43). Cancer mortality was also increased in a large pediatric UK cohort, especially in high-risk patients (44). Moreover, Bell *et al.* reported an increase in the occurrence of second tumors in children with a prior history of malignancy, especially in case of prior exposure to radiotherapy (38). These discrepant results could be possibly explained by the different dosage regime, with higher GH doses in the French study. A recent meta-analysis underlines this hypothesis, establishing that both low and high IGF-1 levels are associated with increased mortality (hazard ratio (HR) 1.18, 95%CI 1.04-1.34) (45). Adult studies did not show an increased mortality or malignancy rate (11;22), except for one study showing moderate increase in overall mortality in females (17). However, the studies do not represent unselected patients, since initiation of rGH replacement is not likely in patients with an a priori increased cancer risk, thus creating a clear selection bias. With respect to cardiovascular outcomes, an increase in cardiovascular deaths and number of CVAs was reported in women, whereas the myocardial infarction rate decreased (11;17;22). The increased

incidence in cardiovascular deaths in women might partly be attributed to the more atypical presentation of cardiovascular diseases in women (46). Other factors such as inadequately substituted hypogonadism causing preterm menopause or hypocortisolism may also have influenced this risk. In this respect, it has to be noted that available data suggest that both mortality risk and the causes for mortality are related in both patients receiving and those not receiving rGH replacement. However, it is of paramount importance to accentuate that all observed increased mortality rates reported to date have been associated only with the presence of hypopituitarism *per se*, but that it is still uncertain if, and to what extent, this can be attributed to GHD.

We assessed the methodological quality of all studies using a modified STROBE list (12), classifying 21 of the 23 included studies as having a good or excellent methodological quality. Several quality criteria were not clearly described, specifically, information on potential bias, handling with missing data and reasons for dropout was lacking in most studies. In addition, the use of co-medication, especially lipid-lowering and anti-diabetic drugs, was not clearly reported. It is of importance to emphasize this limitation, because the effects of rGH therapy on lipid profile, glucose levels and other cardiovascular parameters could not be interpreted without this information. In addition, no randomized controlled trials (RCT) were identified with a follow-up duration >5 years and only a limited number of centers have reported their data, with half of the long-term studies describing (part of) the same patient cohort (Göteborg). Other studies were relatively small and might have been, therefore, underpowered to allow firm conclusions. Due to these limitations, we were not able to perform a meta-analysis.

Another limitation is the report of outcomes only for patients that had completed 5 or 10-years of follow-up, instead of describing the original cohort. This may introduce a significant selection bias, since patients discontinued rGH therapy for specific reasons, and, therefore, are likely to differ from patients with complete follow-up. Furthermore, a major limitation of most studies is the lack of a control group and thereby the lack of control for ageing. This limitation is important to notice when interpreting the long-term effects of rGH replacement on for example body weight, BMI, blood pressure, lipid profile, body composition and BMD (47-49). Consequently, it is very difficult to discriminate between effects as a consequence of natural ageing processes and effects by rGH replacement *per se*. Firm conclusions on the effects of rGH *per se* can therefore not be drawn from present results. Another feature that needs to be addressed is the change in patient characteristics and approach to rGH suppletion over time, which may represent an important confounder for any analysis (50). This emphasizes the need of ongoing monitoring during rGH treatment.

In summary, available literature on long-term rGH replacement in adult GHD patients shows inconsistent results with respect to its expected beneficial effects, in the presence

of several drawbacks to enable a definite interpretation. First, long-term studies were generally uncontrolled and lacked a control group (of non-treated GHD patients) enabling adjustment for subjective changes or changes due to ageing. Second, only a limited number of centers have reported their data, resulting in a low number of evaluable patients with a follow-up duration of ≥ 5 years of rGH replacement with half of the long-term studies describing (part of) the same patient cohort. Especially the course of QoL during ongoing therapy is unestablished. With respect to the metabolic profile, rGH therapy has shown prolonged, beneficial effects on the long-term for body composition, lipid profile, carotid IMT and BMD, but overall cardiovascular risk, as assessed by the prevalence of the metabolic syndrome, glucose levels, BMI appeared not to be influenced or were even negatively affected. Therefore, the benefit of long-term rGH treatment should be a matter of ongoing research to enable adequate risk-benefit analyses and, in clinical daily practice, the benefits of rGH should be considered carefully in each patient.

ACKNOWLEDGEMENTS

The authors thank J.W. Schoones, medical librarian of the Leiden University Medical Center, for his assistance in performing the literature search.

REFERENCE LIST

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990; 336(8710):285-288.
2. Tomlinson JW, Holden N, Hills RK et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet* 2001; 357(9254):425-431.
3. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004; 89(5):2192-2199.
4. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012; 166(5):787-795.
5. Koltowska-Hagstrom M, Mattsson AF, Monson JP et al. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? *Eur J Endocrinol* 2006; 155(1):109-119.
6. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. *J Clin Endocrinol Metab* 2007; 92(4):1442-1445.
7. van der Klaauw AA, Romijn JA, Biermasz NR et al. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. *Eur J Endocrinol* 2006; 155(5):701-708.
8. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rGH) alone and rGH combined with alendronate in GH-deficient adults: a seven-year follow-up study. *Clin Endocrinol (Oxf)* 2004; 60(5):568-575.
9. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001; 86(10):4657-4665.
10. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *Eur J Endocrinol* 2007; 156(1):55-64.
11. van Bunderen CC, van N, I, Arwert LI et al. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. *J Clin Endocrinol Metab* 2011; 96(10):3151-3159.
12. von EE, Altman DG, Egger M, Pocock SJ, Gotsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; 61(4):344-349.
13. Gotherstrom G, Bengtsson BA, Sunnerhagen KS, Johannsson G, Svensson J. The effects of five-year growth hormone replacement therapy on muscle strength in elderly hypopituitary patients. *Clin Endocrinol (Oxf)* 2005; 62(1):105-113.
14. Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Ten years of growth hormone (GH) replacement normalizes muscle strength in GH-deficient adults. *J Clin Endocrinol Metab* 2009; 94(3):809-816.
15. Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Muscle strength in elderly adults with GH deficiency after 10 years of GH replacement. *Eur J Endocrinol* 2010; 163(2):207-215.
16. Svensson J, Sunnerhagen KS, Johannsson G. Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. *J Clin Endocrinol Metab* 2003; 88(5):2061-2069.

17. Gaillard RC, Mattsson AF, Akerblad AC et al. Overall and cause-specific mortality in GH-deficient adults on GH replacement. *Eur J Endocrinol* 2012; 166(6):1069-1077.
18. Clanget C, Seck T, Hinke V, Wuster C, Ziegler R, Pfeilschifter J. Effects of 6 years of growth hormone (GH) treatment on bone mineral density in GH-deficient adults. *Clin Endocrinol (Oxf)* 2001; 55(1):93-99.
19. Giavoli C, Porretti S, Ronchi CL et al. Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. *Metabolism* 2004; 53(6):740-743.
20. van der Klaauw AA, Biermasz NR, Feskens EJ et al. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. *Eur J Endocrinol* 2007; 156(4):455-462.
21. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO. Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. *J Clin Endocrinol Metab* 2002; 87(5):2121-2127.
22. Svensson J, Bengtsson BA, Rosen T, Oden A, Johannsson G. Malignant disease and cardiovascular morbidity in hypopituitary adults with or without growth hormone replacement therapy. *J Clin Endocrinol Metab* 2004; 89(7):3306-3312.
23. Gibney J, Wallace JD, Spinks T et al. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. *J Clin Endocrinol Metab* 1999; 84(8):2596-2602.
24. Spielhagen C, Schwahn C, Moller K et al. The benefit of long-term growth hormone (GH) replacement therapy in hypopituitary adults with GH deficiency: results of the German KIMS database. *Growth Horm IGF Res* 2011; 21(1):1-10.
25. Gilchrist FJ, Murray RD, Shalet SM. The effect of long-term untreated growth hormone deficiency (GHD) and 9 years of GH replacement on the quality of life (QoL) of GH-deficient adults. *Clin Endocrinol (Oxf)* 2002; 57(3):363-370.
26. Roemmler J, Kuenkler M, Schneider HJ, Dieterle C, Schopohl J. Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement. *Metabolism* 2010; 59(3):350-358.
27. Cenci MC, Conceicao FL, Soares DV et al. Impact of 5 years of growth hormone replacement therapy on cardiovascular risk factors in growth hormone-deficient adults. *Metabolism* 2008; 57(1):121-129.
28. Chrisoulidou A, Beshyah SA, Rutherford O et al. Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. *J Clin Endocrinol Metab* 2000; 85(10):3762-3769.
29. Henche SA, Torres RR, Pellico LG. An evaluation of patterns of change in total and regional body fat mass in healthy Spanish subjects using dual-energy X-ray absorptiometry (DXA). *Eur J Clin Nutr* 2008; 62(12):1440-1448.
30. Salomon F, Cuneo RC, Hesp R, Sonksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med* 1989; 321(26):1797-1803.
31. Moller J, Jorgensen JO, Moller N, Hansen KW, Pedersen EB, Christiansen JS. Expansion of extracellular volume and suppression of atrial natriuretic peptide after growth hormone administration in normal man. *J Clin Endocrinol Metab* 1991; 72(4):768-772.
32. Moller J, Moller N, Frandsen E, Wolthers T, Jorgensen JO, Christiansen JS. Blockade of the renin-angiotensin-aldosterone system prevents growth hormone-induced fluid retention in humans. *Am J Physiol* 1997; 272(5 Pt 1):E803-E808.
33. Janssen YJ, Deurenberg P, Roelfsema F. Using dilution techniques and multifrequency bioelectrical impedance to assess both total body water and extracellular water at baseline and during recombinant

- human growth hormone (GH) treatment in GH-deficient adults. *J Clin Endocrinol Metab* 1997; 82(10):3349-3355.
34. Netelenbos JC, Lems WF, Geusens PP et al. Spine radiographs to improve the identification of women at high risk for fractures. *Osteoporos Int* 2009; 20(8):1347-1352.
 35. Holmer H, Svensson J, Rylander L et al. Fracture incidence in GH-deficient patients on complete hormone replacement including GH. *J Bone Miner Res* 2007; 22(12):1842-1850.
 36. Sesmilo G, Biller BM, Llevadot J et al. Effects of growth hormone administration on inflammatory and other cardiovascular risk markers in men with growth hormone deficiency. A randomized, controlled clinical trial. *Ann Intern Med* 2000; 133(2):111-122.
 37. Hoffman AR, Biller BM, Cook D et al. Efficacy of a long-acting growth hormone (GH) preparation in patients with adult GH deficiency. *J Clin Endocrinol Metab* 2005; 90(12):6431-6440.
 38. Bell J, Parker KL, Swinford RD, Hoffman AR, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in children. *J Clin Endocrinol Metab* 2010; 95(1):167-177.
 39. Ogilvy-Stuart AL, Gleeson H. Cancer risk following growth hormone use in childhood: implications for current practice. *Drug Saf* 2004; 27(6):369-382.
 40. Savendahl L, Maes M, bertsson-Wikland K et al. Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during childhood in Belgium, The Netherlands, and Sweden: preliminary report of 3 countries participating in the EU SAGhE study. *J Clin Endocrinol Metab* 2012; 97(2):E213-E217.
 41. Sklar CA, Mertens AC, Mitby P et al. Risk of disease recurrence and second neoplasms in survivors of childhood cancer treated with growth hormone: a report from the Childhood Cancer Survivor Study. *J Clin Endocrinol Metab* 2002; 87(7):3136-3141.
 42. Taback SP, Dean HJ. Mortality in Canadian children with growth hormone (GH) deficiency receiving GH therapy 1967-1992. The Canadian Growth Hormone Advisory Committee. *J Clin Endocrinol Metab* 1996; 81(5):1693-1696.
 43. Carel JC, Ecosse E, Landier F et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. *J Clin Endocrinol Metab* 2012; 97(2):416-425.
 44. Swerdlow AJ, Higgins CD, Adlard P, Preece MA. Risk of cancer in patients treated with human pituitary growth hormone in the UK, 1959-85: a cohort study. *Lancet* 2002; 360(9329):273-277.
 45. Burgers AM, Biermasz NR, Schoones JW et al. Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. *J Clin Endocrinol Metab* 2011; 96(9):2912-2920.
 46. Dey S, Flather MD, Devlin G et al. Sex-related differences in the presentation, treatment and outcomes among patients with acute coronary syndromes: the Global Registry of Acute Coronary Events. *Heart* 2009; 95(1):20-26.
 47. Ettinger WH, Wahl PW, Kuller LH et al. Lipoprotein lipids in older people. Results from the Cardiovascular Health Study. The CHS Collaborative Research Group. *Circulation* 1992; 86(3):858-869.
 48. Nooyens AC, Visscher TL, Verschuren WM et al. Age, period and cohort effects on body weight and body mass index in adults: The Doetinchem Cohort Study. *Public Health Nutr* 2009; 12(6):862-870.
 49. Franklin SS, Gustin W, Wong ND et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation* 1997; 96(1):308-315.
 50. Kreitschmann-Andermahr I, Siegel S, Francis F et al. Variation of the baseline characteristics and treatment parameters over time: an analysis of 15 years of growth hormone replacement in adults in the German KIMS database. *Pituitary* 2012.

APPENDIX 1. SEARCH STRATEGY USED FOR SYSTEMATIC LITERATURE SEARCH

The following combined key words were used: ((*"recombinant growth hormone"* OR *"recombinant growth hormones"* OR *"recombinant human growth hormone"* OR *"recombinant human growth hormones"* OR *"r-bGH"* OR ((*"Growth Hormone"*[mesh] OR *"growth hormone"* OR *"growth hormones"* OR *Somatotropin* OR *Somatotropins* OR *Somatropin* OR *bGH* OR *Serostim* OR *Zomacton* OR *"Cryo-Tropin"* OR *"Cryo Tropin"* OR *CryoTropin* OR *"r-bGH-M"* OR *Humatrope* OR *Umatrope* OR *Maxomat* OR *Norditropin* OR *Norditropine* OR *Nutropin* OR *Omnitrope* OR *Saizen* OR *Genotropin* OR *Genotonorm*) AND (*"Recombinant Proteins"*[mesh] OR *recombinant*[tw])))) AND (*"long term effects"* OR *"long term effect"* OR *"longterm effects"* OR *"longterm effect"* OR *longterm* OR *"long term"* OR ((*"Twenty years"* OR *"Twenty year"* OR *"20 years"* OR *"20 year"* OR *"Nineteen years"* OR *"Nineteen year"* OR *"19 years"* OR *"19 year"* OR *"Eighteen years"* OR *"Eighteen year"* OR *"18 years"* OR *"18 year"* OR *"Seventeen years"* OR *"Seventeen year"* OR *"17 years"* OR *"17 year"* OR *"Sixteen years"* OR *"Sixteen year"* OR *"16 years"* OR *"16 year"* OR *"Fifteen years"* OR *"Fifteen year"* OR *"15 years"* OR *"15 year"* OR *"Fourteen years"* OR *"Fourteen year"* OR *"14 years"* OR *"14 year"* OR *"Thirteen years"* OR *"Thirteen year"* OR *"13 years"* OR *"13 year"* OR *"Twelve years"* OR *"Twelve year"* OR *"12 years"* OR *"12 year"* OR *"Eleven years"* OR *"Eleven year"* OR *"11 years"* OR *"11 year"* OR *"Ten years"* OR *"Ten year"* OR *"10 years"* OR *"10 year"* OR *"Nine years"* OR *"Nine year"* OR *"9 years"* OR *"9 year"* OR *"Eight years"* OR *"Eight year"* OR *"8 years"* OR *"8 year"* OR *"Seven years"* OR *"Seven year"* OR *"7 years"* OR *"7 year"*) AND (*effect*[tiab] OR *effects*[tiab] OR *outcome* OR *outcomes*))) AND (*adult* OR *adults*)) OR ((*"recombinant growth hormone"* OR *"recombinant growth hormones"* OR *"recombinant human growth hormone"* OR *"recombinant human growth hormones"* OR *"r-bGH"* OR ((*"Growth Hormone"*[mesh] OR *"growth hormone"* OR *"growth hormones"* OR *Somatotropin* OR *Somatotropins* OR *Somatropin* OR *bGH* OR *Serostim* OR *Zomacton* OR *"Cryo-Tropin"* OR *"Cryo Tropin"* OR *CryoTropin* OR *"r-bGH-M"* OR *Humatrope* OR *Umatrope* OR *Maxomat* OR *Norditropin* OR *Norditropine* OR *Nutropin* OR *Omnitrope* OR *Saizen* OR *Genotropin* OR *Genotonorm*) AND (*"Recombinant Proteins"*[mesh] OR *recombinant*[tw])))) AND (*"long term effects"* OR *"long term effect"* OR *"longterm effects"* OR *"longterm effect"* OR *longterm* OR *"long term"* OR ((*"Twenty years"* OR *"Twenty year"* OR *"20 years"* OR *"20 year"* OR *"Nineteen years"* OR *"Nineteen year"* OR *"19 years"* OR *"19 year"* OR *"Eighteen years"* OR *"Eighteen year"* OR *"18 years"* OR *"18 year"* OR *"Seventeen years"* OR *"Seventeen year"* OR *"17 years"* OR *"17 year"* OR *"Sixteen years"* OR *"Sixteen year"* OR *"16 years"* OR *"16 year"* OR *"Fifteen years"* OR *"Fifteen year"* OR *"15 years"* OR *"15 year"* OR *"Fourteen years"* OR *"Fourteen year"* OR *"14 years"* OR *"14 year"* OR *"Thirteen years"* OR *"Thirteen year"* OR *"13 years"* OR *"13 year"* OR *"Twelve years"* OR *"Twelve year"* OR *"12 years"* OR *"12 year"* OR *"Eleven years"* OR *"Eleven year"* OR *"11 years"* OR *"11 year"* OR *"Ten years"* OR *"Ten year"* OR *"10 years"* OR *"10 year"* OR *"Nine years"* OR *"Nine year"* OR *"9 years"* OR *"9 year"* OR *"Eight years"* OR *"Eight year"* OR *"8 years"* OR *"8 year"* OR *"Seven years"* OR *"Seven year"* OR *"7 years"* OR *"7 year"*) AND (*effect*[tiab] OR *effects*[tiab]

OR *outcome* OR *outcomes*))) NOT (“*child*”[*mesh*] NOT “*Adult*”[*mesh*])) NOT (“*Animals*”[*mesh*] NOT “*Humans*”[*mesh*])).

APPENDIX 2. LIST OF CRITERIA USED FOR METHODOLOGICAL QUALITY ASSESSMENT, MODIFIED STROBE STATEMENT

Item	Criterion
1.	Clear presentation of key elements of study design
2.	Description of setting, locations, relevant dates including periods of recruitment, exposure, follow-up, and data collection
	Description of eligibility criteria, and the sources and methods of selection of participants
3.	i) Description of methods of follow-up (cohort study) ii) Rationale for the choice of cases and controls (case-control study)
4.	Clear definition of all outcomes, exposures, predictors, potential confounders.
5.	Description of any effects to address potential sources of bias.
6.	Clear description of statistical methods and handling with missing data.
7.	Report of reasons of non-participation at each stage of the study (for example, flow diagram).
8.	Clear description of characteristics of study participants.
9.	Clear description of follow-up time.
10.	Presentation of unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (i.e. 95% confidence interval).

APPENDIX 3. MEAN SERUM IGF-1 LEVELS AND IGF-1 SDS AT START OF RGH SUPPLETION AND DURING FOLLOW-UP, SPECIFIED PER STUDY

Study(ref)	Baseline	1yr	5yr	7yr	10yr	15yr
Götherström 2010(16)						
IGF-1 ug/l	87.0 (54.0)	245.0 (123.0)	200.0 (98.0)	208.0 (98.0)	170.0 (83.0)	NR
IGF-1 SDS	-1.10 (1.08)	2.05 (2.40)	1.17 (2.06)	1.33 (1.76)	1.17 (1.52)	NR
Svensson 2003(17)						
IGF-1 ug/l	109.0 (6.0)	300.0 (13.0)	256.0 (11.0)	NR	NR	NR
IGF-1 SDS	-1.54 (0.12)	2.35 (0.27)	1.53 (0.21)	NR	NR	NR
Götherström 2005(14)						
IGF-1 ug/l	88.0 (10.0)	246.0 (13.0)	202.0 (18.0)	NR	NR	NR
IGF-1 SDS	-1.10 (0.20)	2.06 (0.46)	1.21 (0.39)	NR	NR	NR
Götherström 2009(15)						
IGF-1 ug/l	109.6 (62.4)	300.0 (135.2)	256.0 (114.0)	256.0 (114.0)	202.0 (94.0)	NR
IGF-1 SDS	-1.54 (1.25)	2.35 (2.81)	1.53 (2.18)	1.34 (1.70)	1.12 (2.18)	NR
Roemmler(25)						
IGF-1 ug/l	NR	NR	NR	NR	167.0	NR
IGF-1 SDS	NR	NR	NR	NR	(R74.0-319.0)	NR
Van der Klaauw 2007(29)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	-2.00 (0.8)	NR	0.80 (1.9)	NR	NR	NR
Van der Klaauw 2006(7)						
IGF-1 nmol/l	9.1 (4.6)	NR	NR	25.5 (9.9)	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Giavoli(20)						
IGF-1 nmol/l	8.4 (4.4)	24.8 (9.6)	22.2 (8.0)	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Götherström 2007(6)						
IGF-1 ug/l	NR	NR	NR	NR	NR	NR
IGF-1 SDS	-1.80 (0.12)	3.10 (0.28)	1.88 (0.24)	1.51 (0.19)	1.29 (0.25)	NR
Gibney(23)						
IGF-1 nmol/l	12.3 (3.0)	NR	NR	NR	26.9 (4.0)	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR

Chapter 4

Study(ref)	Baseline	1yr	5yr	7yr	10yr	15yr
Götherström 2001(9)						
IGF-1 ug/l	102.0 (6.0)	313.0 (13.0)	268.0 (11.0)	NR	NR	NR
IGF-1 SDS	-1.73 (0.11)	2.55 (0.25)	1.66 (0.20)	NR	NR	NR
Chrisoulidou(22)						
IGF-1 nmol/l	12.4 (R9.2-18.1)	NR	NR	29.1 (R16.5-41.9)	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Cenci(21)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Svensson 2002(27)						
IGF-1 ug/l	74.0 (14.0)	361.0 (35.0)	NR	260.0 (47.0)	NR	NR
IGF-1 SDS	-2.94 (0.36)	2.90 (0.78)	NR	1.89 (0.89)	NR	NR
Götherström 2007(10)						
IGF-1 ug/l	99.5 (6.6)	341.6 (14.1)	281.0 (13.0)	279.6 (14.5)	223.3 (9.8)	NR
IGF-1 SDS	-1.81 (0.12)	3.10 (0.28)	1.88 (0.24)	1.51 (0.19)	1.29 (0.25)	NR
Spielhagen 2010(26)						
IGF-1 ng/ml (M)	144.3 (11.8)	248.0 (12.3)	224.9 (11.6)	203.1 (12.4)	177.3 (11.7)	NR
IGF-1 ng/ml (F)	114.6 (9.0)	187.6 (10.1)	160.2 (9.2)	155.2 (9.5)	153.4 (NR)	NR
IGF-1 SDS (M)	-1.9 (0.3)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.1 (0.2)	NR
IGF-1 SDS (F)	-2.4 (0.2)	-0.6 (0.2)	-0.6 (0.2)	-0.3 (0.2)	0.0 (0.2)	NR
Elbornsson 2012(4)						
IGF-1 ug/l	103.0 (6.0)	NR	273.0 (10.0)	246.0 (8.0)	206.0 (8.0)	183.0 (7.0)
IGF-1 SDS	-1.69 (0.11)	NR	1.89 (0.21)	1.47 (0.18)	0.84 (0.17)	0.62 (0.16)
Clanget(19)						
IGF-1 ug/l	95.4 (16.2)	304.3 (49.6)	235.0 (24.0)	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Koltowska(5)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR

Gilchrist(24)

IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR

**Svensson
2004(28)**

IGF-1 ug/l (M)	125.0 (6.0)	322.0 (11.0)	283.0 (11.0)	275.0 (16.0)	NR	NR
IGF-1 ug/l (F)	83.0 (6.0)	224.0 (11.0)	229.0 (15.0)	231.0 (21.0)	NR	NR
IGF-1 SDS (M)	-1.39 (0.12)	2.74 (0.21)	2.06 (0.22)	1.88 (0.34)	NR	NR
IGF-1 SDS (F)	-2.12 (0.13)	0.51 (0.22)	0.83 (0.29)	0.87 (0.43)	NR	NR

Van Bunderen(11)

IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR

Gaillard(18)

IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS (M)	NR	0.54 (1.77)	NR	NR	NR	NR
IGF-1 SDS (F)	NR	-0.16 (1.67)	NR	NR	NR	NR

Data are presented as mean (SD), unless specified otherwise.

IGF-1, insulin-like growth; NR, not reported; R, range; M, males; F, females

Chapter 5

Metabolic profile in growth hormone deficient (GHD) adults after long-term recombinant human growth hormone (rGH) therapy

Natasha M. Appelman-Dijkstra, Kim M.J.A. Claessen, Desirée M.M.M. Adoptie,
Ferdinand Roelfsema, Johannes W.A. Smit, Nienke R. Biermasz, Alberto M. Pereira

ABSTRACT

Background: The metabolic effects of recombinant human growth hormone (rGH) therapy in adults are well documented in the short-term. The effects of long-term rGH therapy beyond five years on metabolic parameters are presently unknown.

Objective: To evaluate the long-term effects of rGH treatment on biochemical and anthropometric parameters in a large cohort of GHD adults.

Methods: Ninety-eight adult GHD patients treated with rGH for ≥ 10 years were included (mean age 59.4 years, 50% female). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, anthropometric parameters, IGF-1, and glucose were evaluated at baseline, and after 5, 10 and 15 years of treatment. In addition, the prevalence of the metabolic syndrome (MS) and the incidence of cardiovascular events were assessed.

Results: TC and LDL-C concentrations were lower, and HDL-C levels were significantly higher during long-term rGH replacement when compared to baseline (all $p < 0.001$). Both waist circumference ($p < 0.001$) and BMI ($p = 0.018$) were significantly higher after 10 years, as were fasting plasma glucose levels ($p < 0.001$). No significant changes were observed in TG, WH ratio and blood pressure during follow-up. In the subset of patients with 15-yr rGH treatment ($N = 43$), generally similar metabolic effects were found. MS prevalence was increased after 10 years of rGH treatment (57.1% *vs* 32.7%, $p < 0.001$), especially in males (69.4% *vs* 32.7%, $p < 0.001$).

Conclusion: Despite improvement of several cardiovascular risk factors, MS prevalence increased significantly during rGH treatment. The effect of long-term rGH treatment on overall cardiovascular risk profile needs to be established in a larger cohort.

INTRODUCTION

Recombinant human growth hormone (rGH) replacement therapy has been a regular treatment option for adult growth hormone deficient (GHD) patients since the nineties. Adult GHD is hypothesized to be a cardiovascular risk factor associated with increased mortality, by inducing abdominal obesity, hypercholesterolemia, and hypertriglyceridemia (1;2). In short-term studies, GH replacement reduces some, but not all of these cardiovascular risk factors (3). Consistent ‘short-term’ effects on body composition and lipid metabolism were documented, resulting in reduction of body fat combined with an increase of fat-free mass, and a reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (3). In addition, favorable effects on bone mineral density (BMD) and quality of life (QoL) have been reported (3-6). Sustained improvement of lipid spectrum and diastolic blood pressure (DBP) has been reported over 7-10 years (7-9). However, despite a significant improvement of some, but not all individual cardiovascular risk factors, overall cardiovascular risk profile, as reflected by the prevalence of the metabolic syndrome (MS), was still increased when compared to general population and was not affected by rGH after 5 years of treatment (10). Moreover, a recent study reported an increased risk of cardiovascular death in GHD women treated with rGH when compared to background population (11). A recent review addressing the effects of rGH replacement in elderly patients (>60 years) with GHD, revealed that rGH replacement decreases LDL-C levels and improves QoL, but the effects on other parameters were not unequivocal (12). However, sufficient data concerning the use of long-term rGH therapy in elderly are currently unavailable, as is the case in younger patients. In view of the overall scarce documentation of cardiovascular effects over ten years (8;13;14), the efficacy of ongoing rGH therapy in reducing cardiovascular risk in adult GHD still has to be established.

Therefore, the aim of this study was to evaluate the long-term effects of rGH treatment on biochemical and anthropometric parameters in a large cohort of GHD adults, which were treated with rGH for at least 10 years.

PATIENTS AND METHODS

Patients

Since 1994, consecutive patients diagnosed with GHD at the Endocrinology Department of the Leiden University Medical Center were collected in a database, including both adult-onset (AO) and childhood-onset (CO) GHD. Severe GHD had been defined prior to start of treatment by a GH peak response to the Insulin Tolerance Test (ITT) $<3\mu\text{g/l}$ (glucose nadir $<2.2\text{mmol/l}$) or Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) (with BMI-adjusted GH cut-offs) in case of contraindications for ITT,

according to guidelines (10;15). All patients with rGH treatment during childhood were retested at time of transition to the adult outpatient clinic, after treatment cessation for >3months. Following dose titration, aiming at an IGF-1 level in the normal range, patients were evaluated at least yearly at the outpatient clinic according to a standard protocol.

For the present analysis, we selected patients who started with rGH treatment in 2002 or before (N=184). Exclusion criteria were: 1) rGH treatment duration <10years; 2) cessation for ≥ 2 years; 3) >3 missing visits. Part of this GHD cohort used for present analysis was previously described (9;10;16;17).

Treatment protocol

All patients were treated with s.c. injections of rGH (Genotropin Pharmacia/Pfizer, Zomacton Ferring, or Norditropin NovoNordisk) injected in the evening. The initial dose of rGH was 0.2mg/day, which was individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at SDS between 0 and +2. When stable plasma concentrations were reached, this individualized dose was continued and adjusted as necessary. Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55mmol/l) after a corticotropin-releasing hormone stimulation test or ITT. When secondary amenorrhea was present for >1year, premenopausal women were classified as gonadotropin deficient. In men, gonadotropin deficiency was defined as a testosterone level below the reference range (8.0nmol/l). Thyroid-stimulating hormone (TSH) deficiency was defined as total thyroxine (T_4) or free T_4 level below the reference range (<10pmol/l). Hypopituitarism was supplemented by hydrocortisone, l-thyroxine, testosterone in men, and estrogen in combination with progestagens in premenopausal women only. Dosages were monitored and adjusted as required. Thyroid hormone replacement, lipid-lowering medication and antihypertensive medication was started according to the discretion of their attending physicians.

Efficacy parameters

The following efficacy parameters were assessed at baseline and at the yearly visits at the outpatient clinic:

- i. Biochemical parameters: levels of glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) after an overnight fast. LDL-C concentrations were calculated using the Friedewald formula.

- ii. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressures (SBP and DBP, respectively) were measured. BMI and waist-to-hip (WH)-ratio were calculated. Body weight was measured to the nearest 0.1kg, and body height was measured barefoot to the nearest 0.001m. The BMI was calculated as weight in kilograms divided by the square of height in meters.
- iii. Additional information with respect to medication use, co-morbidity and possible side effects and adverse events was gathered from patient records.

For the present study, we analyzed the efficacy parameters at baseline and after 5, 10 and 15 years of rGH therapy.

Metabolic Syndrome (MS)

The MS was defined according to the updated third report of the 2006 National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) criteria, which required at least three of the following conditions (18;19):

1. Fasting plasma glucose concentration $\geq 100\text{mg/dl}$ or on anti-diabetic drug treatment;
2. TG concentration $\geq 150\text{mg/dl}$ or on drug treatment;
3. HDL-C concentration $< 40\text{mg/dl}$ in men and $< 50\text{mg/dl}$ in women, or on drug treatment;
4. BP $\geq 130/85\text{mmHg}$ or on anti-hypertensive treatment
5. Waist circumference $> 102\text{cm}$ in men and $> 88\text{cm}$ in women.

Assays

Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDA GH protein (detection limit: $0.01\mu\text{g/l}$, interassay coefficient of variation (CV): 1.6-8.4% of $0.01\text{-}15.4\mu\text{g/l}$) from 1992 onwards. For the conversion of $\mu\text{g/l}$ to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), detection limit: 0.5mU/l , with an interassay CV $< 5\%$; for the conversion of $\mu\text{g/l}$ to mU/l, multiply by 2.

From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5nmol/l and an interassay CV less than 11%. IGF-1 is expressed as SD score for age- and sex-related normal levels determined in the same laboratory (20). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8

and 75nmol/l were 5.0 and 7.5%. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (21;22).

A Hitachi 747 autoanalyzer (Roche) was used to quantify serum concentrations of glucose, TC, and TG. HDL-C was measured with a homogenous enzymatic assay (Hitachi 911,Roche). In 2003, the Hitachi 747 was replaced by a modular P800 with no change in the chemistry components.

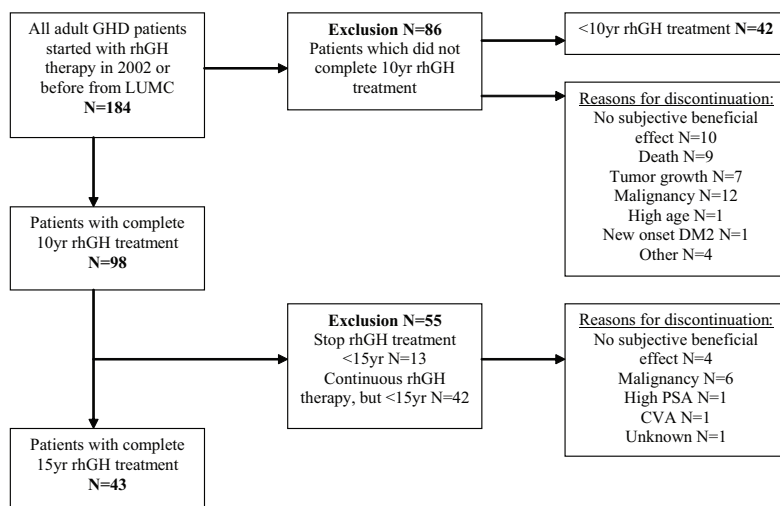
Statistics

SPSS for Windows, Version 17.0 (SPSS,Inc.,Chicago,IL,USA) was used for statistical analysis. Results are presented as mean $\pm$ SD, unless stated otherwise. ANOVA repeated measurements with Bonferroni correction for multiple comparisons were used to compare biochemical and anthropometric parameters between baseline and after rGH treatment. We incorporated age, sex, childhood-onset versus adult-onset GHD, hydrocortisone use, radiotherapy and GH dose in a linear regression model to identify factors influencing the metabolic effects of long-term rGH suppletion. The Friedman test for related fractions was used to assess the effect of rGH treatment on the MS prevalence. Furthermore, we calculated the incidence rate of cardiovascular events (*i.e.* cardiovascular death, myocardial infarction, cerebrovascular attack, intermittent claudication, (progressive) angina pectoris or coronary bypass surgery, pulmonary embolism) during rGH therapy.

RESULTS

One hundred eighty-four adult patients with GHD started rGH suppletion in 2002 or before (*Figure 1*). Forty-two patients are on current rGH treatment, but did not complete 10years of treatment yet. Forty-four patients discontinued rGH treatment for various reasons (*Figure 1*). Reasons for preliminary discontinuation of rGH treatment were: no subjective beneficial effect (N=10), death (N=9), tumor growth (N=7), malignancy (N=12), high age (N=1), new-onset diabetes mellitus type 2 (N=1), other (N=4). Consequently, we included 98 patients (50% female, mean age 59.4 yr) for the present analysis, of which 43 patients completed 15 years of rGH therapy (44% female, mean age 61.3 yr). Baseline characteristics are shown in *Table 1*.

No differences were found between the patients with and without complete ≥ 10 yr rGH treatment, with respect to sex, BMI, age of start rGH therapy, etiological diagnosis, surgery, radiotherapy, pituitary deficiencies, and use of lipid-lowering or antihypertensive medication. Only the number of patients with childhood-onset GHD was higher among non-completers ($p=0.009$) (*data not shown*).

**FIGURE 1.**

Flowchart of patient selection and follow-up

GH dose and IGF-1 concentration

Mean GH dose after dose titration (after 1yr) was 0.46 ± 0.20 mg/day (range 0.20–1.50mg), 0.48 ± 0.25 mg/day at 5years (range 0.15–1.50mg), at 10 and 15years mean GH doses were 0.44 ± 0.26 mg/day (range 0.10–1.50mg) and 0.39 ± 0.21 mg/day (range 0.10–1.50mg), respectively (5, 10 and 15-yr GH doses were not significantly different from the GH dose after dose titration). Serum IGF-1 levels remained significantly higher during rGH replacement for the duration of the study up to 15years compared to baseline (Table 2a). During the entire study period, mean IGF-1 SDS was within the normal range, increased from -0.68 ± 2.27 at baseline to 0.20 ± 2.25 at 15years of rGH suppletion (Figure 2). In males, mean IGF-1 SDS was -0.24 ± 2.26 at baseline and increased to 0.21 ± 2.31 at 15years ($p=0.007$); in females, mean IGF-1 SDS increased from -1.13 ± 2.17 at baseline to 0.19 ± 2.25 after 15years of rGH treatment ($p<0.001$).

Effects on glucose, lipid profile, anthropometric parameters and blood pressure

Fasting plasma glucose levels significantly increased from 4.7 ± 0.8 mmol/l at baseline to 5.0 ± 1.1 mmol/l at 5years (6% increase), thereafter remaining stable at 5.1 ± 1.0 mmol/l after 10years rGH treatment (Table 2a) ($p<0.001$ vs baseline). TC and LDL-C concentrations decreased, and HDL-C levels increased significantly after 5 years when compared to baseline (all $p<0.001$), improving further after 10years of rGH treatment. Both waist circumference and BMI increased after 10years of rGH suppletion ($p<0.001$ and $p=0.018$, respectively). No significant changes were observed in TG, WH-ratio and SBP; only DBP decreased slightly after 5 and 10years ($p<0.01$ and $p=0.016$, resp.).

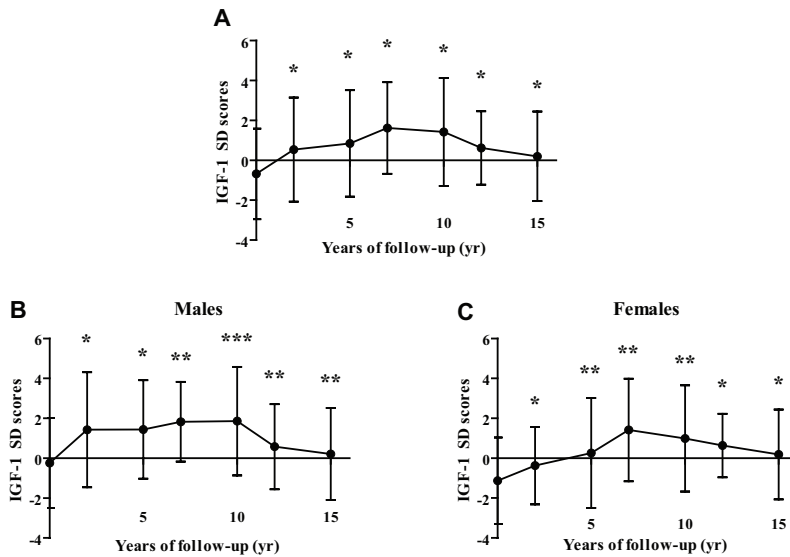
TABLE 1. BASELINE CHARACTERISTICS OF 98 PATIENTS WITH GHD, WHICH COMPLETED MINIMAL 10 YEARS OF RECOMBINANT GROWTH HORMONE (RHGH) REPLACEMENT THERAPY

Patient characteristics	GHD patients with complete 10yr follow-up (N=98)
Sex, female / male (n)	49 / 49
Age at start rhGH therapy, yr (range)	44.9 ± 13.6 (17 – 84)
AO / CO (n)	87 / 11
BMI, kg/m ² (range)	28.2 ± 5.9 (16.9 – 45.4)
Etiological diagnosis of GHD (n)	
NFA	30
Functioning adenoma	25
Craniopharyngeoma	14
Cerebral malignancy	5
Congenital	9
Other causes	15
Surgery, TS / TC (n)	53 / 24
Radiotherapy (n)	37
Pituitary deficiencies (n)	
TSH	89
ACTH	88
FSH/LH	85
ADH	28
Isolated GHD	1
Lipid-lowering drugs (n)	10
Antihypertensive medication (n)	10

Data are presented as mean ± SD, unless specified otherwise. n, number of patients.

GHD, growth hormone deficiency; AO, adult-onset GHD; CO, childhood-onset GHD; NFA, non-functioning adenoma; TSH, thyroid stimulating hormone; ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADH, anti-diuretic hormone

Among the subgroup of patients who completed 15years of rGH treatment (N=43), comparable significant effects of rGH suppletion were found on fasting glucose levels, lipids and waist circumference (*Table 2b*). The effects on TC, HDL-C, LDL-C and TG were not affected when patients using lipid-lowering medication at any time point during follow-up were excluded (N=41, 42%). TG, WH ratio, BMI and DBP did not change when compared to baseline; SBP was significantly higher ($p=0.001$). Within the

**FIGURE 2.**

Mean IGF-1 SD scores during 15 years of rGH therapy, for the total cohort of GHD patients (A, N=98) and separated for males (B) and females (C), respectively

15-yr treated group, there were, in general, no differences between 10 and 15 years of rGH therapy; except for SBP, being significantly higher after 15 years. Excluding patients on antihypertensive medication at any time point (N=41, 42%) revealed no changes in DBP; however, the observed increase in SBP between 10 and 15 years of treatment was not significant anymore.

Influence of sex

The dose of rGH was significantly higher in women in comparison to men at all time points, resulting in a higher GH dose/IGF-1 SDS ratio in female GHD patients. After 10 years of rGH treatment, the GH dose was 0.53 ± 0.30 mg (range 0.20–1.50 mg) and 0.36 ± 0.18 mg (range 0.10–1.20 mg) in females and males, respectively ($p=0.001$); at 15 years 0.47 ± 0.23 mg (range 0.20–1.20 mg) and 0.30 ± 0.14 mg (range 0.10–0.60 mg), respectively ($p=0.009$). We found no differences in the metabolic response to rGH treatment between both sexes.

Other potential influencing factors

Forty-nine patients (50%) were <60 years, 49 patients (50%) were ≥ 60 years of age at baseline. The individualized rGH dose used in older patients did not differ from the dose in younger patients. In addition, there were no significant differences in the rGH response between younger and older patients with respect to IGF-1, glucose, lipid profile,

TABLE 2A. EFFECTS OF 10 YEARS OF RHGH REPLACEMENT IN 98 ADULTS WITH GHD ON BIOCHEMICAL AND ANTHROPOMETRIC PARAMETERS

	Baseline [†]	5 years of rhGH replacement ^{††}	10 years of rhGH replacement ^{†††}
IGF-1, nmol/l	14.8 ± 10.3	25.4 ± 13.3 *	22.2 ± 10.7 *
Fasting glucose, mmol/l	4.7 ± 0.8	5.0 ± 1.1 **	5.1 ± 1.0 *
TC, mmol/l	6.2 ± 1.4	5.5 ± 1.0 *	5.2 ± 1.0 *, ^b
LDL-C, mmol/l	4.5 ± 1.4	3.7 ± 0.9 *	3.3 ± 0.8 *, ^a
HDL-C, mmol/l	1.3 ± 0.5	1.5 ± 0.5 *	1.6 ± 0.6 *
TG, mmol/l	1.8 ± 1.1	1.8 ± 1.0	1.8 ± 1.1
Waist circumference, cm	94.6 ± 13.7	97.0 ± 14.3	98.9 ± 14.2 *, ^a
WH ratio	0.95 ± 0.07	0.97 ± 0.12	0.96 ± 0.06
BMI, kg/m ²	28.2 ± 5.9	28.8 ± 6.3	29.9 ± 6.9 ***, ^c
SBP, mmHg	129.9 ± 14.7	130.4 ± 17.8	130.9 ± 16.6
DBP, mmHg	83.5 ± 9.7	80.4 ± 9.8 **	80.4 ± 9.1 ***

Data are presented as mean ± SD. IGF-1, insulin-like growth factor-1; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; WH ratio, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

†, statins N=10 (10.2%), antihypertensive medication N=10 (10.2%); ††, statins N=32 (32.7%), antihypertensive medication N=32 (32.7%); †††, statins N=34 (34.7%), antihypertensive medication N=33 (33.7%)

*, p<0.001; **, p<0.01; ***, p<0.05 vs baseline

a, p<0.001; b, p<0.01; c, p<0.05 10 vs 5 years of rhGH treatment

WH-ratio, BMI or blood pressure. Furthermore, the number of pituitary insufficiencies did not affect the response to rGH treatment. Mean HC doses were 25.6±7.3mg/day, 23.1±5.8mg/day, 21.4±4.1mg/day, and 21.1±4.3mg/day at baseline and after 5, 10 and 15 years of rGH suppletion, respectively. However, patients with hydrocortisone substitution did not differ from hydrocortisone-independent patients, except for a greater decrease in LDL-C levels after 10 years (-1.35 *vs* -0.70mmol/l, p=0.021). Patients with CO-GHD did not differ from AO-GHD patients in their response to any of the metabolic parameters studied. In addition, there was no difference between patients with or without cranial irradiation, except for a higher waist circumference (p=0.024) among irradiated patients.

When incorporating all factors in a linear regression model, neither age, sex, CO *vs* AO-GHD, nor hydrocortisone use significantly influenced any of the metabolic parameters. Radiotherapy, however, influenced waist circumference and DBP negatively. In addition,

TABLE 2B. EFFECTS OF 15 YEARS OF RHGH TREATMENT IN 43 ADULTS WITH GHD ON BIOCHEMICAL AND ANTHROPOMETRIC PARAMETERS

	Baseline [†]	15 years of rhGH replacement ^{††}
IGF-1, nmol/l	9.6 ± 5.5	19.6 ± 8.2 ^{*,a}
Fasting glucose, mmol/l	4.5 ± 0.7	5.0 ± 0.5 ^{**}
TC, mmol/l	6.6 ± 1.5	5.4 ± 1.1 [*]
LDL-C, mmol/l	4.9 ± 1.4	3.4 ± 0.9 [*]
HDL-C, mmol/l	1.4 ± 0.5	1.7 ± 0.6 [*]
TG, mmol/l	1.7 ± 1.0	1.5 ± 0.6
Waist circumference, cm	91.2 ± 10.5	99.5 ± 11.9 [*]
WH ratio	0.96 ± 0.06	0.95 ± 0.07
BMI, kg/m ²	25.9 ± 3.6	29.2 ± 8.3 ^{***}
SBP, mmHg	128.2 ± 14.0	134.6 ± 15.3 ^{**,b}
DBP, mmHg	83.5 ± 9.1	80.8 ± 9.1

Data are presented as mean ± SD. IGF-1, insulin-like growth factor-1; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; WH ratio, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

†, statins N=4 (9.3%), antihypertensive medication N=5 (11.6%); ††, statins N=20 (46.5%), antihypertensive medication N=20 (46.5%)

*, p<0.001; **, p<0.01; ***, p<0.05 *vs* baseline

^a, p<0.001; ^b, p<0.01; ^c, p<0.05 15 *vs* 10 years of rhGH treatment

higher GH dose was associated with higher BMI and waist circumference after 10years of rGH suppletion.

Prevalence of the MS

The prevalence of the MS increased from 32.7% at baseline to 46.9% after 5years of rGH therapy (p=0.040), further increasing to 57.1% after 10years of rGH treatment (p<0.001 *vs* baseline). As shown in *Figure 3*, this was mainly due to a gradual increase in abdominal obesity, hypertriglyceridemia, and hyperglycaemia.

At baseline, the MS was equally prevalent in men and women (32.7% *vs* 32.7%). After 5years of rGH suppletion, 49.0% of the males and 44.9% of the female GHD patients had MS (p=0.687). After 10years of rGH suppletion, 69.4% of men fulfilled the criteria of MS *vs* 44.9% of the women, p=0.015. This indicates that males especially drove the increase in MS prevalence over time, and that MS prevalence stabilized in females

after 5years. In a logistic regression model incorporating age, sex, hydrocortisone use, radiotherapy and GH dose, only higher GH dose negatively influenced the MS prevalence after 10years of rGH suppletion.

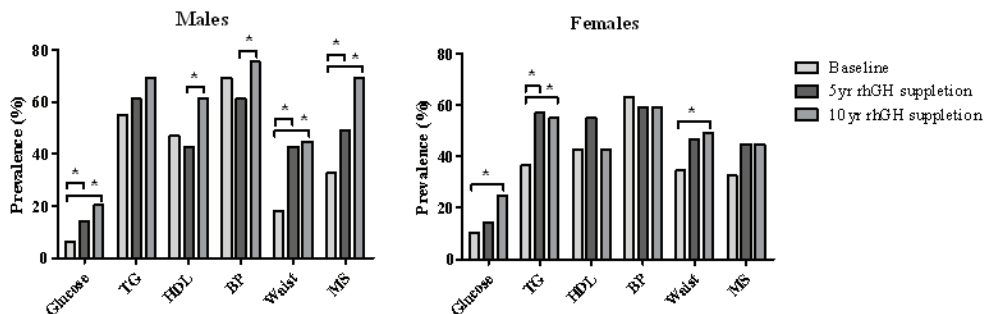


FIGURE 3.

Prevalence of (individual components of) the MS in 98 GHD adults at baseline, and after 5 and 10 years of rGH suppletion, respectively, separated for males and females

Cardiovascular events

We assessed the number of major cardiovascular events in the patients who completed ≥ 10 year rGH treatment ($N=98$). In total, 25 events were reported: myocardial infarction ($N=2$), progressive angina pectoris and/or coronary bypass surgery ($N=7$), cerebrovascular attack ($N=2$), intermittent claudication ($N=1$), pulmonary embolism ($N=1$). No cardiovascular death was reported. Consequently, the incidence rate of major cardiovascular events in our GHD cohort was $25/16552$ (mean duration of rGH therapy 168.9 months $\times 98$ patients) $= 1.5/1000$ py.

In addition, new-onset DM ($N=3$), new-onset hypertension or start of antihypertensive medication ($N=24$), new-onset hypercholesterolaemia or start of lipid-lowering treatment ($N=23$) was reported during rGH therapy.

DISCUSSION

The present study demonstrates ongoing beneficial effects of rGH treatment on lipids in patients with GHD after 10 and 15 years in the presence of an increase in anthropometric parameters such as BMI, waist circumference and SBP. As a consequence, overall cardiovascular risk, as assessed by the prevalence of the MS increased significantly after 10 years of rGH replacement. This increase in MS prevalence was higher than expected as a consequence of ageing alone in non-GHD adults (23). The short-term effects of rGH therapy on cardiovascular risk factors are well documented. A meta-analysis of placebo-controlled studies in GHD adults showed favorable effects of short-term (up

to 1.5yr) rGH replacement therapy on TC and HDL-C levels, DBP, as well as on lean body- and fat mass, but unfavorable effects on glucose and insulin concentrations (3). Data on the long-term effects of adult rGH replacement therapy are limited, and based on observational studies. The metabolic changes after ≥ 10 years of rGH substitution have been reported in only three studies (8;13;14), including a total of 119 patients, and their conclusions were inconclusive. The first study (Gibney *et al.*) reported favorable effects on HDL-C and LDL-C, but no changes in TC, TG, insulin, or blood pressure (13), whereas the second study (Götherström *et al.*) reported a decrease in TC and LDL-C, an increase in HDL-C levels, and an increase in BMI and glucose levels (8). The third study did not demonstrate any effect of treatment on lipids, glucose or anthropometric parameters (14). In this study, we documented a decrease in TC and LDL-C levels, and an increase in HDL-C, in accordance with the findings reported by Gibney *et al.* and Götherström *et al.* (8;13). TG levels, however, did not change during rGH therapy. The pattern of observed changes in lipid concentrations did not change after exclusion of patients using lipid-lowering drugs. The observed mean decrease in TC and LDL-C levels after 15 years of rGH therapy was 1.3 (20%) and 1.6 mmol/l (32%), respectively. In the general population, every 10% decrease in cholesterol levels by statins reduces cardiovascular mortality risk in patients with hypercholesterolemia by 15% (24). Whether lowering of TC levels by rGH replacement is associated with the same magnitude of reduction in cardiovascular mortality remains to be established. Furthermore, it is crucial not only to ascertain whether the beneficial effects of rGH suppletion on lipid profile are comparable to the effects obtained with conventional lipid-lowering drugs, but also if these effects can be superimposed. From a cost-effectiveness point of view, the latter is of paramount importance, but to date, only one study, including 61 GHD patients on statin treatment, has demonstrated such an additional beneficial effect of rGH treatment on lipid profile (25). In addition, Schneider *et al.* reported a cardiovascular risk reduction of approximately 50% after 2 years of rGH treatment, using Framingham and Procam risk scores (26). Since hypopituitarism *per se* is also associated with increased cardiovascular mortality (12,13), any additional effect of rGH substitution next to conventional lipid-lowering drugs may be beneficial. Despite improvement of lipid spectrum, the MS prevalence increased after 10 years of rGH replacement when compared to baseline, especially in males. Previously, we demonstrated an increased MS prevalence in untreated GHD adults, defined by the NCEP-ATP III criteria (27), in comparison to a Dutch historical reference population (data collected between 1993-1997) (38.0% *vs* 15.7%) (10). Subsequently, age-adjusted prevalence rates of 29% for the MS were reported by Attanasio *et al.* before start of rGH treatment in 1420 European adults with AO and CO-GHD (Hypopituitary Control and Complications Study, HypoCCS) (28), and of 41% before start of rGH suppletion by Verhelst and colleagues among 2479 patients with severe adult-onset GHD, using the same updated NCEP-ATP III criteria (27). Two studies have shown a

persistently high MS prevalence after 3 and 5 years of rGH treatment, resp. (10;28). In our study, we found a further increase in MS prevalence to 57% after 10 years of rGH substitution, mainly due to an increase in abdominal obesity, hypertriglyceridaemia and hyperglycaemia.

A limitation of the present study, and a general drawback of long-term follow-up studies, is the lack of a non-treated control group. Since the beneficial effects of rGH therapy are well-established in the short-term, it is unethical to withhold patients with GHD receive rGH in case of no contra-indications. This makes it difficult, if not impossible, to perform long-term randomized, controlled follow-up studies including GHD patients without rGH treatment. However, several Dutch population-based studies reported the MS prevalence in the general population. In a Dutch survey among 4000 subjects, conducted in 2009-2010, an overall MS prevalence of 34% and 24% was reported, in males and females, respectively. When stratified by age (per decade), MS prevalence for males and females, respectively, were 20% and 10% (30-39yr), 29% and 17% (40-49yr), 41% and 29% (50-59yr), and 48% and 44% (60-69yr) (23). Another study involving a cohort of Dutch adults aged ≥ 65 years (Longitudinal Aging Study Amsterdam, LASA) reported an MS prevalence of 37.1% (29). Based on these literature data, the MS prevalence of 57.1% in our cohort of GHD patients (mean age 59yr) is strongly increased, despite 10 years of rGH replacement. As demonstrated previously (8;30;31), we observed a significant increase in fasting glucose levels during rGH replacement. This finding is in accordance with the well-known negative effects of GH on peripheral insulin sensitivity, thereby impairing peripheral glucose uptake. These increased fasting glucose levels may have significantly affected cardiovascular morbidity or mortality, because previous reports have indicated that even when glucose levels were below the diabetic threshold, there was a positive correlation with the occurrence of cardiovascular events (32;33). Previous radiotherapy was shown to negatively influence abdominal obesity and blood pressure in GHD adults. This finding is consistent with earlier studies among long-term survivors of childhood cancer, reporting a higher MS prevalence after cranial irradiation (34;35). A possible explanation might be that, in addition to pituitary damage, cranial irradiation induces damage to the hypothalamic area. Therefore, cranial radiotherapy might be an independent risk factor for cardiovascular disease. Due to the presence of multiple pituitary hormone deficiencies in almost all patients, it is difficult to examine to what extent the reported effects can be attributed to rGH treatment, or whether they are the consequence of suboptimal or excessive replacement therapy of other hormones. Isolated GHD (IGHD) provides the ideal model to characterize GHD without interference from other pituitary deficiencies or their treatment. Abs *et al.* showed generally similar clinical presentation and rGH treatment response in IGHG patients and patients with multiple deficiencies, especially in AO-GHD (36), supporting the concept that GH *per se*, at least in part, affects the metabolic phenotype of substituted patients with multiple pituitary hormone deficiencies. In accordance

with these findings, in a recent meta-analysis, both low and high IGF-1 levels increase mortality in general population (hazard ratio (HR) 1.18, 95%CI 1.04-1.34) (37).

Paradoxically, there are also human studies linking reduced IGF-1 levels/signaling to a reduced cancer risk as well as improved longevity (38). In addition, functional mutations of the IGF-1R gene resulting in altered IGF-1 signaling are more common in centenarians than in younger controls. The activity of the GH/IGF-1 axis decreased with ageing, yet smaller individuals within a species usually live longer (39). Life span was also expanded in mice lacking GHR, resulting in lower IGF-1 levels. Although these mutant mice lack other hormones, their extended longevity is thought to be primarily due to GHD, as restoration of GH levels reverted their longevity to that of non-mutants (40;41). Collectively, these data suggest that optimizing the GH/IGF-1 axis to promote healthy ageing in humans is more complex than originally appreciated, and will require a greater understanding of its array of interactions and tissue specificity.

Another feature that needs to be addressed is the presence of interactions between glucocorticoids and rGH during substitution. In the past, HC doses used in hypopituitarism resulted in supraphysiological cortisol levels, accounting for at least part of the adverse metabolic profile. Decreasing the glucocorticoid dose from 20-30mg/day to 15mg/day had beneficial effects (42). In our study, mean HC dose was lowered during 15years of follow-up from 25.6 ± 7.3 to 21.1 ± 4.3 mg/day. When HC use was incorporated in a regression model, no relation to MS presence/worsening was found. Possible explanations for the absence of an effect may be the lower HC substitution scheme when compared to Danilowicz *et al*, in addition to less variation in the HC doses used. Furthermore, it has been reported that GH accelerates cortisol metabolism by inhibiting 11 β -HSD1 resulting in decreased tissue exposure to cortisol (43;44), thereby reducing HC bioavailability. IGF-1 SD levels were closely monitored and were titrated on a physiological level, adjusted for age during the entire study period. This is one of the most important differences between our GHD cohort and other GHD cohorts, in which an initial high dose resulted high IGF-1 SDS (over +2SDS) and excessive changes in body composition, especially in men. Our data showed no obvious differences in IGF-1 levels or metabolic effects of rGH suppletion between men and women, taken into account the need of a higher rGH substitution dose in women to maintain stable plasma IGF-1 concentrations. In addition, we did not find differences between younger and older GHD patients with respect to response to long-term rGH treatment. However, we cannot exclude lack of power to detect any differences due to the relatively small number of patients within each age group.

In conclusion, the present study showed ongoing beneficial effects of rGH therapy in GHD adults on lipid profile, whereas other cardiovascular risk factors continued to deteriorate after long-term rGH treatment. The increases in glucose levels and BMI were striking, and negatively affected the prevalence of the MS in the long-term. Therefore,

the net beneficial effects of long-term rGH treatment on overall cardiovascular risk still need to be established in further studies that adequately address and balance all these factors involved, including cost-effectiveness and QoL.

REFERENCE LIST

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990; 336(8710):285-288.
2. Tomlinson JW, Holden N, Hills RK et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet* 2001; 357(9254):425-431.
3. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004; 89(5):2192-2199.
4. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012; 166(5):787-795.
5. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *Eur J Endocrinol* 2007; 156(1):55-64.
6. Koltowska-Haggstrom M, Mattsson AF, Monson JP et al. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? *Eur J Endocrinol* 2006; 155(1):109-119.
7. Chrisoulidou A, Beshyah SA, Rutherford O et al. Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. *J Clin Endocrinol Metab* 2000; 85(10):3762-3769.
8. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. *J Clin Endocrinol Metab* 2007; 92(4):1442-1445.
9. van der Klaauw AA, Romijn JA, Biermasz NR et al. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. *Eur J Endocrinol* 2006; 155(5):701-708.
10. van der Klaauw AA, Biermasz NR, Feskens EJ et al. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. *Eur J Endocrinol* 2007; 156(4):455-462.
11. van Bunderen CC, van N, I, Arwert LI et al. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. *J Clin Endocrinol Metab* 2011; 96(10):3151-3159.
12. Kokshoorn NE, Biermasz NR, Roelfsema F, Smit JW, Pereira AM, Romijn JA. GH replacement therapy in elderly GH-deficient patients: a systematic review. *Eur J Endocrinol* 2011; 164(5):657-665.
13. Gibney J, Wallace JD, Spinks T et al. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. *J Clin Endocrinol Metab* 1999; 84(8):2596-2602.
14. Roemmmler J, Kuenkler M, Schneider HJ, Dieterle C, Schopohl J. Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement. *Metabolism* 2010; 59(3):350-358.
15. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008; 18(1):1-16.
16. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. *J Clin Endocrinol Metab* 1997; 82(1):129-135.

17. Janssen YJ, Hamdy NA, Frolich M, Roelfsema F. Skeletal effects of two years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. *J Clin Endocrinol Metab* 1998; 83(6):2143-2148.
18. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; 109(3):433-438.
19. Grundy SM, Cleeman JI, Daniels SR et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005; 112(17):2735-2752.
20. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. *J Clin Endocrinol Metab* 1997; 82(1):129-135.
21. Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990; 44(1):45-60.
22. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 1998; 50(3):166-176.
23. WWW.RIVM.NL/NLDEMAAT. RIVM. 2012.
24. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. *Circulation* 1998; 97(10):946-952.
25. Monson JP, Jonsson P, Koltowska-Haggstrom M, Kourides I. Growth hormone (GH) replacement decreases serum total and LDL-cholesterol in hypopituitary patients on maintenance HMG CoA reductase inhibitor (statin) therapy. *Clin Endocrinol (Oxf)* 2007; 67(4):623-628.
26. Schneider HJ, Klotsche J, Wittchen HU et al. Effects of growth hormone replacement within the KIMS survey on estimated cardiovascular risk and predictors of risk reduction in patients with growth hormone deficiency. *Clin Endocrinol (Oxf)* 2011; 75(6):825-830.
27. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285(19):2486-2497.
28. Attanasio AF, Mo D, Erfurth EM et al. Prevalence of metabolic syndrome in adult hypopituitary growth hormone (GH)-deficient patients before and after GH replacement. *J Clin Endocrinol Metab* 2010; 95(1):74-81.
29. Oosterwerff MM, van Schoor NM, Lips P, Eekhoff EM. Osteocalcin as a predictor of the metabolic syndrome in older persons: a population-based study. *Clin Endocrinol (Oxf)* 2012.
30. Giavoli C, Porretti S, Ronchi CL et al. Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. *Metabolism* 2004; 53(6):740-743.
31. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001; 86(10):4657-4665.
32. Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* 1999; 22(2):233-240.
33. Gerstein HC. Dysglycemia, not just diabetes, is a continuous risk factor for cardiovascular disease. *Evid Based Cardiovasc Med* 1997; 1(4):87-88.
34. Heikens J, Ubbink MC, van der Pal HP et al. Long term survivors of childhood brain cancer have an increased risk for cardiovascular disease. *Cancer* 2000; 88(9):2116-2121.

35. van Waas M, Neggers SJ, Pieters R, van den Heuvel-Eibrink MM. Components of the metabolic syndrome in 500 adult long-term survivors of childhood cancer. *Ann Oncol* 2010; 21(5):1121-1126.
36. Abs R, Mattsson AF, Bengtsson BA et al. Isolated growth hormone (GH) deficiency in adult patients: baseline clinical characteristics and responses to GH replacement in comparison with hypopituitary patients. A sub-analysis of the KIMS database. *Growth Horm IGF Res* 2005; 15(5):349-359.
37. Burgers AM, Biermasz NR, Schoones JW et al. Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. *J Clin Endocrinol Metab* 2011; 96(9):2912-2920.
38. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M et al. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med* 2011; 3(70):70ra13.
39. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012; 61(6):1315-1322.
40. Russell SJ, Kahn CR. Endocrine regulation of ageing. *Nat Rev Mol Cell Biol* 2007; 8(9):681-691.
41. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; 299(5611):1346-1351.
42. Danilowicz K, Bruno OD, Manavela M, Gomez RM, Barkan A. Correction of cortisol overreplacement ameliorates morbidities in patients with hypopituitarism: a pilot study. *Pituitary* 2008; 11(3):279-285.
43. Filipsson H, Johannsson G. GH replacement in adults: interactions with other pituitary hormone deficiencies and replacement therapies. *Eur J Endocrinol* 2009; 161 Suppl 1:S85-S95.
44. Walker BR, Andrew R, MacLeod KM, Padfield PL. Growth hormone replacement inhibits renal and hepatic 11 beta-hydroxysteroid dehydrogenases in ACTH-deficient patients. *Clin Endocrinol (Oxf)* 1998; 49(2):257-263.

Chapter 6

Abnormal metabolic phenotype in middle-aged Growth Hormone Deficient (GHD) adults despite long-term recombinant human GH (rGH) replacement

Natasha M. Appelman-Dijkstra, Kim M.J.A. Claessen , Alberto M. Pereira, Sjoerd.D. Joustra, Renee de Mutsert, Karen B. Gast, Martin den Heijer, Johannes W.A. Smit, Olaf M. Dekkers en Nienke R. Biermasz

ABSTRACT

Background: Adult GH deficiency (GHD) is associated with increased cardiovascular mortality. Recombinant human GH (rGH) replacement has beneficial short-term metabolic effects. Although these positive effects sustain during longer follow-up, the prevalence of the metabolic syndrome (MS) remains increased in comparison with population data not adjusted for the higher mean BMI in GHD adults.

Objective: To explore whether middle-aged patients with proposed physiological rGH replacement have been normalized with respect to MS and its individual components in comparison to the general population, adjusted for age, sex and BMI.

Methods: One-hundred sixty-one GHD patients (aged 40-70yr) were studied before the start and after 5 years of rGH replacement, and were compared with 1671 subjects (aged 45-66yr) from the general population (NEO Study).

Results: MS proportion in GHD patients was 41.0% before the start of rGH suppletion, increasing to 53.4% after 5 years ($p=0.007$). Despite chronic rGH replacement, GHD patients had a 1.3-times higher MS proportion than the general population, independently of age, sex and BMI (95%CI 1.1-1.5, $p=0.008$). The GHD population showed a different metabolic profile than the general population with similar BMI: an increased risk of hypertriglyceridaemia (adjusted prevalence ratio (PR) 2.0, 95%CI 1.7-2.3) and low HDL-C (adjusted PR 1.8, 95%CI 1.5-2.2), but less hyperglycaemia (adjusted PR 0.5, 95%CI 0.4-0.7).

Conclusions: Despite 5 years of rGH replacement, GHD patients still have a different metabolic profile and more frequently MS than the general population. These differences were independent of BMI, and resemble the unfavorable metabolic profile of untreated GHD patients, pointing into question the long-term benefits of rGH replacement.

INTRODUCTION

Growth Hormone Deficiency (GHD) in adults is associated with an adverse metabolic profile, including abdominal obesity, dyslipidemia, and an increased mortality risk (1;2). Short-term follow-up studies have shown that recombinant human GH (rGH) replacement reduced some, but not all of these cardiovascular risk factors (3). Consistent effects were reported on body composition and lipid metabolism, characterized by reduction of body fat and an increase of lean body mass, and a reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (3). Based on these positive short-term metabolic effects, as well as an improvement in quality of life (QoL), rGH replacement has become widely accepted as chronic therapy in adult GHD.

Despite improvement of individual components of the metabolic syndrome (MS), the overall prevalence of the MS, was not normalized in GHD patients after several years of physiological rGH replacement (4;5). At present, data on long-term efficacy and safety of rGH replacement are limited and uncontrolled for the effect of aging (5-18). Recently, we reported a further increase in body mass index (BMI) and MS prevalence despite 10 years of rGH replacement (19). Thus, it can be questioned whether in the long-term rGH replacement is able to improve or even normalize the adverse metabolic profile present in hypopituitary patients. Since an ideal unselected GHD control group without rGH replacement is not available, we selected the Leiderdorp cohort of the Netherlands Epidemiology of Obesity (NEO) Study as representation of the general population for comparison. The NEO Study is a population-based cohort study of middle-aged Dutch adults from the same geographic area as the GHD patients.

The primary aim of the present study was to compare the metabolic profile, as reflected by proportion of the MS and its individual components, between GHD patients after chronic rGH replacement and the general population, adjusted for age and sex, to assess the ability of current rGH replacement strategies to normalize MS features. Second, we critically compared metabolic parameters between treated GHD patients and the general population, while additionally adjusting for BMI. Finally, we compared the MS proportion in GHD patients before start and after chronic rGH replacement.

PATIENTS AND METHODS

Study design

In the present study, we compared the proportion of the MS and its individual components between middle-aged GHD patients and the general population. First, adult GHD patients were studied before the start of rGH suppletion and after 5 years of rGH replacement, to study the metabolic effects of chronic rGH suppletion. Second, we compared chronically treated GHD patients (defined as 5 years) with the general population, using data from the Leiderdorp cohort of the NEO Study (*vide infra*) (20),

adjusting for age and sex and subsequently with additional adjustments for BMI, to assess the ability of current rGH replacement strategies to completely normalize MS features.

Patient selection

From 1994 onwards, all consecutive patients diagnosed with adult-onset (AO) and childhood-onset (CO) GHD at the Endocrinology Department of the LUMC were collected in a database. We investigated the effects of chronic stable rGH replacement in middle-aged GHD patients. Chronic stable replacement was arbitrarily defined as replacement for ≥ 5 years, since in such a period metabolic changes induced by rGH therapy start will have been stabilized (3;21). We selected all GHD patients aged 40-70 years, who started rGH replacement ≤ 2007 , to enable a 5-year follow-up period. The age criterion used was chosen to match the GHD patients with middle-aged persons from the general population from the Leiderdorp cohort (*vide infra*) and to avoid non-positivity. This resulted in a total inclusion of 161 eligible GHD patients (AO 155/6 CO). The 6 patients with CO-GHD received rGH replacement during childhood, and re-started with rGH replacement since this therapy became widely accepted for adult GHD patients.

Treatment protocol

GHD had been defined prior to start of rGH replacement by a GH peak response to the Insulin Tolerance Test (ITT) $< 3\mu\text{g/l}$ (glucose nadir $< 2.2\text{mmol/l}$) according to current guidelines or Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) with BMI-adjusted GH cut-offs, in case of contraindications for ITT (5;22). All patients receiving rGH replacement during childhood were retested at the time of transition to the adult outpatient clinic, after treatment cessation for > 3 months. All patients were treated with rGH (Genotropin Pharmacia/Pfizer, Skokie, IL; Zomacton Ferring, Troy Hills, NJ; or Norditropin NovoNordisk, Princeton, NJ), injected subcutaneously in the evening. In all patients, GH starting dose was 0.2mg/day , which was individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at SDS between 0 and +2. After reaching stable plasma concentrations, this individualized dose was continued and adjusted according to the IGF-1 SDS.

Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55mmol/l) after an ITT or corticotropin-releasing hormone stimulation test in case of contraindications for ITT. When secondary amenorrhea was present for > 1 year, premenopausal women were classified as gonadotropin-deficient. In men, gonadotropin deficiency was defined as

a testosterone level $<8.0\text{nmol/l}$. Thyroid-stimulating hormone (TSH) deficiency was defined as total thyroxine (T_4) or free T_4 level $<10\text{pmol/l}$ in addition to ≥ 2 deficient pituitary axes. Hypopituitarism was adequately supplemented by hydrocortisone, L-thyroxine, testosterone in men, and/or estrogen in combination with progestagens (in premenopausal women only). Dosages of the hormonal replacement therapy were monitored and adjusted as required.

Patients were treated with lipid-lowering medication and antihypertensive medication according to the discretion of their attending physicians. Efficacy and safety parameters were assessed yearly, next to a routine assessment of pituitary function.

General population

The NEO Study is currently performed at the Leiden University Medical Center (LUMC) to investigate pathways that lead to obesity-related diseases. The NEO Study is a population-based prospective cohort study of individuals aged 45-65 years, with an oversampling of individuals with overweight or obesity ($\text{BMI} \geq 27\text{kg/m}^2$) (20). Within the NEO Study, as reference group, all inhabitants aged between 45-65 years from one municipality (Leiderdorp) were invited, irrespective of their BMI. Baseline measurements have been performed between 2008 and 2012. The study was approved by the Medical Ethics Committee of the LUMC and all participants gave informed consent.

We used the Leiderdorp cohort ($N=1671$) for comparison with our chronically rGH-treated GHD patients. GHD patients and subjects from the general population were from the same geographic area.

Study parameters

The following efficacy parameters were studied:

1. Biochemical parameters: fasting glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels. LDL-C concentrations were calculated by the Friedewald formula. Blood samples were taken after an overnight fast.
2. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressures (SBP and DBP, respectively) were measured. BMI (in kg/m^2) and waist-hip ratio were calculated.
3. Additional information on medication use and co-morbidity was gathered from patient records.

Metabolic Syndrome (MS)

The MS was defined according to the updated third report of the 2006 National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) criteria, which required the presence of ≥ 3 of the following conditions (23;24):

1. Fasting plasma glucose concentration ≥ 100 mg/dl or on anti-diabetic drug treatment;
2. TG concentration ≥ 150 mg/dl or on lipid-lowering drug treatment (statins and/or fibrates);
3. HDL-C concentration < 40 mg/dl in men and < 50 mg/dl in women, or on lipid-lowering drug treatment (statins and/or fibrates);
4. BP $\geq 130/85$ mmHg or on anti-hypertensive treatment;
5. Waist circumference ≥ 102 cm in men and ≥ 88 cm in women.

Assays

From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5nmol/l and an interassay CV $< 11\%$. IGF-1 is expressed as SD score for age- and gender-related normal levels determined in the same laboratory (21). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (25;26).

In GHD patients, a Hitachi 747 autoanalyzer (Roche) was used to quantify serum concentrations of glucose, TC, and TG. HDL-C was measured with a homogenous enzymatic assay (Hitachi 911, Roche). In 2003, a Roche Modular Analytics P800 replaced the Hitachi 747 with no change in the chemistry components. In the general population, glucose, TC, HDL-C and TG levels were measured by Roche Modular Analytics P800. Samples in patients and controls were analyzed in the same laboratory. Samples of patients were obtained between 1994 and 2013; samples of controls between 2008 and 2012.

Statistical analysis

SPSS for Windows, Version 20.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Results are presented as mean $\pm$ SD, unless stated otherwise. For comparison of the MS proportion in GHD patients before start and after 5 years of rGH suppletion,

we performed the Friedman Test for related fractions. Although 19 patients did not complete 5 years of rGH replacement for various reasons (*vide infra*), these patients were included using the last observation carried forward method (intention-to-treat approach), thereby including the last available measurements during rGH replacement in the analyses. Independent *T* tests were used to compare crude metabolic data between 5-year rGH-treated GHD patients and the general population. The relationships in MS (components) proportion between GHD patients after 5 years of rGH replacement and the general population are reported as prevalence ratios (PRs), using the log-binominal regression model using STATA Statistical Software version 12.1(Statacorp,College Station,TX), allowing direct estimation of PRs (27;28), which is, because of the high incidence of the MS, more appropriate than logistic models that only allow estimation of odds ratios. Analyses were adjusted for age and sex, and subsequently with additional adjustments for BMI. $P<0.05$ is considered statistically significant.

RESULTS

Characteristics of patients and controls

We studied 161 adult GHD patients, aged 40-70 years at the time of GHD diagnosis (Table 1). Pituitary deficiency was mainly caused by pituitary tumors or their treatment. Patients had been treated with surgery (transsphenoidal N=109/transcranial N=24) and radiotherapy (N=76). Etiological diagnoses of GHD were non-functioning adenoma (N=67), functioning adenoma (N=52), craniopharyngeoma (N=12), cerebral malignancy (N=2), congenital (N=6) and other causes (N=22). Most patients had multiple pituitary hormone deficiencies (ACTH deficiency N=142, TSH deficiency N=132, FSH/LH deficiency N=120, ADH deficiency N=27), whereas only 6 patients had isolated GHD. Sex steroids were supplemented in 21% of female patients (N=15) and 87% of male patients (N=77).

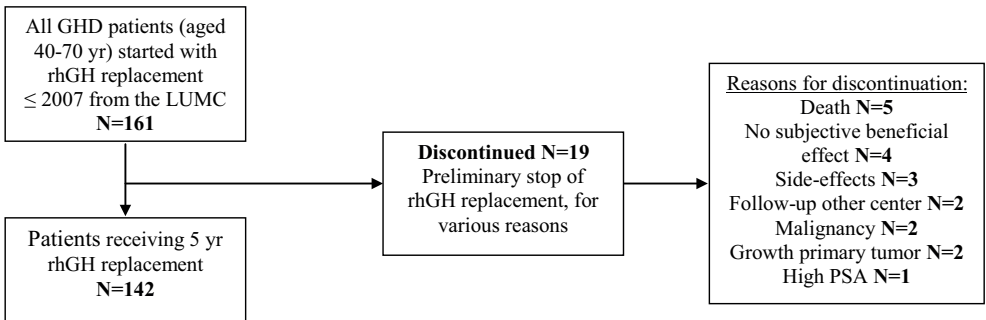


FIGURE 1. Flow chart of selection and follow-up of our middle-aged GHD cohort

One-hundred forty-two patients completed 5 years of rGH replacement (*Figure 1*). Reasons for preliminary discontinuation in 19 patients (11.8%) were: death (N=5), malignancy (N=2), growth of primary tumor (N=2), increase in prostate-specific antigen (PSA) level (N=1), lack of subjective benefit (N=4), side-effects: carpal tunnel syndrome (N=2), weight increase (N=1), and follow-up in other centers (N=2).

At 5 years of rGH replacement, GHD patients were compared with 1671 subjects from the general population (*Table 1*). Proportion of men and mean BMI were higher in patients than in the general population ($p=0.006$ and $p<0.001$, respectively).

TABLE 1. CHARACTERISTICS OF 161 MIDDLE-AGED GHD PATIENTS BEFORE THE START AND AFTER 5 YR OF RHGH REPLACEMENT AND 1671 CONTROLS REPRESENTING THE GENERAL POPULATION

Patient characteristics	GHD patients Before start of rhGH (N = 161)	GHD patients After 5 years of rhGH (N = 161)	General population [#] (N = 1671)
Sex, female (N(%))	72 (44.7%)	72 (44.7%)	937(56.1%)*
Age, years	54.7 ± 8.5 (40 – 70)	59.7 ± 8.5 (45 – 75)	56.0 ± 6.0 (45 – 66)*
BMI, kg/m ² (range)	27.5 ± 4.5 (19.6 – 57.1)	28.0 ± 4.5 (18.5 – 53.8)	26.3 ± 4.5 (17.2 – 57.1)*
Lipid-lowering drugs (N(%))	36 (22.3%)	78 (48.4%)	182 (10.9%)*
Antihypertensive medication (N(%))	36 (22.3%)	77 (47.8%)	402 (24.1%)*
Anti-diabetic medication (N(%))	8 (5.0%)	14 (8.7%)	52 (3.1%)*

Data are presented as mean ± SD, unless specified otherwise.

[#], The Leiderdorp cohort of the NEO Study was used as control group representing the general population (20).

*, $p<0.05$ (Patients after 5 years of rhGH replacement *vs* general population).

N, number of patients; GHD, Growth Hormone Deficiency; rhGH, recombinant human Growth Hormone replacement; BMI, body mass index.

GH dose, IGF-1 SDS and hydrocortisone dose during rGH replacement

Mean GH doses after dose titration (1 year) and 5 years of rGH replacement were, respectively, $0.35\pm0.16\text{mg/day}$ and $0.37\pm0.19\text{mg/day}$ ($p=0.399$). After 5 years of rGH replacement, females received higher mean GH doses than males ($0.40\pm0.23\text{mg/day}$ and $0.34\pm0.17\text{mg/day}$, respectively, $p=0.05$).

During the entire period of rGH suppletion, median of IGF-1 SDS was within the normal range. Median IGF-1 SDS increased significantly from the untreated state from to -1.30 (interquartile range (IQR) -2.43, +0.16) to 0.47 (IQR -0.50, +1.93) after 5 years of rGH suppletion ($p<0.001$). In males, median IGF-1 SDS increased from -1.17 (IQR -2.17, +0.92) before treatment to 0.42 (IQR -0.43, +2.03) after 5 years ($p<0.001$); in females, median IGF-1 SDS increased from -1.63 (IQR -3.04, +0.47) before treatment to 0.51 (IQR -0.57, +1.71) after 5 years ($p<0.001$).

Mean hydrocortisone doses were 17.43 ± 12.40 mg/day and 18.18 ± 10.81 mg/day at the start and after 5 years of rGH suppletion, respectively, indicating no significant change during follow-up. Mean fT4 levels did not significantly change during 5 years of rGH replacement (16.2 ± 3.7 pmol/l at the start and 16.8 ± 3.7 pmol/l after 5 years of rGH).

MS proportion in untreated GHD patients

Before the initiation of rGH replacement, 66 out of 161 patients fulfilled the NCEP-ATP III criteria, resulting in an MS proportion of 41.0% in untreated GHD (37.1% in males, 45.8% in females). Hyperglycaemia, hypertriglyceridaemia, low HDL-C levels, hypertension and abdominal obesity were present in respectively 13.0%, 59.0%, 47.8%, 68.3% and 35.4% of the patients.

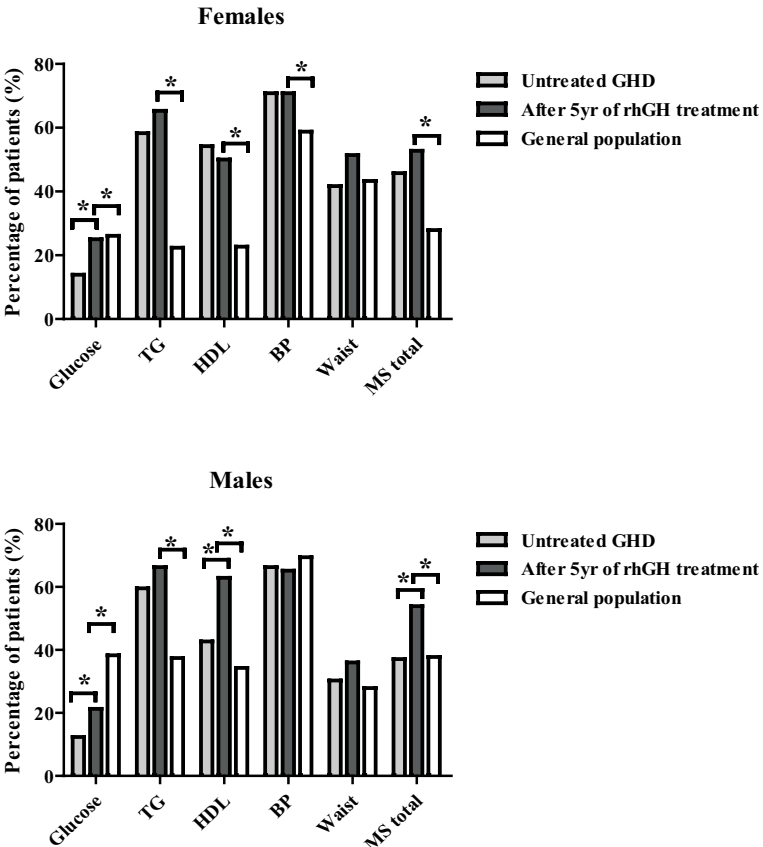
Effects of 5 years rGH replacement on the MS proportion in GHD adults

The MS proportion increased significantly from 41.0% before to 53.4% after 5 years of rGH replacement ($p=0.007$), especially due to an increased proportion of hyperglycaemia ($p=0.02$). In males, HDL-C levels decreased significantly ($p<0.001$). As indicated in *Figure 2*, the increase in MS proportion was most prominent in males (from 37.1% at the start of rGH replacement to 53.9% after 5 years, $p=0.005$). Exclusion of CO-GHD patients ($N=6$) did not significantly change these results. BMI did not significantly change over time, from 27.5 ± 4.5 kg/m² before rGH replacement to 28.0 ± 4.5 kg/m² after 5 years ($p=0.772$).

Comparison of MS proportion between GHD patients on chronic rGH replacement and the general population, adjusted for age and sex (Table 2)

In the general population, we observed an MS proportion of 30.3%. The MS proportion in chronically treated GHD patients was increased when compared to controls, taking into account age and sex (53.4% *vs.* 30.3%) (adjusted PR (95%CI) = 1.4(1.1-1.6), $p=0.001$) (*Table 2B*). For clinical interpretation, mean laboratory values were also presented (*Table 2A*).

FIGURE 2. Proportion of MS (components) in adult GHD patients, before the initiation of rGH replacement and after 5 years, in comparison to the general population, separated for females and males



We studied the MS proportion and its individual components in: (1) 5-year treated GHD patients in comparison to situation before the start of rGH replacement, using the Friedman test for related fractions; and (2) between GHD patients after 5 years of rGH replacement compared to the general population, using a log-binominal regression model with adjustments for age and BMI. Analyses were stratified for sex. MS was defined according to the NCEP-ATP III criteria (23;24).

*, $p < 0.05$

TG, triglycerides; HDL, high-density lipoprotein cholesterol; BP, blood pressure; waist, waist circumference; MS, metabolic syndrome; rGH, recombinant human Growth Hormone.

Comparison of MS proportion between GHD patients after 5 years of rGH replacement and the general population, additionally adjusted for BMI

After additional correction for BMI, besides age and sex, GHD patients still had a 1.3-times increased MS proportion when compared to the general population, despite 5 years of rGH replacement (adjusted PR (95%CI) = 1.3(1.1-1.5), $p=0.008$) (Table 2B). Both in male and female GHD patients, an significant increased MS proportion was seen when compared to the general population (Figure 2). When looking at the individual MS components, treated GHD patients differed from BMI-matched controls with respect to glucose, triglycerides and HDL-C levels (Table 2B).

TABLE 2. A. MEAN VALUES OF THE METABOLIC PARAMETERS OF THE MS AS INCLUDED IN THE NCEP-ATP III CRITERIA, IN ADULT GHD PATIENTS BEFORE THE START AND AFTER 5 YEARS OF RHGH REPLACEMENT, AND THE GENERAL POPULATION

	GHD patients Before start of rhGH (N = 161)	GHD patients After 5 yr of rhGH (N = 161)	General population (N = 1671)
Fasting glucose (mmol/l)	4.8 ± 0.8**	5.2 ± 1.6*	5.5 ± 1.1
Triglycerides (mmol/l)	2.0 ± 1.2	1.9 ± 0.9*	1.2 ± 0.8
HDL-C (mmol/l)	1.4 ± 0.4**	1.5 ± 0.5#	1.6 ± 0.5
Systolic blood pressure (mmHg)	134.0 ± 17.8	135.7 ± 17.6*	130.4 ± 16.9
Diastolic blood pressure (mmHg)	83.9 ± 9.3	83.4 ± 9.5	83.4 ± 10.1
Waist circumference (cm)	97.2 ± 11.8	98.6 ± 11.2*	91.2 ± 12.8

Values are expressed as mean ± SD.

N, number of patients; rhGH, recombinant human Growth Hormone replacement; GHD, Growth Hormone Deficiency; MS, metabolic syndrome; HDL-C, high-density lipoprotein cholesterol.

*, $p<0.01$; #, $p<0.05$ (GHD after 5 years rhGH replacement *vs* the general population).

**, $p<0.001$ (GHD after 5 years of rhGH replacement *vs* GHD before the start of rhGH suppletion).

TABLE 2. B. COMPARISON OF MS PROPORTION, ACCORDING TO THE NCEP-ATP III CRITERIA, BETWEEN MIDDLE-AGED GHD PATIENTS ON CHRONIC rhGH REPLACEMENT AND THE GENERAL POPULATION

	GHD patients (N = 161)	General population (N = 1671)	PR (95%CI) ¹	PR (95%CI) ²
Fasting glucose	37 / 161 (23.0%)	539 / 1663 (32.4%)	0.5 (0.4 – 0.7)*	0.5 (0.4 – 0.7)*
Triglycerides	106 / 161 (65.8%)	427 / 1666 (25.6%)	2.0 (1.7 – 2.4)*	2.0 (1.7 – 2.3)*
HDL-C	92 / 161 (57.1%)	418 / 1666 (25.1%)	2.0 (1.7 – 2.3)*	1.8 (1.5 – 2.2)*
Blood pressure	109 / 161 (67.7%)	1054 / 1668 (63.2%)	0.9 (0.8 – 1.0)	0.9 (0.8 – 1.1)
Waist	69 / 161 (42.9%)	597 / 1669 (35.8%)	1.1 (0.9 – 1.4)	1.0 (0.8 – 1.3)
Metabolic syndrome	86 / 161 (53.4%)	503 / 1660 (30.3%)	1.4 (1.1 – 1.6)*	1.3 (1.1 – 1.5)*

Values are expressed as number (percentage). Chronic rhGH replacement was defined as 5 years. MS was defined according to the NCEP-ATP III criteria (23;24). Log-binominal regression models were applied with robust standard errors, allowing direct estimations of PRs adjusted for age and sex, and, subsequently, with additional adjustments for BMI. N, number of patients; rhGH, recombinant human Growth Hormone replacement; GHD, Growth Hormone Deficiency; HDL-C, high-density lipoprotein cholesterol; waist, waist circumference; BMI, body mass index; PR, prevalence ratio; 95%CI, 95% confidence interval. RR1, adjusted for age and sex. RR2, adjusted for age, sex and BMI.

*, p<0.01.

DISCUSSION

The present study demonstrates a high MS proportion of 41.0% in middle-aged GHD patients before the start of treatment, which further increases to 53.4% after 5 years of rGH replacement. Especially a higher proportion of hyperglycaemia contributed to this increase, whereas the lipid profile was not affected. Despite chronic rGH replacement, GHD patients still have a 1.3-times increased MS proportion when compared to the general population, independently of age, sex and BMI. GHD patients remain to have a different metabolic profile, characterized by hypertriglyceridaemia and low HDL-C, whereas the proportion of hyperglycaemia is lower than that observed in the general population.

In middle-aged GHD patients, the increased cardiovascular risk profile is a main justification of rGH replacement. Important components of this risk are dyslipidemia, manifesting as raised levels of LDL-C and triglycerides, and reduced HDL-C levels (29). In addition, increased fat mass, reduced insulin sensitivity, and increases in inflammatory

markers are often present (29;30). Mortality is substantially higher in GHD adults than in general population, particularly among women (1;2). RGH replacement has beneficial metabolic effects in the short-term, including improvement in the lipid profile, body composition and cardiac function (3), which were reported to be sustained during longer follow-up (14;18), although glucose and insulin levels increase. However, despite improvement of several cardiovascular risk factors, MS prevalence remains increased (4;5;19). Obvious limitations of the few available long-term studies are the lack of an untreated GHD control population and difficulties with physiological rGH replacement.

This study is the first to investigate the metabolic profile of chronically treated GHD patients in a controlled manner, taking into account not only age and sex, but also BMI. In this respect, the Leiderdorp cohort represents the general population with comparable BMI distribution to that observed in other Dutch population-based studies (<http://www.rivm.nl/nldemaat.rivm.2012>). We demonstrated that, independent of BMI, chronically treated GHD patients have a persistently different metabolic profile than the general population, with a lower prevalence of insulin resistance, but an adverse lipid profile. It is remarkable that although this GHD cohort is thought to be adequately substituted, as reflected by mean IGF-1 SDS within the physiological range as well as no hydrocortisone oversubstitution, the metabolic profile after chronic rGH replacement resembles the profile of an untreated GHD patient, with predominantly lipid abnormalities. Additional analyses in which we compared the GHD patients before starting rGH replacement with general population show similar results, suggesting that the metabolic profile is not significantly influenced by long-term rGH replacement (*data not shown*).

These findings give rise to several questions. First, there is an increasing awareness of intrinsic imperfections of endocrine replacement therapy in general (31), that may also well apply to GHD patients. It is questioned whether circulating total IGF-1 levels truly reflect peripheral IGF-1 activity, since several earlier reports indicate that measuring free unbound IGF-1 levels and IGF-1 bioactivity better reflect GH/IGF-1 status (32). Further study is needed to identify sensitive biomarkers to monitor rGH replacement therapy. Second, it is previously shown that there is an optimum for GH dosing with respect to insulin resistance (33). In this respect, further lowering of the GH dose is probably needed to minimize insulin resistance. On the other hand, the MS proportion in GHD patients remained increased despite chronic rGH replacement, and resembles the metabolic profile of untreated GHD, suggesting that GHD may have irreversible effects on the cardiovascular system. In the context of increasing evidence for, on the one hand, a limited or even negative role of GH and IGF-1 in cancer, longevity and cardiovascular disease (34-36), and, on the other hand, the limited evidence for benefit of rGH substitution in the elderly GHD population and long-term positive effects on QoL (37), long-term rGH use in GHD adults should be critically re-evaluated. Third, we cannot exclude co-existing hypothalamic damage, especially in case of a history of

large tumors with suprasellar extension (38). Obesity is a well-recognized complication in patients treated for tumors in the hypothalamic-pituitary region, which is attributed to compression of surrounding tissues (39). This hypothalamic damage could be an independent factor influencing the metabolic profile in GHD patients. Finally, most patients had multiple pituitary hormone deficiencies, making distinction between effects of rGH replacement or effects of suboptimal or excessive replacement therapy of other hormones difficult.

Several validated definitions can be applied for the definition of the MS, precluding direct comparisons between studies (40). We used the NCEP-ATP III criteria (23;24) to be comparable to our previous studies on MS (5;19). In the NCEP-ATP III criteria, the use of antihypertensive, anti-diabetic and lipid-lowering medication is incorporated. In the original description of the NCEP-ATP III criteria, statins are not specifically mentioned. However, the lipid-lowering effects of statins are well-established in the general population, and thereby significantly influencing the lipid criteria of the MS (41).

We found sex-specific differences in MS proportion and the metabolic response to rGH replacement. In untreated GHD females, MS proportion was very high compared to the general population. This might be in line with a recent report of Van Bunderen *et al.*, describing an increased risk of cardiovascular death in GHD females, despite rGH substitution (42). The increased incidence in cardiovascular deaths in females might partly be attributed to the more atypical presentation of cardiovascular diseases (43) or inadequately substituted hypogonadism causing preterm menopause or hypocortisolism. The most predominant increase of the MS proportion during rGH replacement in males further supports that the cardiovascular risk pattern in GHD patients is sex-specific.

When compared to other population-based control cohorts, such as the MORGEN cohort (1993-1997) or the Tromsø Study (1994-1995) (44), the MS proportion found in the Leiderdorp cohort was nearly doubled (30.3% in Leiderdorp cohort *vs* 15.7% in MORGEN cohort and 19.2% (males) and 17.8% (females) in the Tromsø Study) (5). Since the same criteria for MS were applied, we expect that this increase in MS proportion most likely reflects the increase in BMI and an increased use of antihypertensive medication in the general population over 15 years, and emphasizes the importance for a contemporary comparison group from the general population.

A strength of the present study is the availability of large recent population-based control data and the inclusion of analysis on the crude metabolic laboratory data in addition to the age-, sex, and BMI-adjusted analysis using the NCEP-ATP III criteria of MS. Our relatively large middle-aged GHD cohort reflects the best-case scenario in which hormonal supplementation of all pituitary axes is optimized and adequately monitored. A potential limitation could be the fact that we evaluated the MS proportion

in the untreated GHD situation only in patients in whom rGH replacement was actually initiated. This could introduce a selection bias, since GHD patients without receiving rGH replacement are likely to differ from patients that actually received rGH replacement. Second, it has to be noted that the patients in our GHD cohort are very heterogeneous with respect to the cause of GHD, failure of other pituitary hormone axes and history of cranial irradiation and/or surgery, which may have influenced the results. Third, we only reported the metabolic parameters included in the MS according to the NCEP-ATP III criteria, not for example LDL-C or body fat percentages, which are also GH-dependent parameters. Another feature that needs to be addressed is the presence of interactions between glucocorticoids and rGH during substitution. In the past, hydrocortisone suppletion in hypopituitary patients frequently resulted in supraphysiological cortisol levels, which accounted for at least part of the adverse metabolic profile in these patients. However, since most patients received hydrocortisone doses of 20 mg/day or less during the entire follow-up period and cortisol levels were highly monitored, we expect that in our cohort hydrocortisone oversuppletion is not likely to have negatively influenced the results. In this respect, it has also be noted that minor thyroid dysfunction and insufficient thyroid replacement has a significant independent negative impact on cardiovascular risk factors in hypopituitary adults (45), further emphasizing the importance of adequate hormonal suppletion of all deficient pituitary axes. In addition, it would be ideally preferred to include a longitudinally follow-up control group for comparison; however, such a control group was not available in the Netherlands. Finally, it has to be noted that a priori GHD patients are more likely to receive anti-diabetic and/or lipid-lowering medication than controls in case of lipid and/or glucose abnormalities, since these patients are regularly followed up at the Dept. of Endocrinology, which is not the case for controls without a history of pituitary disease.

In conclusion, despite 5 years of rGH replacement, GHD patients still have a different metabolic profile and a higher MS proportion than the general population. These differences were independent of BMI, and resemble the metabolic profile of untreated GHD patients. This emphasizes the need for further study to establish whether rGH replacement is actually beneficial in the long-term, adequately incorporating the cost-effectiveness, QoL, and the potential negative effects of GH/IGF-1 on cancer, longevity and cardiovascular risk.

ACKNOWLEDGEMENTS

We would like to thank Dr. N. van Duinen, Dr. N.E. Kokshoorn, Dr. A.A. van der Klaauw, E. de Lange and D.M.M.M. Adoptie for their help with the data collection of GHD patients during the yearly GH control visits at the outpatient clinic.

In addition, we would like to thank the NEO Study Group, the NEO research team, and participants of the NEO Study. The NEO Study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Centre, and by the Leiden University, Research Profile Area 'Vascular and Regenerative Medicine'.

REFERENCE LIST

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990;336:285-288.
2. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet* 2001;357:425-431.
3. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004;89:2192-2199.
4. Attanasio AF, Mo D, Erfurth EM, Tan M, Ho KY, Kleinberg D, Zimmermann AG, Chanson P. Prevalence of metabolic syndrome in adult hypopituitary growth hormone (GH)-deficient patients before and after GH replacement. *J Clin Endocrinol Metab* 2010;95:74-81.
5. van der Klaauw AA, Biermasz NR, Feskens EJ, Bos MB, Smit JW, Roelfsema F, Corssmit EP, Pijl H, Romijn JA, Pereira AM. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. *Eur J Endocrinol* 2007;156:455-462.
6. Cenci MC, Conceicao FL, Soares DV, Spina LD, Brasil RR, Lobo PM, Michmacher E, Vaisman M. Impact of 5 years of growth hormone replacement therapy on cardiovascular risk factors in growth hormone-deficient adults. *Metabolism* 2008;57:121-129.
7. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG. Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. *J Clin Endocrinol Metab* 2000;85:3762-3769.
8. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012;166:787-795.
9. Giavoli C, Porretti S, Ronchi CL, Cappiello V, Ferrante E, Orsi E, Arosio M, Beck-Peccoz P. Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. *Metabolism* 2004;53:740-743.
10. Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D. The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. *J Clin Endocrinol Metab* 1999;84:2596-2602.
11. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001;86:4657-4665.
12. Gotherstrom G, Bengtsson BA, Sunnerhagen KS, Johannsson G, Svensson J. The effects of five-year growth hormone replacement therapy on muscle strength in elderly hypopituitary patients. *Clin Endocrinol (Oxf)* 2005;62:105-113.
13. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *Eur J Endocrinol* 2007;156:55-64.
14. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. *J Clin Endocrinol Metab* 2007;92:1442-1445.
15. Gotherstrom G, Elbornsson M, Stibrant-Sunnerhagen K, Bengtsson BA, Johannsson G, Svensson J. Ten years of growth hormone (GH) replacement normalizes muscle strength in GH-deficient adults. *J Clin Endocrinol Metab* 2009;94:809-816.

16. Roemmler J, Kuenkler M, Schneider HJ, Dieterle C, Schopohl J. Comparison of glucose and lipid metabolism and bone mineralization in patients with growth hormone deficiency with and without long-term growth hormone replacement. *Metabolism* 2010;59:350-358.
17. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO. Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. *J Clin Endocrinol Metab* 2002;87:2121-2127.
18. van der Klaauw AA, Romijn JA, Biermasz NR, Smit JW, van DJ, Dekkers OM, Roelfsema F, Pereira AM. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. *Eur J Endocrinol* 2006;155:701-708.
19. Claessen KM, Appelman-Dijkstra NM, Adoptie DM, Roelfsema F, Smit JW, Biermasz NR, Pereira AM. Metabolic profile in growth hormone-deficient (GHD) adults after long-term recombinant human growth hormone (rGH) therapy. *J Clin Endocrinol Metab* 2013;98:352-361. doi:10.1210/jc.2012-2940 [pii];10.1210/jc.2012-2940 [doi].
20. de Mutsert R, den Heijer M, Rabelink AJ, Smit JWA, Romijn JA, Rabe KF, Jukema JW, de Roos A, le Cessie S, Middelborg, Rosendaal. The Netherlands Epidemiology of Obesity (NEO) Study: study design and data collection.
21. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. *J Clin Endocrinol Metab* 1997;82:129-135.
22. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008;18:1-16.
23. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433-438.
24. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-2752.
25. Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990;44:45-60.
26. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 1998;50:166-176.
27. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;159:702-706.
28. Thompson ML, Myers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done? *Occup Environ Med* 1998;55:272-277.
29. Rosen T, Eden S, Larson G, Wilhelmsen L, Bengtsson BA. Cardiovascular risk factors in adult patients with growth hormone deficiency. *Acta Endocrinol (Copenh)* 1993;129:195-200.
30. Johansson JO, Landin K, Tengborn L, Rosen T, Bengtsson BA. High fibrinogen and plasminogen activator inhibitor activity in growth hormone-deficient adults. *Arterioscler Thromb* 1994;14:434-437.
31. Romijn JA, Smit JW, Lamberts SW. Intrinsic imperfections of endocrine replacement therapy. *Eur J Endocrinol* 2003;149:91-97.
32. Varewijck AJ, Lamberts SW, Uitterlinden P, Hofland LJ, Janssen JA. IGF-I bioactivity better reflects growth hormone deficiency than total IGF-I. *J Clin Endocrinol Metab* 2011;96:2248-2254. doi:10.1210/jc.2011-0051 [pii];10.1210/jc.2011-0051 [doi].

33. Brugts MP, van Duijn CM, Hofland LJ, Witteman JC, Lamberts SW, Janssen JA. Igf-I bioactivity in an elderly population: relation to insulin sensitivity, insulin levels, and the metabolic syndrome. *Diabetes* 2010;59:505-508. doi:db09-0583 [pii];10.2337/db09-0583 [doi].
34. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012;61:1315-1322.
35. Hartman ML, Xu R, Crowe BJ, Robison LL, Erfurth EM, Kleinberg DL, Zimmermann AG, Woodmansee WW, Cutler GB, Jr., Chipman JJ, Melmed S. Prospective safety surveillance of GH-deficient adults: comparison of GH-treated vs untreated patients. *J Clin Endocrinol Metab* 2013;98:980-988. doi:jc.2012-2684 [pii];10.1210/jc.2012-2684 [doi].
36. Rozing MP, Westendorp RG, Frolich M, de Craen AJ, Beekman M, Heijmans BT, Mooijaart SP, Blauw GJ, Slagboom PE, van HD. Human insulin/IGF-1 and familial longevity at middle age. *Aging (Albany NY)* 2009;1:714-722.
37. Kokshoorn NE, Biermasz NR, Roelfsema F, Smit JW, Pereira AM, Romijn JA. GH replacement therapy in elderly GH-deficient patients: a systematic review. *Eur J Endocrinol* 2011;164:657-665.
38. Karavitaki N, Cudlip S, Adams CB, Wass JA. Craniopharyngiomas. *Endocr Rev* 2006;27:371-397. doi:er.2006-0002 [pii];10.1210/er.2006-0002 [doi].
39. Iughetti L, Bruzzi P. Obesity and craniopharyngioma. *Ital J Pediatr* 2011;37:38. doi:1824-7288-37-38 [pii];10.1186/1824-7288-37-38 [doi].
40. Nilsson PM, Engstrom G, Hedblad B. The metabolic syndrome and incidence of cardiovascular disease in non-diabetic subjects--a population-based study comparing three different definitions. *Diabet Med* 2007;24:464-472. doi:DME2142 [pii];10.1111/j.1464-5491.2007.02142.x [doi].
41. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. *Circulation* 1998;97:946-952.
42. van Bunderen CC, van N, I, Arwert LJ, Heymans MW, Franken AA, Koppeschaar HP, van der Lely AJ, Drent ML. Does growth hormone replacement therapy reduce mortality in adults with growth hormone deficiency? Data from the Dutch National Registry of Growth Hormone Treatment in adults. *J Clin Endocrinol Metab* 2011;96:3151-3159.
43. Dey S, Flather MD, Devlin G, Brieger D, Gurfinkel EP, Steg PG, Fitzgerald G, Jackson EA, Eagle KA. Sex-related differences in the presentation, treatment and outcomes among patients with acute coronary syndromes: the Global Registry of Acute Coronary Events. *Heart* 2009;95:20-26.
44. Herder M, Arntzen KA, Johnsen SH, Mathiesen EB. The metabolic syndrome and progression of carotid atherosclerosis over 13 years. The Tromso study. *Cardiovasc Diabetol* 2012;11:77. doi:1475-2840-11-77 [pii];10.1186/1475-2840-11-77 [doi].
45. Klose M, Marina D, Hartoft-Nielsen ML, Klefter O, Gavan V, Hilsted L, Rasmussen AK, Feldt-Rasmussen U. Central hypothyroidism and its replacement have significant influence on cardiovascular risk factors in adult hypopituitary patients. *J Clin Endocrinol Metab* 2013;98:9. doi:10.1210/jc.2013-1610.

Chapter 7

**Effects of up to 15 years treatment with recombinant
human GH (rGH) on bone metabolism of adults with
Growth Hormone Deficiency (GHD):
The Leiden Cohort Study**

Natasha M. Appelman-Dijkstra, Kim M.J.A. Claessen,
Neveen A.T. Hamdy, Alberto M. Pereira en Nienke R. Biermasz

Clin Endo 2014 Nov;81(5):727-35.

ABSTRACT

Background: Growth Hormone Deficiency (GHD) in adulthood is associated with a decreased bone mineral density (BMD), a decreased bone mineral content (BMC) and an increased fracture risk. Recombinant human GH (rGH) replacement induces a progressive increase in BMD under up to 5-7 years of treatment. Data on longer follow-up are however scarce.

Methods: Two-hundred and thirty adult GHD patients (mean age 47.1 years, 52.6% female) receiving rGH replacement for ≥ 5 years were included in the study. Bone turnover markers, BMC and BMD and T-scores at the lumbar spine and femoral neck were evaluated at baseline, and after 5, 10 and 15 years of rGH supplementation. In addition, clinical fracture incidence was assessed.

Results: Mean lumbar spine BMD gradually increased during long-term rGH replacement therapy ($p=0.002$). Lumbar spine BMC and T-scores increased during the first 10 years of rGH replacement, but remained stable thereafter. Largest effects of rGH supplementation were found in men. Use of bisphosphonates did not impact additional beneficial effects in the long-term. Low baseline BMD and high GH dose at 1 year significantly affect the change in BMD and BMC over time. Clinical fracture incidence during long-term rGH supplementation was 20.1 / 1000 py.

Conclusions: Fifteen years of rGH supplementation in GHD adults resulted in a sustained increase in BMD values at the lumbar spine, particularly in men. Clinical fracture incidence was observed to be similar to the incidence in age- and sex-matched controls from general population.

INTRODUCTION

Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) are important regulators of bone growth and metabolism during the life span (1). IGF-1 mediates most of the effects of GH on skeletal metabolism via the IGF-1 receptor (2), although GH also exerts direct effects on bone (3). GH and IGF-1 act as anabolic hormones on bone by stimulating proliferation, and to some extent, differentiation of osteoblasts. Furthermore, osteoclastic bone resorption is stimulated, resulting in an overall increase in bone remodeling (3;4).

Untreated Growth Hormone Deficiency (GHD) is characterized by low bone turnover, decreased bone mineral density (BMD) and bone mineral content (BMC) (5-7). Recombinant human GH (rGH) replacement therapy was shown to increase bone turnover as reflected by an increase in bone formation and bone resorption markers, resulting in an initial decline in (or unchanged) BMD, followed by a small increase of about 1-2% in the first 2 years of rGH replacement due to a net positive balance in bone formation (3;8).

Data on the effects of long-term rGH replacement on BMD are, however, scarce. Previous studies reported that rGH supplementation induces a progressive increase in BMD and BMC up to 5-7 years of treatment, which is followed by a plateau phase (9-12). Götherström *et al.* found an increase of total body and lumbar spine BMD and BMC even up to 10 years of rGH replacement, whereas femoral neck BMD and BMC reached a peak value after 5-7 years (13). Only one study reported the effects of 15 years of rGH supplementation, showing a sustained increase in total body and lumbar BMD and BMC over 15 years, whereas femoral neck BMD and BMC returned towards baseline values after 7 years (14).

The primary aim of our study was to assess the effects of 5, 10 and 15 years of rGH replacement on bone metabolism in a well-characterized cohort of adult GHD patients. In addition, we aimed to identify factors influencing the bone response to rGH replacement and we studied the incidence of clinical fractures during long-term rGH supplementation in GHD adults.

PATIENTS AND METHODS

Patients

Consecutive patients with an established diagnosis of childhood-onset (CO) or adulthood-onset (AO) GHD who were followed up at the Department of Endocrinology and Metabolic Diseases of the Leiden University Medical Center were collected in a database from 1994 onwards. In the present study, we selected all GHD patients who started rGH replacement therapy in or before 2007, and, therefore, had the potential to receive rGH replacement therapy for ≥ 5 years. We arbitrarily defined chronic treatment as replacement for ≥ 5 years, since such a period will reflect a stable situation without confounding by ongoing changes induced by the start and titration of rGH replacement (15;16). This strategy resulted in a total inclusion of 230 eligible GHD patients, of which part of the included patients has been previously described (17).

GHD was diagnosed on the basis of a GH peak response to the Insulin Tolerance Test (ITT) $< 3\mu\text{g/l}$ (glucose nadir $< 2.2\text{mmol/l}$) according to current guidelines or Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) with BMI-adjusted GH cut-offs, in case of contraindications for ITT (18;19). All patients receiving rGH replacement during childhood were retested at the time of transition to the adult outpatient clinic, after treatment cessation for > 3 months. GHD was treated according to a standardized Leiden protocol, in which patients received rGH replacement (Genotropin Pharmacia/Pfizer, Skokie, IL; Zomacton Ferring, Troy Hills, NJ; or Norditropin Novo Nordisk, Princeton, NJ), injected subcutaneously in the evening. GH starting dose was 0.2mg/day , which was individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at SDS between 0 and +2 (*i.e.* titration phase). When stable plasma concentrations were reached, this individualized dose was continued during the entire study period and adjusted according to the IGF-1 standard deviation score (SDS). After dose titration, patients were evaluated at least yearly at the outpatient clinic, according to a standard protocol.

Adrenocorticotrophic hormone (ACTH) deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55mmol/l) after an ITT or a corticotropin-releasing hormone stimulation test in case of an ITT could not be performed. Standard hydrocortisone replacement was 20 mg divided in 3 doses, individually adjusted when necessary. Premenopausal women were classified as gonadotropin-deficient when secondary amenorrhea persisted for > 1 year. In men, gonadotropin deficiency was defined as a testosterone level $< 8.0\text{nmol/l}$. Thyroid-stimulating hormone (TSH) deficiency was defined as total thyroxine (T_4) or free T_4 levels below the normal laboratory reference range ($< 10\text{pmol/l}$). Hypopituitarism was supplemented by hydrocortisone, L-thyroxine, testosterone in men, and/or estrogen in combination with

progestagens (in premenopausal women only) and adequacy of supplementation was regularly controlled and adjusted as required.

Patients were treated with bone-modifying medication according to the discretion of their attending physicians.

Methods

Data were collected from the patients' electronic hospital records on the following efficacy parameters which were assessed on a yearly basis after establishment of diagnosis and start of treatment at the outpatient clinic:

1. Anthropometric parameters: body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).
2. Biochemical parameters: data were collected on serum concentrations of IGF-1, GH, TSH, fT4, LH, FSH, estradiol, testosterone and prolactin, which were collected after an overnight fast. Serum GH was measured with a sensitive IFMA (Wallac, Turku, Finland), specific for the 22 kDa GH protein (detection limit: $0.01\mu\text{g}/\text{l}$, interassay coefficient of variation (CV): 1.6-8.4% of $0.01\text{-}15.38\mu\text{g}/\text{l}$) from 1992 onwards. For the conversion of $\mu\text{g}/\text{l}$ to mU/l, multiply by 2.6. Before 1992, GH was measured by RIA (Biolab, Serona, Coissins, Switzerland), detection limit: $0.5\text{mU}/\text{l}$, with an interassay CV $<5\%$; for the conversion of $\mu\text{g}/\text{l}$ to mU/l, multiply by 2. From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of $1.5\text{ nmol}/\text{l}$ and an interassay CV less than 11%. IGF-1 is expressed as SD score for age- and gender-related normal levels determined in the same laboratory (Janssen et al. JCEM 1997). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and $75\text{nmol}/\text{l}$ were 5.0 and 7.5%, respectively. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (21;22).
3. Markers of bone metabolism: Data on serum creatinine, calcium, albumin, alkaline phosphatase, vitamin D, PTH, procollagen type 1 amino-terminal propeptide (P1NP, bone formation) and β -crosslaps concentrations (bone resorption) (the latter two markers available from 2006 onwards). B-crosslaps and P1NP concentrations were measured by an electrochemolumiscent immunoassay with a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). Vitamin D25OH was measured by RIA (Incstar/DiaSorin, Stillwater, MN, USA).

4. BMD and BMC: Data on BMD and BMC measurements were collected. BMD was measured yearly at the lumbar spine (L1-L4) and femoral neck using dual energy X-ray absorptiometry (DXA) (Hologic QDR 1000 (until 2008) and Hologic QDR 4500 (thereafter), Hologic Inc., Waltham, MA, USA) equipped with reference values based on the National Health and Nutrition Examination Survey (NHANES III) from the time of start of rGH replacement onwards. Coefficient of variation of lumbar spine and femoral neck BMD measurements was 1%. An in-house comparison using 300 measurements provided a conversion formula for all Hologic 1000 measurements which were converted for comparison for the laterly acquired Hologic 4500 BMD measurements. BMD T-scores were used to reflect the number of standard deviations (SD) above or below the mean for a healthy age- and sex-matched adult. World Health Organization (WHO) criteria were used to define osteopenia (T-score between -1.0 and -2.5) and osteoporosis (T-score $\leq$ -2.5). BMC was measured at the lumbar spine using the same DXA apparatus.
5. Fracture data: Specific information on clinical fractures was retrieved from the medical charts. No routine spine radiographs were performed to assess the presence of vertebral fractures; however, additional radiographs were made in case of height loss >3cm or specific complaints at the discretion of the treating physician. Vertebral fractures were defined as clinical fractures (i.e. pain or decrease in height) and/or radiographic fractures in case of available radiographs, defined as the presence of Genant $\geq$ 2(20).
6. Data on medication use and co-morbidity was also obtained from electronic files.

Statistical analysis

SPSS for Windows, Version 20.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Results are presented as mean $\pm$ SD, unless stated otherwise. ANOVA repeated measurements with Bonferroni correction for multiple comparisons were used to compare anthropometric parameters, bone markers, BMD, T-scores and BMC between baseline and after rGH treatment. Although 19 patients did not complete 5 years of rGH replacement for various reasons (*vide infra*), these patients were included using the last observation carried forward method according to the intention-to-treat approach used. In addition, we identified factors influencing the response to long-term rGH supplementation on bone by incorporating age, sex, childhood-onset *versus* adult-onset GHD, hydrocortisone use, GH dose, hypogonadism, number of pituitary deficiencies (<2 *vs* $\geq$ 2), bisphosphonate use, baseline Vitamin D levels and baseline BMD values in a linear regression model. We repeated the analyses after exclusion of patients using bisphosphonates at any time point during the follow-up period. Finally, we calculated the incidence rate of clinical fractures during rGH replacement.

RESULTS

Description of patients

We studied 230 adult GHD patients (202 adulthood-onset (AO) / 28 childhood-onset (CO)) of whom baseline characteristics are shown in *Table 1*. Pituitary deficiency was mainly caused by pituitary tumors or their treatment. Most patients had multiple pituitary hormone deficiencies. Two-hundred and eleven patients completed 5 years of rGH replacement therapy (*Figure 1*). Reasons for earlier discontinuation in 19 patients (8.3%) were: death (N=5), malignancy (N=2), growth of primary tumor (N=2), increase in prostate-specific antigen (PSA) level (N=1), lack of subjective benefit (N=4), side-effects: carpal tunnel syndrome (N=2), weight increase (N=1), and follow-up in other centers (N=2). There was no difference between patients completing 5 years of rGH replacement and non-completers with respect to age, sex, pituitary hormone deficiencies or co-morbidity. Ninety-eight and 43 patients completed, respectively, 10 and 15 years of rGH replacement.

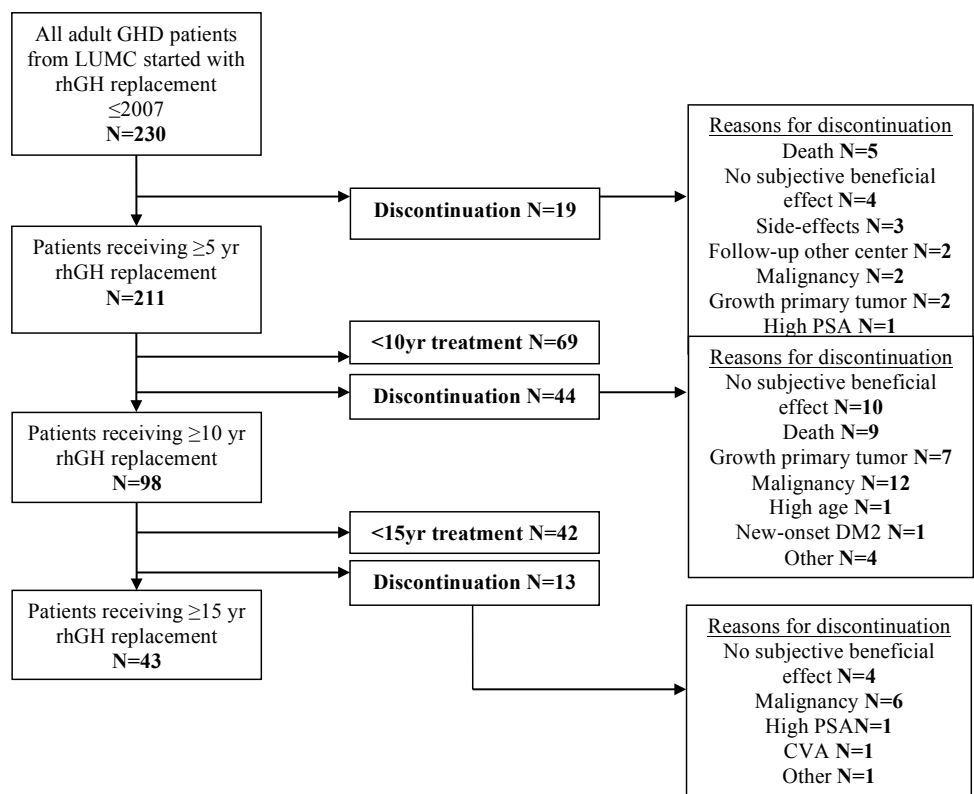


FIGURE 1.
Flow chart of selection and follow-up of our adult GHD cohort (N=230)

TABLE 1. Baseline characteristics of 230 adult GHD patients

Patient characteristics	GHD patients (N = 230)
Sex, female / male (N)	121 / 109
Age at start rhGH replacement, years (range)	47.1 ± 14.9 (15 – 81)
AO / CO (N)	202 / 28
BMI, kg/m ² (range)	27.3 ± 5.1 (16.9 – 57.1)
Etiological diagnosis of GHD (N)	
NFA	76
Functioning adenoma	68
Craniopharyngeoma	21
Cerebral malignancy	12
Congenital	27
Other causes	26
Surgery, TS / TC (N)	136 / 38
Radiotherapy (N)	102
Pituitary deficiencies (N)	
TSH	190
ACTH	197
FSH / LH	173
ADH	51
Isolated GHD	10
Bisphosphonates (N)	10
Calcium supplementation (N)	84
Vitamin D supplementation (N)	85

Data are presented as mean ± SD, unless specified otherwise.

N, number of patients; GHD, Growth Hormone Deficiency; rhGH, recombinant human Growth Hormone replacement; AO, adult-onset GHD; CO, childhood-onset GHD; BMI, body mass index; NFA, non-functioning adenoma; TS, transsphenoidal; TC, transcranial; TSH, thyroid stimulating hormone; ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADH, anti-diuretic hormone; Vitamin D, vitamin D.

With respect to concomitant medication, at baseline, 10 patients (4.3%) received bisphosphonates, and, respectively, 84 (36.5%) and 85 patients (37.0%) received calcium and Vitamin D supplements. After respectively 5, 10 and 15 years of rGH replacement 28 (12.2%), 19 (19.4%) and 8 (18.6%) patients used bisphosphonates. Thirty-eight patients used bisphosphonates at any time point during the study. Mean duration of bisphosphonate use was 6.9 ± 4.3 years. Indications for bisphosphonate use

were osteoporosis (N=23), clinical fractures (N=10), osteoporosis and clinical fractures (N=8) and other (N=7). Mean age at start of bisphosphonate-therapy was 52.6 ± 13.5 years.

GH dose, IGF-1 SDS and hydrocortisone dose during rGH replacement

Mean GH doses after dose titration (*i.e.* after 1 year) and 5 years of rGH replacement were, respectively, 0.39 ± 0.20 mg/day and 0.43 ± 0.27 mg/day ($p=0.016$ compared to 1 yr dose). Mean doses after, respectively, 10 and 15 years of rGH replacement were 0.44 ± 0.26 mg/day (NS compared to 1 yr dose) and 0.39 ± 0.21 mg/day ($p=0.041$ compared to 1 yr dose).

Mean IGF-1 SDS was within the normal range during the entire study period, and increased significantly from -1.09 ± 2.46 at baseline to 0.66 ± 2.01 after 5 years and was 1.24 ± 2.45 after 10 years (both $p<0.01$ compared to baseline). In males, mean IGF-1 SDS increased significantly from -0.60 ± 2.21 at baseline to 0.95 ± 1.88 after 5 years ($p=0<001$) and to 1.64 ± 2.57 after 10 years ($p=0.011$). In females, mean IGF-1 SDS increased significantly from -1.73 ± 2.63 at baseline to 0.35 ± 2.11 after 5 years of rGH replacement ($p<0.001$) and to 0.83 ± 2.29 after 10 years ($p=0.003$) (Figure 2).

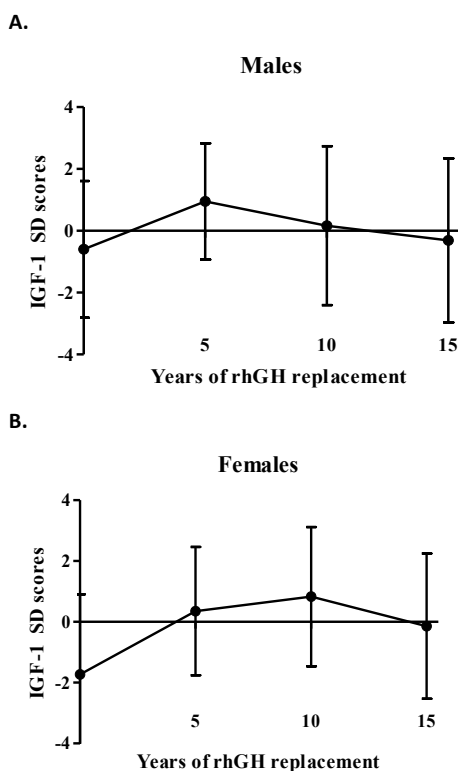


FIGURE 2. Mean IGF-1 SD scores during 15 years of rhGH replacement, stratified for males (A) and females (B)

Mean hydrocortisone doses were 24.1 ± 7.3 mg/day, 21.4 ± 4.10 mg/day and 21.1 ± 4.3 mg/day at baseline, after 10 and after 15 years of rGH replacement, respectively, indicating no change in hydrocortisone dose during rGH replacement.

Biochemical bone markers

Baseline calcium and Vitamin D levels were, respectively, 2.40 ± 0.24 mg/dL and 60.3 ± 26.7 nmol/L. After 5 years, mean calcium and Vitamin D levels were 2.40 ± 0.11 mg/dL (NS compared to baseline) and 63.3 ± 24.5 nmol/L ($p=0.02$ compared to baseline), respectively, and 2.39 ± 0.26 mg/dL and 61.8 ± 26.0 nmol/L (both NS compared to baseline) after 10 years of rGH replacement.

At baseline, P1NP and β -crosslaps concentrations were only available in 12 and 30 patients and were therefore not reported. After 5 years of rGH replacement, mean P1NP and β -crosslaps concentrations were 45.3 ± 26.6 ng/mL and 0.42 ± 0.24 ng/mL, respectively, and 48.4 ± 25.4 ng/mL and 0.71 ± 2.19 ng/mL after 10 years. After 15 years of rGH replacement, mean P1NP and β -crosslaps concentrations were 42.1 ± 24.7 ng/mL and 0.37 ± 0.20 ng/mL. These data indicate normal bone turnover throughout the entire study period.

BMC and BMD

Mean height, weight and BMI at the different time points during rGH replacement are shown in *Table 2*. Mean lumbar spine BMD gradually increased during 15 years of rGH replacement, from 0.98 ± 0.15 g/cm² at baseline to 1.02 ± 0.14 g/cm² after 15 years ($p=0.002$) (*Table 2*). After 15 years of rGH supplementation, mean lumbar spine BMD was 4% above the baseline value. Mean lumbar spine T-scores increased during the first 10 years of rGH replacement from -0.69 ± 1.30 to -0.43 ± 1.33 ($p<0.001$), and then stayed at a constant level. Lumbar spine BMC increased throughout the first 10 years of rGH replacement being 4% above the baseline level after 10 years (from 63.2 ± 15.4 kg to 65.8 ± 18.0 kg), and then reached a plateau. Similar results were obtained after stratification for bisphosphonate use, indicating that in the long-term there was no beneficial effect impacted from additional bisphosphonate use (*Figure 3*).

Left femoral neck BMD did not change during 15 years of rGH replacement (*Table 2*), also after exclusion of patients with additional bisphosphonate therapy.

Osteopenia and osteoporosis

At baseline, 29.1% and 31.7% of the patients has osteopenia (T-score <-1.0 SD) in the lumbar spine and femoral neck, respectively. After 10 years of rGH replacement, 37.8% of the patients has osteopenia at the lumbar spine and 35.7% at the femoral

TABLE 2. Effects of 15 years of rhGH replacement in 230 adults with GHD on BMC, BMD and T-scores measured using DXA, and anthropometric parameters

	Baseline (N=230)	5 years rhGH (N=230)	10 years rhGH (N=98)	15 years rhGH (N=43)
DXA lumbar (L1-L4) spine				
BMC (kg)	63.2 ± 15.4	65.5 ± 15.4**	65.8 ± 18.0*	64.4 ± 14.2
BMD (g/cm ²)	0.98 ± 0.15	1.01 ± 0.14**	1.03 ± 0.16**	1.02 ± 0.14*
T-score (SD)	-0.69 ± 1.30	-0.58 ± 1.29**	-0.43 ± 1.33**	-0.55 ± 1.06
DXA left femoral neck				
BMD (g/cm ²)	0.83 ± 0.14	0.83 ± 0.15	0.84 ± 0.16	0.84 ± 0.14
T-score (SD)	-0.94 ± 1.30	-0.94 ± 1.06	-0.77 ± 1.18	-0.90 ± 1.03
Height (cm)	171.1 ± 13.0	171.6 ± 11.2	170.8 ± 11.1	168.4 ± 14.7
Weight (kg)	81.7 ± 19.1	82.4 ± 18.6	84.6 ± 20.6	81.9 ± 17.1
BMI (kg/m ²)	27.3 ± 5.1	27.9 ± 5.3*	28.8 ± 5.9*	29.6 ± 8.4*

Statistical analyses were done by performing a Repeated Measurements ANOVA. To take into account multiple testing, $p < 0.01$ was considered statistically significant.

N, number of patients on rhGH replacement at different time points; rhGH, recombinant human Growth Hormone; GHD, Growth Hormone Deficiency; BMC, bone mineral content; BMD, bone mineral density; DXA, dual energy X-ray absorptiometry; BMI, body mass index; SD, standard deviation.

*, $p < 0.01$ (versus baseline)

**, $p < 0.001$ (versus baseline)

neck. The patients with persisting osteopenia at the femoral neck and/or lumbar spine at the study end, were older (68.5yr vs 59.2 yr, $p = 0.014$) and included more females (66.7% vs 46.7%, although NS). No differences were found with respect to 1 year GH dose or number of pituitary deficiencies between patients with and without persisting osteopenia and/or osteoporosis at the study end.

At baseline, 11 GHD patients (4.8%) had osteoporosis at the lumbar spine (4 females, 7 males), with a mean age of 54.1 ± 15.2 yr. Six of these patients used bisphosphonates and respectively 7 and 5 patients had calcium and Vitamin D supplementation. Seventeen patients (7.4%) had baseline osteoporosis at the femoral neck (7 females, 10 males), of whom nine patients used bisphosphonates, 11 calcium and 9 Vitamin D supplementation. In this small subgroup of patients with osteoporosis, no change in femoral neck BMD was found during rGH supplementation. However, lumbar spine BMD increased during the first 5 years of rGH supplementation, without a further increase afterwards. After 10 years of rGH supplementation, osteoporosis was found in, respectively, 7.1% and 1.1% of patients at the femoral neck and the lumbar spine.

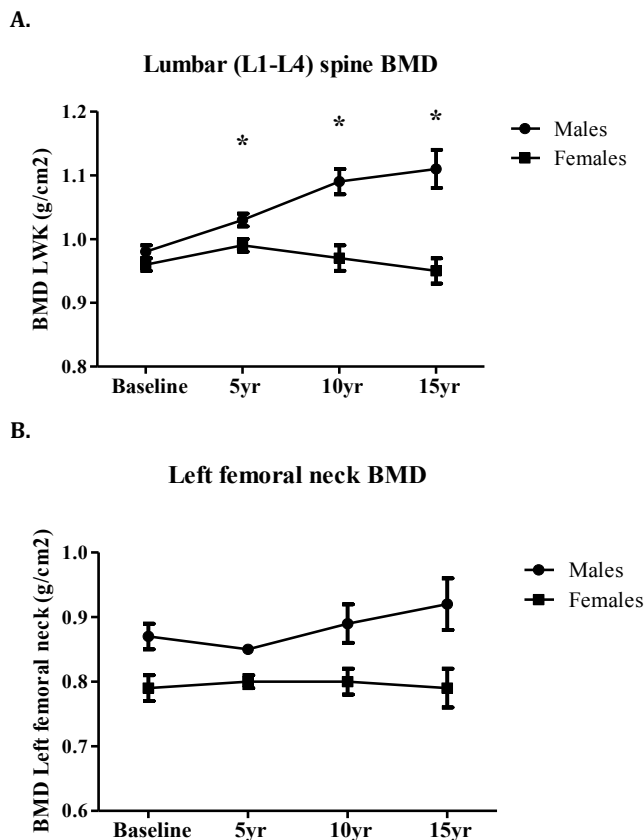


FIGURE 3. Sex differences in the effects of 15 years of rhGH replacement in 230 adult GHD patients on lumbar (L1-L4) spine BMD (A) and left femoral neck BMD (B)

Data are presented as mean BMD values (g/cm²), stratified by sex. Vertical bars indicate SEM values. Men had a greater increase in lumbar spine BMD than women at all time points when compared to baseline (all $p < 0.001$).

rhGH, recombinant human Growth Hormone therapy; GHD, Growth Hormone Deficiency; BMD, bone mineral density; SEM, standard error of the mean.

*, $p < 0.001$ (versus baseline)

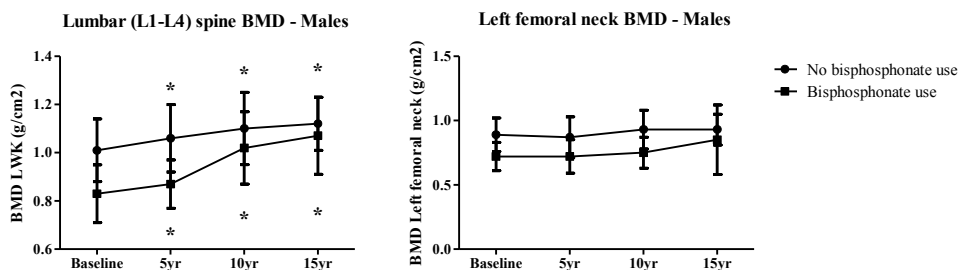
Influence of sex

Mean GH dose was significantly higher in women compared to men for the first 10 years of rGH replacement, resulting in a higher GH dose/IGF-1 SDS ratio in female GHD patients. After 5 years of rGH treatment, mean GH dose in men and women was respectively 0.37 ± 0.26 mg/day and 0.49 ± 0.28 mg/day ($p = 0.001$) and at 10 years respectively 0.36 ± 0.18 mg/day and 0.53 ± 0.30 mg/day ($p = 0.001$).

Men had a greater increase in lumbar spine BMD at all time points ($p < 0.01$ compared to baseline), however not in femoral neck BMD (Figure 3A+3B). These data were similar between patients with and without bisphosphonate use (Figure 4A-D). Similar sex differences, with highest response to rGH replacement in men, were seen

for lumbar spine BMC. Also in lumbar spine T-scores highest increase was seen in men after 10 and 15 years of rGH replacement, although this was not shown for femoral neck T-scores (*data not shown*).

A.



B.

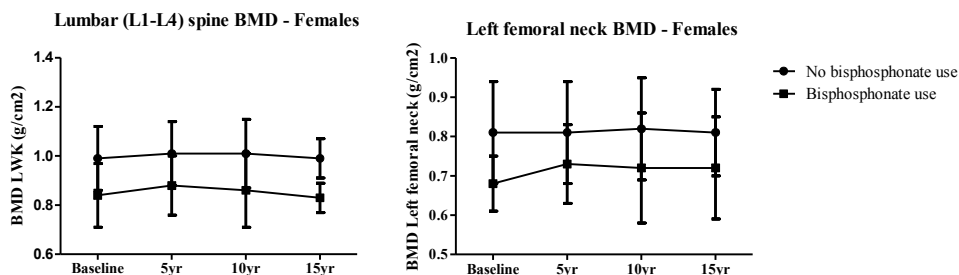


FIGURE 4. BMD changes during 15 years of rhGH replacement at the lumbar spine and the left femoral neck, stratified for the use of bisphosphonates in male (A) and female GHD adults (B)

Data are presented as mean BMD values (g/cm²), stratified by the use of bisphosphonates. Vertical bars indicate SD values.

rhGH, recombinant human Growth Hormone therapy; GHD, Growth Hormone Deficiency; BMD, bone mineral density; SD, standard deviation.

*, $p < 0.01$ (versus baseline)

Other potential influencing factors

Subsequently, we identified factors influencing the response to rGH replacement on BMD and BMC, in GHD females and males. GH dose at 1 year, low Vitamin D levels, hydrocortisone use, use of bisphosphonates, presence of ≥ 2 pituitary or hypogonadism were not found to influence the effects of rGH replacement on femoral neck BMD, lumbar spine BMD or BMC. A lower baseline lumbar BMD was found to be associated with a higher increase in lumbar BMD during 15 years of rGH replacement, in both sexes. Bone response to rGH replacement did not differ between patients with a history of functioning *vs* non-functioning adenoma or between patients with childhood- *vs* adulthood-onset GHD.

When incorporating all factors in a linear regression model, we found only higher GH dose at 1 year was associated with a decreased response to rGH on BMC over 15 years. No other influencing factors were found.

Fracture data

Twenty-three patients (10%, 11 females / 12 males) had a history of clinical fractures, of which 12 patients had fractures at ≥ 2 sites. Fifteen patients had vertebral fractures (10 patients had ≥ 2 fractures) and 9 patients suffered from non-vertebral fractures (hip N=6 (2 patients bilateral hip fracture), toe N=2, clavícula N=1). Consequently, the incidence rate of fractures during rGH replacement was 46 / 2288.5 (mean duration of rGH therapy 9.95 years x 230 patients) = 20.1 / 1000 py in our GHD cohort.

DISCUSSION

The present study describes the long-term effects of rGH replacement on bone metabolism in a well-defined cohort of adult patients with GHD. We found a sustained increase in BMD at the lumbar spine, but not at the femoral neck, during 15 years of rGH replacement. In addition, lumbar spine BMC and T-scores increased during the first 10 years of rGH replacement, but remained stable thereafter. At all time points, the largest effects of rGH supplementation on lumbar spine BMD and BMC were observed in men. Use of bisphosphonates did not impact additional beneficial effects beyond an initial early effect (34-35). With respect to potential influencing factors, lower baseline BMD and high GH dose at 1 year significantly influenced the bone response to 5, 10 or 15 years of rGH replacement. Clinical fracture incidence during long-term rGH supplementation was 20.1 / 1000 py.

GH and IGF-1 are important anabolic hormones. Most of the effects of GH are mediated by systemic and/or local IGF-1, enhancing the differentiated function of osteoblasts and bone formation, although GH may also act directly on bone cells(1). On the other hand, IGF-1 induces receptor activator of nuclear factor κ B ligand (RANK-L) synthesis and thereby osteoclastogenesis. In contrast to IGF-1, GH stimulates the production of osteoprotegerin, which competes with the RANK-L receptor and impairs osteoclastogenesis(2). Untreated GHD in adults is characterized by low bone turnover, decreased BMD and BMC(5-7), which was shown to be increased or even normalized by short-term rGH replacement(3;5;8). We have previously shown that also excessive GH production, as found in patients with acromegaly, is associated with increased fracture risk despite normal or high BMD, which is likely to be due to altered bone quality(23). These data indicate that there is a U-shaped association between GH/IGF-1 levels and bone quality/quantity.

We have previously shown a significant increase in BMD after 2 years of low physiological dose of rGH in GHD adults(33) that sustained after 7 years of treatment(34). In addition, during the first 4 years of rGH supplementation, a significant greater increase in BMD was found in GHD patients with osteoporosis with additional bisphosphonate treatment(34). These additional beneficial effects of bisphosphonates were maintained during another 3 years of rGH supplementation(35). In the present study investigating skeletal effects of 15 years of rGH replacement therapy, we observed a sustained increase of lumbar spine BMD in the long-term, but not femoral neck BMD. These results are in line with previous reports showing sustained increase in BMD during 5-10 years of rGH replacement with a peak value after 5-7 years of rGH replacement(10;12-14). When stratifying analyses for sex, lumbar spine BMD increases in both sexes during the first 5 years of rGH replacement. Afterwards, a further increase in BMD was observed in males, whereas in females the BMD decreased towards baseline values. Gender-specific differences in the bone response to rGH supplementation were also demonstrated in several other studies, in which rGH effects were also only found in males(24;25). It has been suggested that the lack of GH treatment effect on BMD in females with adult GHD may be due to the inhibition of the effect of GH on IGF-1 production by orally administered estrogens(26;27) or by inhibiting direct actions of GH on both osteoblasts and osteoclasts by estrogen(3). Differences in bone structure between males and females may also contribute to the gender-specific bone response to rGH supplementation.

Literature on fracture risk in adult GHD patients treated with rGH supplementation is scarce and, to date, only three studies have addressed fracture risk during rGH supplementation. Holmer *et al.* studied clinical fracture incidence in 832 rGH-treated patients compared to 2581 matched healthy controls and reported a two-fold increase in fracture risk in CO-GHD women, although in AO-GHD men fracture risk was significantly lowered. No increased risk was found in AO-GHD women and CO-GHD men(28). Elbornsson *et al.* reported clinical fractures in a Swedish cohort of GHD adults, and observed only 1 hip fracture and 1 symptomatic vertebral fracture (14). Mazziotti *et al.* reported a high prevalence of radiographic vertebral fractures of 53.8% in rGH-treated GHD adults when compared to a prevalence of 78.6% in untreated GHD patients(29), indicating that rGH supplementation is able to decrease fracture risk in adult GHD patients. In our cohort of especially AO-GHD patients, 10% of patients sustained clinical fractures during rGH replacement, resulting in an incidence rate of fractures of 20.1 / 1000py during rGH supplementation. This a incidence rate is comparable to the incidence rate in the general population, as described by Donaldson *et al.* among a middle-aged population in England, showing a fracture incidence rate of 36 / 1000py(30). Taking into account the younger age of our cohort, our data do not suggest that fracture incidence is increased in rGH-treated AO-GHD patients.

Our study has strengths as well as limitations. Its main strength is the size of our cohort study consisting of GHD adults, who were regularly followed and stringently controlled over the years according to internationally, used guidelines. Care for attention was given to Vitamin D status as reflected by normal average levels of vitamin D and normal bone turnover rates throughout the entire study period. In addition, we did a subanalysis among patients with osteoporosis at the start of rGH supplementation and investigated fracture incidence during rGH supplementation. Limitations of the present study are, first, the lack of a non-rGH treated control group. Since the beneficial effects of rGH therapy are well established in the short-term, rGH replacement became a widely available therapy for adult GHD in the Netherlands in case of no contra-indications. We believe it is therefore no longer possible or ethical to perform long-term randomized placebo-controlled studies in GHD patients. A second limitation is the potential underestimation of the fracture incidence in our patients as spinal X-rays were only performed in case of a clinical fracture. Another feature that needs to be addressed that due to the presence of multiple pituitary hormone deficiencies in almost all patients, it is difficult to examine to what extent the reported effects can be attributed to rGH treatment, or whether they are the consequence of suboptimal or excessive replacement therapy of other hormones. In this respect, the presence of interactions between glucocorticoids and rGH during substitution and the negative effects of cortisol excess, either endogenous or supraphysiological supplementation, on bone has to be noted(31;32). However, in our study, there were no signs of hydrocortisone over supplementation and when incorporating hydrocortisone use in a regression model, we found no significant association with BMD response. Finally, bisphosphonates, calcium and Vitamin D supplementation were initiated at the discretion of the treating physician and not given using a standardized protocol. In this respect, little is known on the combined use of bisphosphonates and rGH supplementation(34), which should be a topic for future research.

In conclusion, findings from our cohort study of GHD patients suggest ongoing beneficial effects of long-term (≥ 5 years) rGH replacement therapy, particularly in men. Beneficial effects are most marked on BMD at the lumbar spine. Fracture incidence is similar to that of age- and sex-matched general population. Sex-specific differences in bone response to rGH supplementation is asked to be elucidated and long-term beneficial effects of bisphosphonates on fracture risk remain to be established.

REFERENCE LIST

1. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev* 2008; 29(5):535-559.
2. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. *N Engl J Med* 2007; 357(9):905-916.
3. Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Slootweg MC. Growth hormone and bone. *Endocr Rev* 1998; 19(1):55-79.
4. Manelli F, Carpinteri R, Bossoni S et al. Growth hormone in glucocorticoid-induced osteoporosis. *Front Horm Res* 2002; 30:174-183.
5. Bouillon R, Koledova E, Bezlepkina O et al. Bone status and fracture prevalence in Russian adults with childhood-onset growth hormone deficiency. *J Clin Endocrinol Metab* 2004; 89(10):4993-4998.
6. Rosen T, Hansson T, Granhed H, Szucs J, Bengtsson BA. Reduced bone mineral content in adult patients with growth hormone deficiency. *Acta Endocrinol (Copenh)* 1993; 129(3):201-206.
7. Verhelst J, Abs R. Long-term growth hormone replacement therapy in hypopituitary adults. *Drugs* 2002; 62(16):2399-2412.
8. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; 96(6):1587-1609.
9. Appelman-Dijkstra NM, Claessen KM, Roelfsema F, Pereira AM, Biermasz NR. Long-term effects of recombinant human GH replacement in adults with GH deficiency: a systematic review. *Eur J Endocrinol* 2013; 169(1):R1-14.
10. Clanget C, Seck T, Hinke V, Wuster C, Ziegler R, Pfeilschifter J. Effects of 6 years of growth hormone (GH) treatment on bone mineral density in GH-deficient adults. *Clin Endocrinol (Oxf)* 2001; 55(1):93-99.
11. Drake WM, Rodriguez-Arnan J, Weaver JU et al. The influence of gender on the short and long-term effects of growth hormone replacement on bone metabolism and bone mineral density in hypopituitary adults: a 5-year study. *Clin Endocrinol (Oxf)* 2001; 54(4):525-532.
12. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001; 86(10):4657-4665.
13. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *Eur J Endocrinol* 2007; 156(1):55-64.
14. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012; 166(5):787-795.
15. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. *J Clin Endocrinol Metab* 1997; 82(1):129-135.
16. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004; 89(5):2192-2199.
17. Claessen KM, Appelman-Dijkstra NM, Adoptie DM et al. Metabolic profile in growth hormone-deficient (GHD) adults after long-term recombinant human growth hormone (rhGH) therapy. *J Clin Endocrinol Metab* 2013; 98(1):352-361.
18. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008; 18(1):1-16.

19. van der Klaauw AA, Biermasz NR, Zelissen PM et al. Administration route-dependent effects of estrogens on IGF-I levels during fixed GH replacement in women with hypopituitarism. *Eur J Endocrinol* 2007; 157(6):709-716.
20. Genant HK, Wu CY, van KC, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. *J Bone Miner Res* 1993; 8(9):1137-1148.
21. Cole TJ. The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 1990; 44(1):45-60.
22. Rikken B, van DJ, Ringeling A, Van den Brande JL, Massa G, Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 1998; 50(3):166-176.
23. Claessen KM, Kroon HM, Pereira AM et al. Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study. *J Clin Endocrinol Metab* 2013.
24. Bex M, Abs R, Maiter D, Beckers A, Lamberigts G, Bouillon R. The effects of growth hormone replacement therapy on bone metabolism in adult-onset growth hormone deficiency: a 2-year open randomized controlled multicenter trial. *J Bone Miner Res* 2002; 17(6):1081-1094.
25. Snyder PJ, Biller BM, Zagar A et al. Effect of growth hormone replacement on BMD in adult-onset growth hormone deficiency. *J Bone Miner Res* 2007; 22(5):762-770.
26. Cook DM, Ludlam WH, Cook MB. Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. *J Clin Endocrinol Metab* 1999; 84(11):3956-3960.
27. Janssen YJ, Helmerhorst F, Frolich M, Roelfsema F. A switch from oral (2 mg/day) to transdermal (50 microg/day) 17beta-estradiol therapy increases serum insulin-like growth factor-I levels in recombinant human growth hormone (GH)-substituted women with GH deficiency. *J Clin Endocrinol Metab* 2000; 85(1):464-467.
28. Holmer H, Svensson J, Rylander L et al. Fracture incidence in GH-deficient patients on complete hormone replacement including GH. *J Bone Miner Res* 2007; 22(12):1842-1850.
29. Mazzotti G, Bianchi A, Bonadonna S et al. Increased prevalence of radiological spinal deformities in adult patients with GH deficiency: influence of GH replacement therapy. *J Bone Miner Res* 2006; 21(4):520-528.
30. Donaldson LJ, Reckless IP, Scholes S, Mindell JS, Shelton NJ. The epidemiology of fractures in England. *J Epidemiol Community Health* 2008; 62(2):174-180.
31. Chiodini I, Torlontano M, Carnevale V, Trischitta V, Scillitani A. Skeletal involvement in adult patients with endogenous hypercortisolism. *J Endocrinol Invest* 2008; 31(3):267-276.
32. Danilowicz K, Bruno OD, Manavela M, Gomez RM, Barkan A. Correction of cortisol overreplacement ameliorates morbidities in patients with hypopituitarism: a pilot study. *Pituitary* 2008; 11(3):279-285.
33. Janssen YJH, Hamdy NA, Frolich M, Roelfsema F. Skeletal effects of two-years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. *J Clin Endocrinol Metab* 1998; 83(6):2143-2148.
34. Biermasz NR, Hamdy NA, Janssen YJ, Roelfsema F. Additional beneficial effects of alendronate in growth hormone (GH)-deficient adults with osteoporosis receiving long-term recombinant human GH replacement therapy: a randomized controlled trial. *J Clin Endocrinol Metab* 2001; 86(7):3079-3085.
35. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rhGH) alone and rhGH combined with alendronate in GH-deficient adult: a seven-year follow-up study. *Clin Endocrinol* 2004; 60: 568-575.

Chapter 8

Discontinuing recombinant growth hormone replacement in adult GH deficient patients: a cohort study and systematic review of literature.

Natasha M. Appelman-Dijkstra, Marnick Rijndorp, Nienke R. Biermasz,
Olaf M. Dekkers, Alberto M. Pereira

Submitted

ABSTRACT

Background: Growth hormone (GH) replacement is advocated in adult Growth hormone deficient (GHD) patients to increase bone mass, to improve lipid profile and body composition, and Quality of Life. However, data on the consequences of discontinuation of GH replacement are scarce in adult GHD patients.

Design: Cohort study and a systematic literature search aimed at the evaluation of the metabolic effects of GH discontinuation on anthropometry, glucose and lipid metabolism, and bone mass in adult GHD patients.

Methods: We included 64 adult GHD patients who discontinued GH replacement for > 12 months after a duration of rhGH replacement of $8.7 \text{ years} \pm 4.6 \text{ years}$. AGE range! Data on anthropometry, lipids, glucose, and bone mass were assessed until 3 years after discontinuation of therapy. In addition, a systematic literature review was performed using the electronic databases Pubmed, Embase, and Web of Science.

Results: Fat mass increased from $31.5 \pm 9.5\%$ to $33.8 \pm 9.0\%$ ($p < 0.001$) after 3 years of discontinuation. Total - and LDL-cholesterol levels decreased from $5.4 \pm 0.5 \text{ mmol/L}$ to $5.1 \pm 0.7 \text{ mmol/L}$ ($p = 0.03$), and $3.2 \pm 0.4 \text{ mmol/L}$ to $2.9 \pm 0.6 \text{ mmol/L}$ ($p = 0.016$) after discontinuation in the presence of an increase in the use of lipid lowering medication. Fasting glucose levels and bone mass did not change. In patients aged > 60 years ($n = 34$) no significant changes were observed in any of the studied parameters. These observations, except for the changes in lipid profile, were in line with the results of the systematic review, which included 7 studies with a maximum duration of follow up of 18 months.

Conclusions: We did not observe obvious adverse metabolic changes in the 3 yrs following discontinuation of GH replacement for various reasons in adult patients with GHD. Further research is needed to determine optimal strategies for long-term GH replacement including the optimum time point for GH discontinuation.

INTRODUCTION

Adult GHD is associated with an adverse metabolic profile, including abdominal obesity, dyslipidemia and an increased cardiovascular risk and mortality (1,2). Several studies have demonstrated that GHD patients have lower bone mass, increased fracture risk, and an impaired quality of life (QoL) (3,4). Recombinant human growth hormone (rhGH) replacement therapy has shown to improve lipid profile, body composition, quality of life, and to increase bone mass, and has become available for adult patients with GHD since 2 decades (5-10). However, long-term follow up studies have shown that the prevalence of the metabolic syndrome does not ameliorate and BMI increases while on rhGH replacement. In addition, the metabolic profile of long-term treated GHD patients remains significantly different from the general population (8, 11,12).

At present, many issues regarding long-term treatment are not well established, for example the optimal duration of rhGh replacement with advancing age, the long-term efficacy and the consequences of rhGH withdrawal in adulthood are not well established. Therefore, replacement is continued indefinitely in many cases. However, in case of an active malignancy or lack of subjective benefit it is advised to stop rhGH replacement. In addition, there are anecdotal data questioning the beneficial effects of rhGH replacement with advancing age, since GH secretion and IGF-1 levels decrease in healthy adults with advancing age, and low IGF-1 levels are even associated with increased longevity and decreased morbidity both in animal models and man (13,14). In addition, studies reporting on the efficacy of rhGH replacement in elderly GHD patients are limited and show inconclusive results, and data on rhGH discontinuation in adult GHD are scarce (15). Therefore, we aimed to evaluate the long-term metabolic effects of discontinuation of rhGH replacement in our cohort of adult GHD patients in general, and in the subset of patients aged 60 years or older. At our center, the duration of rhGH replacement is individualized and reasons for discontinuation of rhGH in case of lack of subjective benefit, malignancy, side effects, and at older age.

Furthermore, we performed a systematic review of studies reporting on the effects of discontinuing rhGH therapy in adults.

PATIENTS AND METHODS

Patients

Patients were identified from the database of our cohort of adult patients with GHD. This cohort comprised all patients starting rhGH since 1994, with standardized evaluation of clinical and metabolic parameters annually at the outpatient clinic of the Center for Endocrine Tumors (8, 11,12, 16-18). For the present analyses, we included all GHD patients treated continuously for at least 12 months with rhGH during adulthood, and who discontinued rhGH replacement therapy for at least for 12 months for various

reasons. Patients with a baseline visit before discontinuation and at least one follow up visit after discontinuation were included.

Treatment protocol

Severe GHD was documented prior to start of rhGH replacement and defined as a GH peak response to the Insulin Tolerance Test (ITT) $<3\mu\text{g/l}$ (glucose nadir $<2.2\text{mmol/l}$) according to current guidelines, or, in case of contraindications for ITT, by combined Growth Hormone Releasing Hormone/Arginine-stimulation (GHRH/Arg) with BMI-adjusted GH cut-offs, (19-24). According to protocol patients receiving rhGH replacement during childhood were retested at the time of transition to the adult outpatient clinic, after treatment cessation for at least 3 months. Hormone deficiencies were diagnosed and treated as previously reported (8,25). Patients were treated with bisphosphonates, calcium and vitamin D replacement, lipid-lowering and antihypertensive medication according to the discretion of their attending physicians, in accordance with national guidelines, which was continued after withdrawal of rhGH replacement. Efficacy and safety parameters were assessed yearly, next to a routine assessment of pituitary function.

STUDY DESIGN AND METHODS

A) Cohort Study

We performed a cohort study, aimed at evaluation of the metabolic effects of rhGH discontinuation. The baseline visit was defined as the last visit prior to discontinuation of rhGH replacement. Data were assessed at baseline, and at 1, 2, and 3 years after stop.

During the periodical visits, the following data were assessed:

1. Anthropometric parameters: body weight was measured in kilograms with 1 decimal precision, and body height was measured barefoot to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).
2. Biochemical parameters: serum concentrations of IGF-1, GH, TSH, fT_4 , LH, FSH, estradiol, testosterone, prolactin, bone turnover markers, PTH, vitamin D, glucose, parameters for renal and kidney function, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were collected after an overnight fast. Details on the different assays used were reported previously (8).
3. Bone Mineral Density and Bodycomposition: Data on BMD, BMC and fatpercentage were collected. BMD was measured yearly at the lumbar spine (L1-L4) and femoral neck using dual energy X-ray absorptiometry (DXA) (Hologic QDR 1000 until 2008)

and Hologic QDR 4500 thereafter) (25). Fatpercentage was assessed by calculating the total mass of fat divided by total body mass.

4. Data on medication use and co-morbidity was obtained from electronic patient files.

B) Literature search

We composed a search strategy focusing on discontinuation of long-term rhGH treatment, GHD adults, bone metabolism, Quality of life, metabolic effects, and body composition. Only articles reporting original data were included, studied reporting duplication of cohorts were excluded. References of relevant articles were checked for additional articles. The search in Pubmed, Web of Science, and Embase was performed on 05/09/2014 in collaboration with a trained clinical librarian, using the following search string: *“Human Growth Hormone”[Majr] OR “Growth Hormone”[ti] OR “GH”[ti] AND (“discontinuing”[ti] OR “discontinuation”[ti] OR “discontinued”[ti] OR “withdrawal”[ti] OR “withdrawing”[ti] OR “cessation”[ti] OR “deprivation”[ti]) AND (“therapy”[Subheading] OR “therapy”[ti] OR “treatment”[ti] OR “therapeutics”[Majr Terms] OR “therapeutics”[ti] OR “replacement”[ti] OR “Hormone Replacement Therapy”[Majr] OR “administration”[ti])*

Studies were eligible when all of the following criteria were met: (1) they investigated effects on biochemical, metabolic/anthropometric parameters, bone metabolism, QoL, muscle strength, mortality, or adverse events, (2) had a duration of follow up > 1 month and included > 10 patients, (3) were performed in patients with confirmed GHD in adulthood, and (4) were written in English.

The selection of studies was done by two reviewers (N.M.A-D.en M.R.). The two reviewers evaluated studies independently, and disagreements were resolved by consensus. Data extraction was based on data from each study provided at the population level. The following data were extracted: (1) study population (patient characteristics, population size, GHD assessment, control population); (2) exposure to rhGH (duration of rhGH treatment, rhGH dose), (3) outcome (biochemical and anthropometric parameters, QoL, bone metabolism) (4) risk of bias.

STATISTICAL ANALYSIS

SPSS for Windows, version 20.0 (SPSS, Inc.) was used for statistical analysis. Data are presented as mean $\pm$ SD, unless stated otherwise. ANOVA accounting for repeated measurements with Bonferroni correction was used to compare parameters between baseline and T= 1, 2 and 3 years, SPSS Bonferroni adjusted p-values are given unless stated otherwise. A linear regression model was used, including age, sex, radiotherapy, hydrocortisone use, BMI, to identify factors influencing the metabolic effects of discontinuing rhGH replacement therapy. In addition, we included the use

of antihypertensive medication, use of lipid-lowering medication, anti-diabetic agents, calcium and vitamin D supplementation, and the use of bisphosphonates. We also conducted a sub-analysis in elderly subjects (age >60, n=34). The age of 60 was chosen as cut-off, because the effects of GH replacement therapy in GHD patients aged >60 years is not well established (15).

RESULTS

Clinical characteristics

A total of 102 patients who discontinued therapy were identified. Of these, 4 patients died before the first annual visit after cessation of rhGH replacement, 10 patients re-initiated rhGH replacement within one year, 6 patients were lost to follow up, and 18 patients did not meet the inclusion criteria. See *Figure 1* for flow-chart of patient inclusion.

A total of 64 patients, mean age 59.7 ± 15.7 years, were included in the study. Duration of rhGH replacement was $8.7 \text{ years} \pm 4.6 \text{ years}$. Thirty-four patients (53% of total) were 60 years or older. Mean age in the subgroup of elderly patients was 71.3 ± 7.0 years. Mean period of discontinuation was 3.7 years (range 1-18 years). The main reasons for discontinuation were adverse events (n=25), or a lack of subjective benefit (n=10).

Table 1

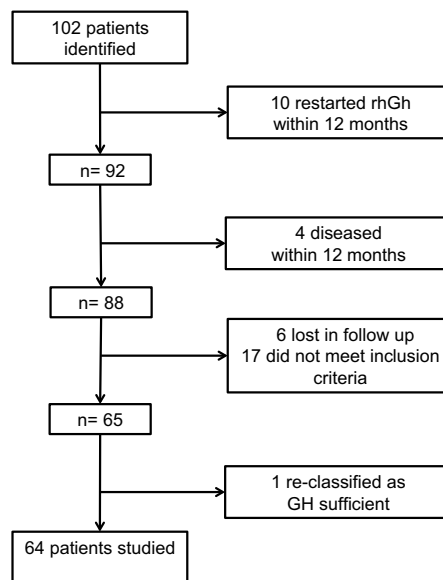


FIGURE 1.

Flow chart patient selection

rhGH; recombinant human growth hormone, GH ; growth hormone

TABLE 1 . Baseline characteristics (n=64)

Age at baseline (yr)	59 ±15 (21-88)
Sex (M/F)	34/30
Cause of GHD (%)	
NFA	21 (33%)
Prolactinoma	6 (9%)
Acromegaly	10 (16%)
Cushing's disease	7 (11%)
Craniopharyngioma	4 (6%)
Other	16 (25%)
Onset (%)	
AO	55 (86%)
CO	9 (14%)
Other deficiencies (n)	
Corticotropic	43 (67%)
Thyreotropic	45 (69%)
Gonadotropic	46 (72%)
Diabetes insipidus	10 (16%)
Isolated GHD	2 (5%)
Rh-GH duration (yr)	9 ± 5 (1-18)
Cumulative Dose (mg)	22.6 ± 5,7
Radiation (%)	33 (51.6%)
Medication (%)	
Antidiabetic	6.3%
Antihypertensive	43.8%
Lipid-lowering	39.1%
Oral contraceptives	1.6%
Bisphosphonates	9.4%
Calcium supplements	40.6%
Vitamin D supplements	46.9%
Thyroxine replacement	70.3%
Dosage (µg)	118.7 ± 41.4

AO = adult onset, CO = childhood onset, GHD = Growth hormone deficiency, n = number of patients, NFA = non-functioning pituitary adenoma, rhGH = recombinant human growth hormone, yr = years.

Anthropometry and blood pressure

Both systolic and diastolic blood pressure did not change significantly after discontinuation of growth hormone replacement therapy, 134 ± 10.1 mmHg *vs* 134.9 ± 13.1 mmHg and 81.1 ± 6.7 *vs* 81.2 ± 4.9 mmHg respectively.

BMI decreased in the first years after cessation of rhGH therapy (Figure 2 A & B). In elderly subjects, BMI did not significantly decrease, mean difference 0.8 kg/m², 95% CI -0.3 - 1.8 . Fat percentage increased from 31.5 ± 9.5 % to 33.8 ± 9.0 % ($p < 0.001$) after 3 years. Subgroup analyses in patients > 60 years of age did not show any significant change in fat percentage after 3 year, mean difference -0.6% with 95%CI -2.3 - 1.0 .

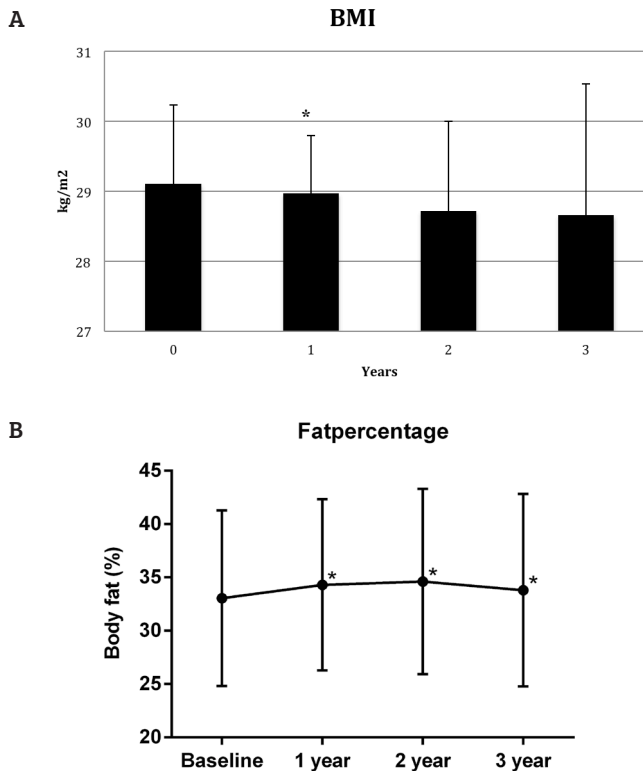


FIGURE 2A&B. Changes in BMI up to 3 years after discontinuation.

BMI: Body Mass Index kg/m²; * $p < 0.05$ compared to baseline

Lipid and glucose profile Figure 3A & B.

After 3 years of discontinuation serum total- and LDL-cholesterol (TC), decreased significantly from 5.4 ± 0.5 mmol/L to 5.1 ± 0.7 mmol/L ($p = 0.03$), and from 3.2 ± 0.4 mmol/L to 2.9 ± 0.6 mmol/L ($p = 0.016$). HDL-C concentration after 3 years did not change, 1.4 ± 0.2 *vs* 1.5 ± 0.2 mmol/L.

The percentage of patients on lipid lowering agents, predominantly statins, was 39 %at baseline and increased during follow up to 44%.

Glucose concentration and the number of patients on glucose lowering therapy did not change after discontinuation, 4.9 ± 0.5 mmol/L vs 5.4 ± 1.7 mmol/L. In the elderly patients, no changes were observed.

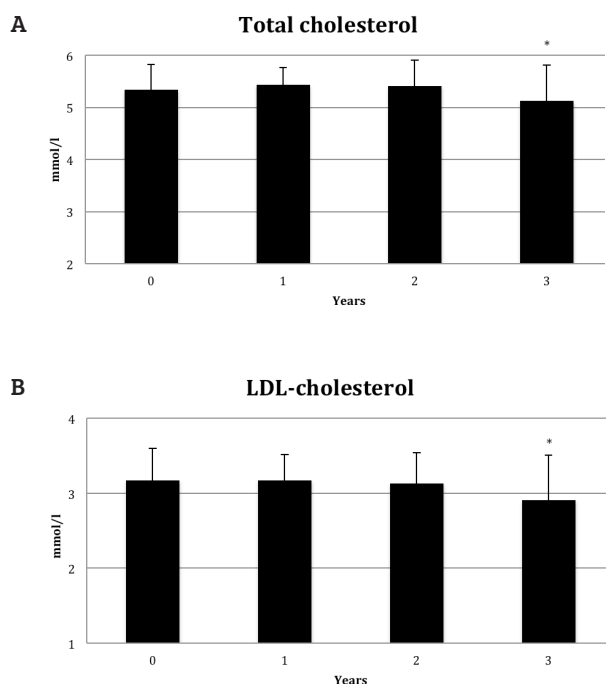


FIGURE 3A&B. Changes in total and LDL cholesterol levels up to 3 years after discontinuation

* = $P < 0.05$ compared to baseline

Bone metabolism

Lumbar spine BMD and femoral neck BMD remained stable, 1.0 ± 0.1 g/cm² vs 1.1 ± 0.2 g/cm² and 0.8 ± 0.1 g/cm² vs 0.8 ± 0.1 g/cm² respectively. Bisphosphonate use increased from 9.4% at baseline to 12.1% after 3 years. In a small subgroup ($n=17$) bone markers were available at baseline and after 3 years. P1NP decreased from 42.2 ± 8.8 ng/mL to 37 ± 13.7 ng/mL ($p=0.064$). Beta crosslaps decreased from 0.41 ± 0.06 ng/mL to 0.36 ± 0.16 ng/mL ($p=0.056$).

SYSTEMATIC REVIEW

The initial search resulted in a total of 95 articles. Of these, 26 publications were retrieved for full assessment, 17 papers were excluded since they reported on patients

< 18 years, 1 because of duration of follow up of only 5 days and 1 because of a low number of patients. Seven articles (that included a total number of 166 patients) met the inclusion criteria. The number of included patients per study ranged from 10 to 42.

Table 2

Study characteristics

Details of the included studies are summarized in Table 2. Included studies were published between 1992 and 2012. Six studies (n= 90 patients in total) were randomized controlled trials, of which 3 had a crossover design, there was one retrospective cohort study. The mean duration of withdrawal was 3-18 months in all studies except one. This was a cohort study including 42 patients with duration of follow up between 0-21 Years (26). Two out of 7 studies did not clearly report the duration of rhGH therapy at time of discontinuation (26,29). Overall quality of the studies was fair with a low risk of bias since there were designed as RCT, although none of the studies mention handling of statins, bisphosphonates and glucose lowering medication or even excluded patients on these medications. In addition data reported in the studies are heterogeneous and therefore precluded a formal meta-analyses.

Anthropometry and Body composition:

One study reported on BMI, waist and hip circumference: no statistically significant changes were observed after 6 months of follow up (30). Body composition was measured in three studies, and all reported an increase in fat mass and decrease in lean body mass after a follow up of 3-18 months (27,29,30).

Lipid metabolism:

Total Cholesterol and LDL increased in 3 studies but was did not change in another study (26,28,30). Two studies also reported an increase of hsCRP after discontinuation of rhGH therapy (27,28)

Glucose metabolism:

Two studies reported on glucose metabolism and insulin sensitivity after discontinuation (27,30). Fasting glucose, insulin levels, c-peptide and HBA1c were lower in 2, however one study reported an increase of glucose levels.

TABLE 2.

Author	Study Design	Time (months)	N (%)	Age (years)	Duration of replacement at baseline (years)	Bone Metabolism	Body Composition	Glucose Metabolism	Cholesterol Metabolism	Other reported effects
M/F										
Billir	RCT, crossover	18	20	48.9	NR	Increase of Hip BMD: +0.0014 ±0.0042 g/cm ² (p 0.0051)	LBM decreased: 2133 gram ±539 gram (p 0.0016)	NR	NR	NR
Colao	RCT, crossover	6	17	39	6 months during trial	Stable BMD of the spine: +0.0018 ±0.00097 g/cm ² (p 0.087)	Fat mass increased: 3.18%±0.44% (p 0.0001)	NR	TG increased: 19.9 mg/dl (p 0.007)	Diastolic blood pressure increase: 76.7 ±3.8 to 80.0±6.1 (p 0.0007)
Filipsson	RCT, crossover	4	29	60	10.1 ±3.4 (3-16)	BMC increased: 0.002±0.11 g/cm (CI 0.004-0.006) p 0.0047	Fat mass increased: 2.05 kg±2.32 (CI 1.45-2.56) (p <0.001)	Glucose levels stable	TC mmol/l increase: 0.2 (p<0.05)	AGHDA scores worsened, NHP/PGWB subscores on emotional and positive well being worsened (p< 0.05)
			68/32	(28-73)			BMI and WH circumference no change	-0.36 ±1.62 (CI -0.53-0.18) HbA1c (%) decreased -0.18±0.42 (CI0.30 -0.07) (p 0.02)	HDL mmol/l increased 0.05 (p<0.05)	
									LDL mmol/l increase 0.3 (p<0.01)	
									TG mmol/l decrease -0.2	
									CRP mg/L increase 1.3 (p< 0.05)	

Author	Study Design	Time (months)	N	Age years	Duration of replacement at baseline (years)	Bone Metabolism	Body Composition	Glucose Metabolism	Cholesterol Metabolism	Other reported effects
Fukuda	Retrospective	0-252	42	19	NR	NR	NR	NR	TC increased as well as triglycerides	NR
			52/48	(14-38)						
Gomez	Observational crossover	12	20	40.2	18-24 months	Stable BMD in L2 — L4 + 0.008 g/cm ²	NR	NR	NR	NR
			70/30	(22-56)		femoral neck no change				
Holmes	Observational crossover	12	10	20.4	12 months	forearm cortical BMC increase	NR	NR	NR	NR
			80/20	±2.2		0.1 g/cm				
						(p 0.00)				
						vertebral trabecular BMD 0.0063 g/cm ²				
Mc Millan	RCT	3	12	44.9	5	NR	NR	NR	NR	QoL questionnaires (SF-36, GWB, HBQoL) decrease in energy, tiredness. More profound in younger male participants, almost no complaints in subgroup > 53 years
			50/50	(25-68)		NS				
Yuen	RCT	6	16	49	1	NR	Decrease LBM, increase Fat mass, ,	glucose remained stable	increase in CRP?	
			50/50	(31-60)						

* median; BMD: Bone Mineral Density, BMC :Bone Mineral Content, LBM: Lean Body Mass, RCT: Randomized Controlled Trial, WH circumference: waist hip circumference. GHID: Growth Hormone Deficient, TC: Total Cholesterol, LDL-C: LDL- cholesterol, HDL-C: HDL- Cholesterol, rhGH: recombinant human Growth Hormone, QoL: Quality of Life.

Bone metabolism:

Several parameters were reported. One study found an increase in BMC during the study period where a second study did not report any difference in BMC (29,30). Two studies with a duration of follow up of 6 months and 18 months respectively, reported a stable or increase of BMD at the hip and spine (29,30). No study reported on fracture prevalence or bone markers.

Quality of Life:

Only 2 studies reported on quality of life (30,31). After discontinuation of rhGH for a period of 3-4 months patients reported worsened scores on emotional reactions and well being. Furthermore they reported an increase in tiredness. Noteworthy, these effects were more profound in younger male participants, and almost absent in older patients (31).

In conclusion most studies investigated the effects of growth hormone discontinuation in the follow up course after short-term rhGH suppletion. Only 2 studies reported on patients treated for more than 5 years of rhGH suppletion and their follow up was < 6 months (30,31). Therefore firm conclusions on the effects of discontinuation rhGH suppletion cannot be made.

DISCUSSION

In this study we demonstrated that adult GHD patients who discontinued rhGH replacement for various reasons do not show important adverse metabolic changes in the long-term. Fat mass increased without negatively affecting lipid profile, glucose metabolism, or bone mass. In addition, in older patients no changes were observed at all. It thus appears that rhGH replacement can be discontinued without negative metabolic effects in the long term, provided that routine cardiovascular clinical guidelines for the treatment of lipid abnormalities and hypertension are properly followed. We found a decrease in LDL- and total cholesterol in the presence of an increase in the use of lipid lowering medication after discontinuation. In the subgroup of patients aged > 60 years no significant metabolic changes after cessation of therapy were observed at all. Furthermore, we observed no changes in lumbar spine BMD or fasting glucose level. BMI even slightly decreased within the 3 year follow up period as did bone turnover markers, although the latter changes were not significant possibly due to the sample size. The only parameter that significantly deteriorated after discontinuation was fatpercentage as measured by DXA.

It is questionable whether the observed statistically significant changes are clinically relevant. At all time points, lipid- and fasting glucose concentrations remained within the

normal range in both groups and BMI even decreased after discontinuation of rhGH. In the general population, for every 1 mmol/L LDL-C reduction, the risk of major vascular events decreases by 20%(33). Given the adverse lipid profile in adult GHD patients that is associated with increased cardiovascular risk, it might be beneficial to start lipid-lowering medication in all middle-aged GHD patients. The clinical implications of the average 0.4 mmol/L increase in fasting glucose levels after discontinuation are less clear since fasting glucose levels remained within the normal range at all time points. It has been shown that the hazard ratio for coronary heart disease does not differ between patients with fasting glucose concentrations ranging between 3.9 and 5.6 mmol/L and the incidence of coronary heart disease increases with increasing BMI (34, 35). On average, all subjects would be classified as overweight at all time points, however since BMI might not be the best anthropometric predictor for cardiovascular events, the observed increase in fatpercentage might counteract the decrease in BMI after discontinuation (36). Treatment of adult GHD is mainly indicated for improvement of cardiovascular risk factors and quality of life. Short- and long-term studies have demonstrated that rhGH replacement is associated with improvement of cardiovascular risk factors like lipid profile, bone mass, and quality of life (5,7,9,25, 32). However, data also show that the metabolic phenotype of GHD patients remains abnormal compared to the general population despite long-term replacement. Therefore, it is very well possible that the classic GHD phenotype might not be completely attributable to growth deficiency and/or that not all effects are reversible (11,12).

Since this is an observational study it obvious has limitations. There are no guidelines for discontinuation of rhGH replacement; consequently the observed group was clearly pre-selected by the treating physician. Also, in this study we did not evaluate the effects on quality of life, another important indication for rhGH replacement. However, since this was an observational study, patients who might have benefitted most from rhGH replacement were less likely to have discontinued therapy for a prolonged period, and patients were also always asked for their physical and psychological well being during the visits. In case of severe deterioration of quality of life after discontinuation rhGH would have been re-initiated, as was reflected by the 10 patients that were identified prior to inclusion that re-initiated treatment within the first year.

In order to put our observations in the best perspective, we additionally performed a systematic literature search on studies reporting on discontinuation of rhGH replacement in adults. A total of 144 patients were reported, of whom 84 were included in randomized controlled studies. The duration of follow up was limited and ranged between 3 to 18 months. Overall, most studies reported that a parameter did not change without mentioning the outcome. This makes it hard to interpret these literature results since this implies that there was no change at all whereas the study only can report that there is no evidence of a difference (37). The majority of included patients had been on rhGH replacement for only 6-12 months prior to discontinuation whereas

some studies did not report the duration of therapy. This makes it difficult to interpret the results since the effects of rhGH are time dependent. For instance, a decrease in BMD might be observed within the first year affecting the effects of withdrawal when this occurs within the first year (7,9,18). Nevertheless, all studies reporting on body composition, including ours, have consistently shown an increase in fat percentage after discontinuation (27,20,30). Data on lipid profile after discontinuation were not conclusive, also because data on the use of lipid-lowering medication could not be extracted or subjects on lipid lowering agents were excluded. Biller et al. reported an ongoing increase of BMD after 18 months of discontinuation, although data on bone modifying agents including calcium and vitamin D were not available in this study (29). One study reported on patients (n=29) who used rhGH during a longer period of time (30). In the follow up period of 4 months in this study BMC and fatmass increased, lipid profile and quality of life scores however worsened and glucose level remained grossly unchanged. From all studies selected this study resembled our patient population at most and the reported results are comparable with ours although handling of statins and bisphosphonates were not mentioned.

The rationale for being restrictive with GH replacement in elderly patients is based upon the following. The GH/IGF-1 axis declines physiologically with ageing and smaller individuals within a species usually live longer (38). Additionally, the GH/IGF-1 axis has been linked with cancer risk and improved longevity (14), and functional mutations of the IGF-1R gene resulting in altered IGF-1 signalling appear to be more common in centenarians than in younger controls. Life span is also extended in mice lacking GHR, resulting in lower IGF-1 levels. Although these mutant mice lack other hormones, restoration of GH levels reverted their longevity to that of non-mutants (39, 40). Collectively, these results suggest that optimizing the GH/IGF-1 axis to promote healthy ageing in humans is more complex than originally appreciated, requires a greater understanding of its array of interactions and tissue specificity.

In conclusion, the results of this study indicate that discontinuation of rhGH replacement in routine clinical practice does not result in apparent negative effects on overall metabolic profile. Since long-term replacement with rhGH generally will not normalize the metabolic profile and the beneficial effects of unrestricted GH replacement therapy especially in elderly GHD are yet inconclusive, we advise to reconsider the need to continue rhGH treatment of long-term treated GHD patients, and also explore alternative treatment strategies in patients, like a more stringent treatment of dyslipidaemia and hypertension, especially in those aged > 60 years. We advocate the initiation of further studies evaluating the effects of discontinuing GH replacement in the long-term enabling to determinate the optimal time point for withdrawal of growth hormone replacement.

REFERENCES

1. Rosen T, Bengtsson BA. Premature mortality due to cardiovascular disease in hypopituitarism *Lancet* 1990;336:285-288.
2. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewar PM. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. *Lancet* 2001;357:425-431.
3. Carroll PV, Christ ER, Bengtsson BA, Carlsson L, Christiansen JS, Clemmons D, Hintz R, Ho K, Laron Z, Sizonenko P, Sönksen PH, Tanaka T & Thorner M. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. *Journal of Clinical Endocrinology and Metabolism* 1998; 83 (2): 382-95
4. Rosén T, Wilhelmsen L, Landin-Wilhelmsen K, Lappas G & Bengtsson BA. Increased fracture frequency in adult patients with hypopituitarism and GH deficiency. *European Journal of Endocrinology* 1997; 137: 240-5
5. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P. Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *J Clin Endocrinol Metab* 2004;89:2192-2199.
6. Cenci MC, Conceicao FL, Soares DV, Spina LD, Brasil RR, Lobo PM, Michmacher E, Vaisman M. Impact of 5 years of growth hormone replacement therapy on cardiovascular risk factors in growth hormone-deficient adults. *Metabolism* 2008;57:121-129.
7. Elbornsson M, Gotherstrom G, Bosaeus I, Bengtsson BA, Johannsson G, Svensson J. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol* 2012;166:787-795.
8. Claessen KM, Appelman-Dijkstra NM, Adoptie DM, Roelfsema F, Smit JW, Biermasz NR, Pereira AM. Metabolic profile in growth hormone-deficient (GHD) adults after long-term recombinant human growth hormone (rhGH) therapy. *J Clin Endocrinol Metab* 2013;98:352-361.
9. Gotherstrom G, Svensson J, Koranyi J et al. A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. *J Clin Endocrinol Metab* 2001; 86(10):4657-4665.
10. Koltowska-Haggstrom M, Mattsson AF, Monson JP et al. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? *Eur J Endocrinol* 2006; 155(1):109-119.
11. Claessen KMJA, Appelman-Dijkstra NM, Pereira AM, Joustra SD, de Mutsert R, Gast KB, den Heijer M, Smit JWA, Dekkers OM & Biermasz NR. Abnormal metabolic phenotype in middle-aged GH-deficient adults despite long-term recombinant human GH replacement. *European Journal of Endocrinology* 2014; 170: 263-72
12. van der Klaauw AA, Romijn JA, Biermasz NR et al. Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. *Eur J Endocrinol* 2006; 155(5):701-708.
13. Corpas E, Harman SM & Blackman MR. Human growth hormone and human aging. *Endocrine Reviews* 1993; 14 (1): 20-399.. *European Journal of Endocrinology* 2011; 164 (5): 657-65
14. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, Hwang D, Martin-Montalvo A, Saavedra ated with a major reduction in pro-aging signalling, cancer and diabetes in humans. *Science Translational Medicine* 2011; 3 (70): 1-9
15. Kokshoorn NE, Biermasz NR, Roelfsema F, Smit JWA, Pereira AM & Romijn JA. GH replacement therapy in elderly GH-deficient patients: a systematic review.. *European Journal of Endocrinology* 2011; 164 (5): 657-65
16. Janssen YJ, Frolich M, Roelfsema F. A low starting dose of genotropin in growth hormone-deficient adults. *J Clin Endocrinol Metab* 1997; 82(1):129-135.

17. Janssen YJH, Hamdy NA, Frolich M, Roelfsema F. Skeletal effects of two-years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. *J Clin Endocrinol Metab* 1998; 83(6):2143-2148
18. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F. Long-term skeletal effects of recombinant human growth hormone (rhGH) alone and rhGH combined with alendronate in GH-deficient adult: a seven-year follow-up study. *Clin Endocrinol* 2004; 60: 568-575.
19. Ghigo E, Aimaretti G, Corneli G. Diagnosis of adult GH deficiency. *Growth Horm IGF Res* 2008, Feb;18(1):1-16.
20. Aimaretti G, Corneli G, Rovere S, Granata R, Baldelli R, Grottoli S, Ghigo E, Insulin-Like Growth Factor I Levels and the Diagnosis of Adult Growth Hormone Deficiency. *Horm Res Paediatr* 2004;62(suppl 1):26-33
21. Grinspoon SK, Biller BM. Clinical review 62: laboratory assessment of adrenal insufficiency. *J Clin Endocrinol Metab.* 1994;79:923-931.
22. Fish HR, Chernow B, O'Brian JT. Review Endocrine and neurophysiologic responses of the pituitary to insulin-induced hypoglycemia: a review. *Metabolism.* 1986 Aug; 35(8):763-80.
23. Courtney CH, McAllister AS, McCance, Hadden, Leslie H, Sheridan B, Atkinson AB. The insulin hypoglycaemia and overnight metyrapone tests in the assessment of the hypothalamic-pituitary-adrenal axis following pituitary surgery. *Clin Endocrinol (Oxf)* 2000;53:309-312.
24. Maghnie M, Uga E, Temporini F, Di IN, Secco A, Tinelli C, Papalia A, Casini MR, Loche S. Evaluation of adrenal function in patients with growth hormone deficiency and hypothalamic-pituitary disorders: comparison between insulin-induced hypoglycemia, low-dose ACTH standard ACTH and CRH stimulation tests. *Eur J Endocrinol.* 2005;152:735-741.
25. Appelman-Dijkstra NM, Claessen KMJA, Hamdy NAT, Pereira AM, Biermasz NR. Effects of up to 15 years of recombinant human GH (rhGH) replacement on bone metabolism in adults with Growth Hormone Deficiency (GHD): The Leiden Cohort Study. *Clin Endo Epub* 2014 Nov;81(5):727-35.
26. Fukuda I, Hizuka N, Yasumoto K, Morita J, Kurimoto M & Takano K. Metabolic co-morbidities revealed in patients with childhood-onset adult GH deficiency after cessation of GH replacement therapy for short stature. *Endocrine Journal* 2008; 55: 977-84
27. Yuen KCJ, Dunger DB. Persisting effects on fasting glucose levels and insulinsensitivity after 6 months of discontinuation of a very low-dose GH therapy in adults with severe GH deficiency *Clinical Endocrinology* (2006) 64, 549-555
28. Colao A, di Somma C, Rota F, Pivonello R, Savanelli MC, Spiezia S & Lombardi G. Short-term effects of growth hormone (GH) treatment or deprivation on cardiovascular risk parameters and intima-media thickness at carotid arteries in patients with severe GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2005; 90 (4): 2056-62
29. Biller BMK, Sesmilo G, Baum HBA, Hayden D, Schoenfeld D & Klibanski A. Withdrawal of long-term physiological growth hormone (GH) administration: differential effects on bone density and body composition in men with adult-onset GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2000; 85 (3): 970-6
30. Filipsson Nyström H, Barbosa EJJ, Nilsson AG, Norrman L, Ragnarsson O & Johannsson G. Discontinuing long-term GH replacement therapy – A randomized, placebo-controlled crossover trial in adult GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 2012; 97 (9): 3185-95
31. McMillan CV, Bradley C, Gibney J, Healy ML, Russell-Jones DL, Sönksen PH. Psychological effects of withdrawal of growth hormonotherapy from adults with growth hormone deficiency *Clinical Endocrinology* (2003)59, 467-475
32. Mo D, Blum MD, Rosilio M, Webb SM, Qi R, Strassburger CJ. Ten-Year Change in Quality of Life in Adults on Growth Hormone (GH) Replacement for GH Deficiency: An Analysis of the Hypopituitary

- Control and Complications Study (HypoCCS). *Journal Clinical Endocrinology and Metabolism*. 2014 Sep 18. [Epub ahead of print]
33. Cholesterol Treatment Trialists' (CTT) Collaborators: Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R & Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005; 366 (9493): 1267-78
 34. Emerging Risk Factors Collaboration: Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, Stehouwer CD, Lewington S, Pennells L, Thompson A, Sattar N, White IR, Ray KK & Danesh J. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* 2010; 375 (9733): 2215-22
 35. Brixner DI, Bron M, Bellows BK, Ye X, Yu J, Raparla S & Oderda GM. Evaluation of cardiovascular risk factors, events, and costs across four BMI categories. *Obesity* 2013; 21 (6): 1284-92
 36. Romero-Corral A, Montori VM, Somers VK, Korinek J, Thomas RJ, Allison TG, Mookadam F & Lopez-Jimenez F. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. *Lancet* 2006; 368 (9536): 666-78
 37. Altman DG, Bland JM. Absence of evidence is not evidence of absence *BMJ*. 1995 Aug 19;311(7003):485
 38. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012; 61(6):1315-1322.
 39. Russell SJ, Kahn CR. Endocrine regulation of ageing. *Nat Rev Mol Cell Biol* 2007; 8(9):681-691.
 40. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; 299(5611):1346-1351.
 41. Holmes SJ, Whitehouse RW, Economou G, O'Halloran DJ, Adams JE, Shalet SM. Further increase in forearm cortical bone mineral content after discontinuation of growth hormone replacement. *Clin Endocrinol (Oxf)*. 1995 Jan;42(1):3-7.
 42. Gómez JM, Gómez N, Fiter J, Soler J. Effects of long-term treatment with GH in the bone mineral density of adults with hypopituitarism and GH deficiency and after discontinuation of GH replacement. *Horm Metab Res*. 2000 Feb;32(2):66-70.

Chapter 9

Discussion and Conclusions

SECTION I

Cranial radiation therapy for head- and neck tumours, and for non-pituitary cerebral tumours may affect pituitary function. This is because the pituitary gland often lies within the radiation field of these tumours, more specifically; the hypothalamus and pituitary are often exposed to dosages equivalent to, or even higher than standard radiation schemes used for (non)-functioning pituitary adenomas. Consequently, the prevalence of hypopituitarism in these patients may be high and of clinical relevance, but clinical guidelines for the evaluation of pituitary function after cranial radiation therapy for these tumours are not available. **Chapters 2, 3 and the appendix**, report on the effects of cranial irradiation on pituitary function in patients treated for head and neck and non-pituitary tumours. In chapter 2 we have performed a meta-analysis of available data reported in literature on pituitary function after cranial radiotherapy for head and neck and non-pituitary tumours. In this study, we found a 0,66 (CI 0,55-0,76) prevalence of hypopituitarism of any kind in a group of studies with a high level of heterogeneity. The studies, that included a total number of 813 patients, differed tremendously both in the number and in the type of patients that were included. In addition, the evaluation of pituitary function and the definitions used to define hypopituitarism were not uniform. For instance, for the diagnosis of growth hormone- and cortisol deficiency more than 6 different tests and different cut-off values were used. Due to this variation in quality of the studies we also performed a sensitivity analysis among the 4 studies rated as high quality that showed the same results as for the whole group; furthermore, no differences were seen between tumour types. This implicates that a high prevalence of pituitary insufficiency is likely to occur after cranial radiotherapy for these tumours regardless of tumour type. Because periodical evaluation of pituitary function has not yet been implemented in the routine clinical care of patients irradiated for these tumours, we decided, in view of these results, to perform a cross-sectional analysis of the presence of any hypopituitarism in the patients that were treated in the Leiden University Medical Center (charts of the departments of Endocrinology and Radiotherapy). These results are presented and discussed in chapter 3. We found that not all treated patients, alive had been screened for endocrine pathology. Therefore, in an attempt to obtain the best possible cross-sectional evaluation reflecting pituitary function after cranial irradiation in current clinical practice, we actively recruited all consecutive patients that fulfilled pre-defined inclusion criteria. These patients underwent state-of-the art endocrine testing using stringent cut-off values for hormone deficiencies. Of all patients tested, including those already known at the outpatient clinic of the Endocrinology department, we found that 62% of these had developed hypopituitarism during a variable period after cranial irradiation. Pituitary insufficiency of any form was present in 55% of cases within 5 years after radiotherapy, and amounted to 75% and 90% of patients after 10 and 15 years, respectively.

We have carefully described the origin of our cohort, aiming at a consecutive cohort of patients that underwent cranial radiotherapy for non-pituitary tumours as based on radiotherapy records, avoiding selection bias when patients had already been referred to the endocrine ward. However, since not all patients could be assessed, selection of patients is unavoidable. Next, it is important to accentuate that only patients with a significant dose delivered to the pituitary were included, therefore the overall prevalence of any form of hypopituitarism in our study was high (62% of any pituitary insufficiency). However, these results are in line with our meta-analysis on this topic that demonstrated a prevalence of 0.66 (CI 0.55-0.76) in the same patient groups. Besides selection bias there are other explanations for the discrepant results with some but not all previous studies, such as the use of different tests and differences in test procedures, which, amongst others, reflects the complex, fragile and heterogeneous patient group. Unfortunately, we were not able to explore possible differences between tumours, dose and mode of radiotherapy due to the limited number of patients. In our study, we excluded patients treated with radiation doses < 20 Gy to the pituitary region. A sensitivity analyses revealed no hypopituitarism in any of these patients (data not shown). Consequently, we proceeded with the threshold of 20 Gy. We selected this threshold based on 2 findings: 1) Data from historical cohorts that have indicated that a total dose of 7- 24 Gy delivered to the pituitary/hypothalamus region causes, if any, growth hormone deficiency only, whereas dosages as high as 50-60 Gy will most certainly cause multiple pituitary hormone deficiencies. Using the threshold of 20 Gy delivered into multiple fractions to the pituitary region will generally exclude patients treated with TBI for the need of follow-up for pituitary function, of course these patients should receive follow up for primary endocrinopathies like hypothyroidism, premature testicular or ovarian failure and other metabolic consequences of the previous radiation and chemotherapy. Whether such a threshold is accurate enough should be, of course, be evaluated prospectively in future studies.

Because life expectancy after treatment for the underlying diseases is increasing and hypopituitarism *per se* strongly influences quality of life, it is important to correctly identify hypopituitarism in these patients. In particular, it is crucial to identify those with hypocortisolism, since these patients are exposed to additional, potentially life-threatening risks, like Addisonian crises in case of intercurrent illnesses, and decreased Quality of Life. Noteworthy, current guidelines for the follow-up of patients treated with radiotherapy for NPC tumours include the routine checking of TSH levels to screen for primary hypothyroidism because of high prevalence rates of 0,48 whereas the risk for pituitary insufficiency, like TSH deficiency, in NPC patients may be at least as high but will be easily missed when only TSH is measured.

For a critical evaluation of the likelihood of development of pituitary deficiencies after cranial radiotherapy for the tumours, we advocate the following: there should be an awareness that calculating the dose to the pituitary provides essential information on the

probability of hypopituitarism in the future and should therefore be always performed. To date, this is not reported on a regular basis.

Follow up after cranial irradiation of patients treated with 20 Gy or more delivered to the pituitary region should include the following aspects (*see also Figure 1*). First: a baseline endocrine evaluation prior to radiotherapy, of baseline hormone concentrations. Baseline levels of FT4 and IGF-1 will serve as individual reference values for follow up after treatment, since for example a decrease >20% in FT4 might be indicative for the development of central hypothyroidism. A second step includes measurement of fasting morning cortisol concentration. In case of a cortisol concentration >500–550 nmol/l, in the absence of concurrent corticosteroid use, the activity of the HPA-axis is considered intact making further dynamic testing unnecessary. Also, when cortisol concentrations are <80 nmol/l, the diagnosis of hypocortisolism is evident and dynamic testing will also not be necessary. When dynamic endocrine testing is indicated, the timing of the test in relation to the time of radiation should be taken into account since this may affect the outcome of the tests. Tests like stimulation with ACTH, and the combined GHRH + Arginine test will reveal normal results in the first years after radiation and will become more reliable after a longer period of time, approximately 5 years. Therefore, one of the most reliable tests reflecting the integrity of the hypothalamus-pituitary- end organ axes, is the insulin tolerance test (ITT). However, the use of an insulin tolerance test may not be appropriate in all cases because this is an intensive test in a fragile patient group. In addition, GH deficiency might not be clinically relevant for patients treated for a malignant tumour, because malignancy is generally considered a contraindication for GH replacement in adults after anecdotal reports of tumour growth in craniopharyngeomas, and of increased risk for bone tumours in children treated with rGH, and the association between IGF-1 and cancer risk. However, the documentation of the presence of GHD will be useful to identify patients at risk for radiation induced hypothalamic/pituitary damage and therefore we still recommend testing of the pituitary- GH-IGF-1 axis. When patients are treated with dexamethasone, the HPA-axis cannot be evaluated, and GHD can be tested using the combined GHRH+ARg-test. Nevertheless, it might be decided that diagnosing GHD is not relevant in a specific patient and than no test should be performed. In all other patients we would advice to perform the best available test, taken into account the patients WHO performance classification and life expectancy. It is not possible to only rely on the patients' symptoms with respect to hypopituitarism for the decision to whether or not to perform endocrine function tests. Such an approach would underestimate the presence of hypopituitarism since patients may have various complaints that could also be attributable to cranial radiation/chemotherapy or to the impact of the diagnosis of cancer itself. Therefore, how to proceed within the first years after radiation has to be tailored individually in each patient, since this is a very complex and fragile patient cohort.

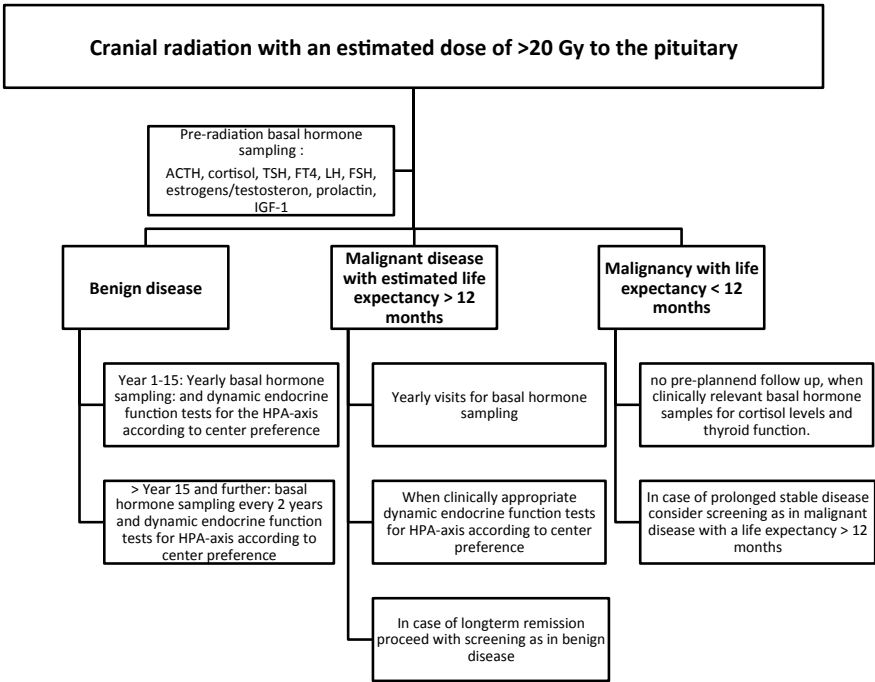


FIGURE 1.
Proposed follow up

The incidence of multiple pituitary deficiencies increases both in time and with higher doses. *See also figure 2 in the Introduction of this thesis.* Considering these and our findings that 90% of patients will be diagnosed with hypopituitarism after 15 years we recommend periodical screening for this entire period. Whether this should be done annually or with greater intervals is at the discretion of the physician.

The protocolled follow-up algorithms needs to be validated prospectively, and should also include periodical evaluation of quality of life.

SECTION II

Chapters 4-8 describe the effects of long-term recombinant human growth hormone replacement in adult patients with proven severe growth hormone deficiency (GHD). In **Chapter 4** we performed a systematic review of the literature of studies that have evaluated the effects of growth hormone replacement beyond 5 years of treatment. In the short term, several studies have shown beneficial effects of growth hormone replacement therapy on lipid metabolism, muscle mass and strength, bone mass density and quality of life. The systematic review identified a total of 23 studies. We encountered several problems in this search. Generally, most reports lacked a representative control

group (of non-treated GHD patients) not allowing adjustment for potential subjective changes or changes due to ageing. Secondly, only a limited number of centers have reported their data, resulting in a limited number of evaluable patients with a follow-up duration beyond 5 years of rGH replacement. This means that long-term follow-up results of rGH replacement are reported only by a very limited number of centers, and consequently extending follow-up results derive by the same centers. Thus, most studies describe (part of) the same patient cohort. With respect to the metabolic profile, rGH therapy has shown prolonged, beneficial effects on the long-term for body composition, lipid profile, carotid IMT and BMD. However, overall cardiovascular risk, as assessed by the prevalence of the metabolic syndrome, glucose levels, and BMI appeared not to be influenced or were even negatively affected. The course of changes in QoL during ongoing therapy is not established. Therefore, the benefit of prolonged rGH treatment should be a matter of ongoing research to enable adequate risk-benefit analyses. Consequently, the benefits of rGH in clinical daily practice in the long-term should be critically re-evaluated in each patient.

Chapter 5 describes the evaluation of the 10 and 15 years results of rGH replacement therapy on the metabolic syndrome in our own Leiden adult GHD patient cohort. For this study, a total of 98 adult patients with GHD with extended follow up were prospectively followed at the outpatient clinic of the Leiden University Medical Centre. Cardiovascular risk factors, such as lipids, glucose, blood pressure, and anthropometric parameters (waist-hip ratio and BMI) were checked annually in these patients. Even after more than 10 years of treatment, we demonstrated ongoing beneficial effects on lipid profile, with a significantly decrease in LDL and increase in HDL compared to baseline. However, when we attempted to extrapolate these individual changes in cardiovascular risk factors into the commonly used risk profile of the metabolic syndrome (MS), the net result did not appear to have any beneficial effects at all. On the contrary, the MS prevalence increased significantly, especially in males, to 57,1% after 10 years of replacement (from 32.7 % at baseline). Since in the Netherlands rGH replacement is widely available and all eligible patients will be treated with rGH, no control group of untreated GHD patients is available and the use of historical cohorts is not completely representative for present optimal care due to improvements in hormonal replacement, and consequently, the prevalence of adverse metabolic profile. Therefore, we selected a control group of the Netherlands Epidemiology of Obesity (NEO) study. In the NEO-study, a contemporary cohort of healthy middle-aged controls was sampled from the Leiderdorp area, which is in the vicinity of Leiden. In **Chapter 6** we report the results of the comparison of the metabolic phenotype of the patients from the GHD cohort treated with growth hormone for at least 5 years with these healthy controls from the NEO cohort. A total of 161 middle-aged patients were included in the study. When adjusted for age, sex and BMI, GHD patients were classified significantly more often with a diagnosis of the MS when compared with the control population (53.4%

vs 30.3%). Most importantly, it was shown that starting rGH replacement affects the different components of the MS, resulting in more hypertriglyceridemia and lower HDL. Despite hormonal treatment in concurrence with modern guidelines, their metabolic profile does not seem to differ much from non-treated GHD at the expense of marked increases in BMI/weight. Although the study was not designed to detect differences in cardiovascular events, these results indicate that it still has to be established that rGH replacement is more effective than commonly advised lipid lowering treatment with statins in the prevention of cardiovascular events in this specific patient group.

In **Chapter 7**, the effects on bone were investigated in 230 GHD patients. We assessed bone mineral density at baseline, and annually thereafter during follow-up while on rGH replacement. In addition, we reported the incidence of clinical fractures. Intriguingly, no clear beneficial changes were seen in female patients, whereas in male patients, we observed a sustained increase in lumbar spine BMD during the entire period of 15 years of treatment. Bisphosphonate use did not alter the net outcomes on BMD in these patients. Clinical fracture incidence was 20,1/1000 patients per year and was not higher when compared to literature controls. Long-term rGH replacement does seem to be beneficial for the bone mass of males suffering from GHD. In females the effect of rGH may be blunted due to estrogen deficiency and due to sex specific geometrical differences since women tend to have more trabecular loss due to aging. Furthermore if rGH also decreases the risk of fractures is yet to be debated since all studies performed, including ours, were not powered to detect fracture difference. It might very well be that the additional calcium/d3 medication already is responsible for a drop in fracture incidence.

In **Chapter 8** we evaluated the effects of discontinuation of rGH therapy within the Leiden GHD cohort and additionally performed a systematic literature search on the topic. In a sub-analysis, we also specifically aimed to evaluate the effects of discontinuation of rGH replacement in elderly patients. We found that discontinuation of rGH replacement therapy was associated with an increase in body fat. However, in clinical practice this did not negatively affect lipid profile, and we also did not observe any changes in glucose metabolism, in the presence of a decrease in BMI. In patients older than 60 yrs, rGH withdrawal did not affect the metabolic profile at all. It thus appears that the net metabolic effects of discontinuing rGH replacement after long-term use is not by definition disadvantageous. This may reflect ongoing beneficial effects of the rGH treatment, a lack of significant effects (especially at advanced age), or possibly a combination of both.

In adult patients with growth hormone deficiency data from long-term studies indicate that recombinant growth hormone replacement has beneficial effects on cardiovascular risk factors and quality of life in a pre-selected group of patients with hypopituitarism. However, one of the main indications, amelioration of the adverse cardiometabolic

risk, as profiled in the general population with the metabolic syndrome phenotype, is not achieved by this treatment. Conversely, in our cohort, we even observed an increase in the prevalence of the metabolic syndrome during long-term therapy. In addition, the effects of rGH replacement therapy on bone seem gender dependent. However, as stated before, ideally long-term comparisons should be made with a comparable untreated GHD cohort and a longitudinal study for the control population, which were not available. Unfortunately, there are no longitudinal population control data available to assess the expected trend for weight gain in the period of assessment for the study described in chapter 5. In addition, there are no large-scale longitudinal data in representative GH deficient patients not treated with rGH. However, the Dutch Ministry of Health conducted two large population-based studies: the ‘MORGEN Study’, which was conducted during the period 1993-1997, and ‘Nederland de Maat genomen’ in 2009-2010, both reporting control data for BMI. In 1993-1997, mean BMI was 25.8 kg/m² and 25.0 kg/m² in males and females, respectively, and fifteen years later 26.0 kg/m² in males and 24.9 kg/m² in females in 2009-2010, aged 30-59 years.

In the absence of ongoing RCTs of rGH treatment we have to deal with a heterogeneous group of GH patients and findings are likely a resultant of the imperfections of hormone replacement in those with abnormal function. All the patients in our GHD cohort have failure of multiple pituitary axes, and this results in important interactions between the hormones used for replacement. It is of importance that awareness and proper monitoring of these interactions are important in order to reach an optimal replacement therapy. Isolated GHD (IGHD) provides the ideal model to characterize GHD without interference from other pituitary deficiencies or their treatment. It is reported that baseline clinical characteristics and rGH treatment response in patients with IGHD in comparison to patients with multiple axis failure show a generally similar pattern and rGH treatment response in IGHD patients as well as in patients with multiple deficiencies, especially in AO-GHD. These findings support the concept that GH *per se* is responsible for at least part of the metabolic aspects of patient that receive hormone replacement for multiple pituitary hormone deficiencies. Furthermore, it has been shown in a recent meta-analysis that both very low and high IGF-1 levels increase mortality in general population (hazard ratio (HR) 1.18, 95%CI 1.04-1.34). This U-shape relationship was present for both cancer and cardiovascular mortality. In addition, several other reports show an association of both low and high IGF-1 levels with DM2, sarcopenia, fracture risk and cardiovascular diseases. However there are also human studies linking reduced IGF-1 levels/signaling to a reduced cancer risk as well as improved longevity. For example, in an Ecuadorian family with severe GHD due to GHR gene mutations, type 2 diabetes and cancer risk were strongly reduced. In addition, functional mutations of the IGF-1R gene resulting in altered IGF-1 signaling are more common in centenarians than in younger controls. The GH/IGF-1 axis declines physiologically with ageing and smaller individuals within a species usually live longer

(including ponies and small dogs). Life span was also expanded in mice lacking GHR, resulting in lower IGF-1 levels. Although these mutant mice lack other hormones, their extended longevity is thought to be primarily due to GHD, as restoration of GH levels reverted their longevity to that of non-mutants. Collectively, these results suggest that optimizing the GH/IGF-1 axis to promote healthy ageing in humans is more complex than originally appreciated, and will require a greater understanding of its array of interactions and tissue specificity.

Thus, one can conclude that rGH replacement can be safely initiated in adult patients with severe GHD and has to be monitored throughout the years, enabling to make individual adaptations based upon alteration in e.g. MS and bone mass for each patient every year. It has also to be noted that the long-term effects on quality of life should be more carefully addressed in future research, a topic that has not been investigated in the studies presented in this thesis. In view of an aging population the question if growth hormone replacement should be continued lifelong should also be an important topic for future research. Growth hormone secretion declines during life and therefore it is logical to withdraw growth hormone replacement, or at least to decrease the dosage to a minimum when reaching advanced age, especially since some of the effects maybe long-lasting or could be treated at least equally effective with other drugs, like for instance with statins. Finally, we advocate that future studies on growth hormone replacement should also focus on value based outcomes and that centers that treat adult patients with rGH replacement continue to critically evaluate and report on long-term outcome.

Chapter 10

Nederlandse Samenvatting

De hypofyse en hypothalamus

De hypofyse is een klein orgaan dat zich onderaan de hersenen bevindt en in een benige holte ligt, de sella turcica genoemd. De hypofysesteel verbindt de hypofyse met een andere hersenkern, de hypothalamus. Gezamenlijk zorgen de hypofyse en hypothalamus voor de juiste ‘vertaalslag’ van externe signalen door afgifte van hormonen. De hypofyse is, ondanks zijn kleine afmeting (ongeveer de grootte van een doperwt), één van de belangrijkste klieren van het lichaam. Onder de invloed van hypothalamische hormonen maakt de hypofysevoorkwab groeihormoon (GH), prolactine, adreno-corticotroop hormoon (ACTH), thyroid stimulerend hormoon (TSH) en de gonadotrofines LH en FSH. Deze hormonen sturen weer hun eigen doelorganen aan zoals de bijnierschors, schildklier en eierstokken/zaadballen. De hypofyseachterkwab bestaat uit axonale uitlopers van hypothalamische neuronen die het hormoon vasopressine (ADH) maken.

Uitval van de hypofysefunctie kan ontstaan als gevolg van een tumor in de (buurt van de) sella turcica, of als gevolg van schade aan de hypofyse en/of hypothalamus door operatie of bestraling. Uitval van de hypofysefunctie gaat gepaard met specifieke klachten zoals vermoeidheid en algeheel onwel bevinden, maar kan ook leiden tot gevaarlijke situaties zoals een Addisonse crisis met lage bloeddrukken als gevolg van ernstig cortisoltekort. Hypofyse uitval kan enkel vastgesteld worden door het meten van de betreffende hypofysehormonen, als ook de hormonen van de doelorganen, zoals bijvoorbeeld testosteron en oestradiol als maat voor LH/FSH activiteit. Gezien het sterk pulsatiele karakter van de secretie van hypofysehormonen en de sterke circadiane variatie is het meten van enkel ochtendwaarden voor met name groeihormoon en ACTH/cortisol niet voldoende. Om te kunnen bepalen of de hypofyse voldoende reservecapaciteit heeft om tijdens situaties met een sterk verhoogde behoefte (zoals stress en koorts) genoeg hormonen te kunnen maken, is het noodzakelijk om een zogenaamde functietest of dynamische test uit te voeren. Hierbij wordt de ACTH en GH secretie gestimuleerd door het inspuiten van de hypothalamische hormonen (CRH en GHRH) of door middel van insuline, wat de meest natuurlijke prikkel is voor het opwekken van een stressrespons, namelijk een lage bloedsuiker.

DEEL I: HYPOFYSE UITVAL NA BESTRALING VAN HET HOOFD VOOR HERSEN-OF KNO-TUMOREN

In de hoofdstukken 2, 3 en de bijlage (appendix) worden de effecten van bestraling op de hypofysefunctie beschreven zoals deze kunnen ontstaan na bestraling van het hoofd als behandeling van hersen-/Keel-Neus en Oor(KNO) tumoren. Bij deze behandeling ligt de hypofyse vaak midden in het bestralingsveld, waardoor deze ook veel straling krijgt. Hierdoor wordt de hypofyse blootgesteld aan een stralingsdosis die gelijk, of zelfs hoger is dan de dosering die men gebruikt voor de behandeling van

hypofysetumoren. Hierdoor is het een reële kans dat enkele jaren na de bestraling verlies van de hypofysefunctie kan ontstaan. De levensverwachting van patiënten met hersentumoren en met KNO-tumoren is in de loop der jaren toegenomen, hierdoor kan hypofyse uitval, voor de patiënt een relevant probleem worden. Hypofyse uitval kan de kwaliteit van leven nadelig beïnvloeden, maar ook medische problemen geven variërend van vermoeidheid tot een Addisonse crise bij cortisoltekort of seksuele dysfunctie bij vermindering van het geslachtshormoon. Omdat deze klachten vaak moeilijk te herkennen zijn bij mensen die behandeld zijn voor kanker, kan dit gemist worden indien er niet specifiek naar gezocht wordt. Met name cortisoltekort kan tot potentiële levensbedreigende problemen leiden zoals lage bloeddruk. In **Hoofdstuk 2** worden de resultaten van een meta-analyse beschreven over het voorkomen van hypofyse uitval na bestraling van de hersenen. In deze studie laten wij zien dat de prevalentie van hypofyse uitval erg hoog is bij patiënten na behandeling voor KNO tumoren en hersentumoren. In totaal zijn in deze analyse 813 patiënten geïncludeerd in 18 studies. Er is een prevalentie van hypofyse uitval van 0,66 (95% betrouwbaarheidsinterval 0,55-0,76) gevonden. Zowel bij hersentumoren, als ook bij KNO tumoren is de prevalentie verhoogd en de getallen ontlopen elkaar niet veel. Verder valt in deze meta-analyse op dat er veel verschillen bestaan in de methodologie tussen de diverse studies. Zo verschilt de wijze van testen van hypofysefuncties en de definitie van hypofyse uitval tussen de studies. Voor de diagnose groeihormoon deficiëntie en cortisol deficiëntie zijn meer dan 6 verschillende testen gebruikt met ook nog verschillende afkapwaarden. Vanwege het grote verschil in kwaliteit tussen de studies is ook een sensitiviteitsanalyse gedaan waarbij de 4 'beste' studies qua methodologie zijn vergeleken met de resultaten van de hele groep. Deze sensitiviteitsanalyse toont dezelfde resultaten als voor de gehele groep. Gezien de hoge prevalentie van hypofyse uitval in deze meta-analyse is besloten de stand van zaken te onderzoeken m.b.t (de evaluatie van) de hypofysefuncties van patiënten die in het LUMC bekend zijn bij de afdelingen Radiotherapie of Endocrinologie over een periode van 20 jaar. De resultaten van dit onderzoek staan beschreven in **hoofdstuk 3**. Uit dit onderzoek is gebleken dat in de eerste plaats een groot gedeelte van de patiënten binnen het LUMC niet gescreend zijn op hypofyse uitval. Van deze groep niet-gescreende patiënten was de meerderheid reeds overleden of had een dermate slechte gezondheid dat zij niet opgeroepen konden worden voor hormonale evaluatie. De overige patiënten zijn uitgenodigd voor evaluatie van de hypofysefunctie. Van alle onderzochte patiënten in het LUMC is gebleken dat 62% van hen enige vorm van hypofyse uitval had ontwikkeld na bestraling. Na de bestraling was in de helft van de gevallen na 5 jaar functieverlies van de hypofyse opgetreden. Na 10 jaar en na 15 jaar was dit respectievelijk 75% en 90%.

Op dit moment zijn erg geen landelijke richtlijnen voor het vervolgen van de hypofysefuncties na bestraling. Voor een juiste risicoschatting van de kans op het ontwikkelen van hypofysefunctie uitval is het van belang dat de radiotherapeut

bij de planning van de bestraling aangeeft wat de geschatte dosis op de hypofyse is. Patiënten met een dosis van 20 Gy of meer op de hypofyse kunnen verwezen worden voor follow up. Gezien het frequent voorkomen van hypofyse uitval en de met de uitval gepaard gaande problemen op het gebied van kwaliteit van leven en andere gezondheidsproblemen, adviseren wij periodieke hormonale evaluatie na behandeling voor deze aandoeningen mee te nemen in de richtlijn ontwikkeling. De combinatie van de geschatte bestralingsdosis op de hypofyse met de verwachte levensverwachting zal uit moeten wijzen of jaarlijkse/tweejaarlijkse follow-up wenselijk is. Daarnaast zal gekeken moeten worden bij welke arts dit moet gebeuren, bijvoorbeeld een endocrinoloog of via de behandelend radiotherapeut- of internist-oncoloog. Indien besloten wordt tot follow-up adviseren we dit in ieder geval tot 15 jaar na afronden van de radiotherapie te doen. De belasting van het vervolgen van patiënten en het herhalen van hypofysefunctie-testen moet voor elke patiënt individueel afgewogen worden. In ieder geval wordt geadviseerd de endocrinologische tractus na te vragen en dient bij alle patiënten een eenvoudig bloedonderzoek in de ochtend tussen plaats te vinden voor cortisolbepaling en bepaling van de schildklierfunctie (TSH en FT4). Aanvullend kunnen tevens IGF-1, prolactine, geslachtshormonen in combinatie met een hypofysefunctie-test gedaan worden bij patiënten met benigne ziekte of een levensverwachting van meer dan 12 maanden. Hiervoor kunnen verschillende testen gebruikt worden, maar de Insuline Tolerantie Test (ITT, de gouden standaard) heeft de voorkeur. Bij contra-indicaties voor deze test kan gekozen worden voor de CRH- of ACTH stimulatietest. In ons cohort hebben wij geen bijwerkingen of complicaties gezien van de ITT, behalve de obligate passagere klachten van hypoglykemie tijdens de test zelf. Bij patiënten die corticosteroiden gebruiken moet duidelijk genoteerd worden dat deze medicatie niet zomaar gestopt mag worden, aangezien men zeker moet zijn dat de Hypofyse-Bijnier-as nog intact is.

Bij patiënten die met een cumulatieve dosis van minder dan 20 Gy bestraald zijn op de hypofyse is de kans op hypofyse uitval zo klein dat een standaard controle hierop niet zinvol wordt geacht.

CONCLUSIE

Hypofyse uitval na bestraling komt frequent voor, sterker nog, meer dan 60% van de behandelde volwassen patiënten heeft enige vorm van hypofyse uitval. Daarom adviseren wij bij mensen met een levensverwachting van meer dan 12 maanden om de hypofysefuncties regelmatig te controleren voor een periode van 15 jaar. Dit kan gedaan worden door een internist-endocrinoloog of door de behandelende internist-oncoloog of radiotherapeut-oncoloog. Voor elke patiënt zal individueel gekeken moeten worden wat de belasting is van bloedonderzoek en hypofysefunctie-testen. Dit voorstel voor follow up is gebaseerd op de resultaten van cross-sectionele analyses en zal prospectief

geëvalueerd moeten worden. Geadviseerd wordt om metingen naar de kwaliteit van leven hierbij mee te nemen.

DEEL II: GROEIHORMOON

Hoofdstukken 4-8 beschrijven het effect van langdurig groeihormoongebruik bij volwassen patiënten met groeihormoon deficiëntie (GHD). **Hoofdstuk 4** is een systematische review van de literatuur over de effecten van recombinant humaan groeihormoontherapie (rGH) na een gebruik van meer dan 5 jaar. In deze review werden in totaal 23 studies geanalyseerd. We zagen dat langdurige suppletie met rGH gunstige effecten had op de lichaamssamenstelling, lipidenprofiel, op intima media dikte en op de botmassa. Andere uitkomstmaten, zoals het metabool syndroom, de bloedsuikerwaarden en BMI werden niet beïnvloed of verslechterden juist. Dit zijn juist factoren zijn die alom beschouwd worden als factoren die het cardiovasculaire risicoprofiel beïnvloeden. Veranderingen in kwaliteit van leven na meer dan 5 jaar groeihormoon gebruik zijn niet beschreven. Vrijwel geen enkele studie had een goede controlegroep van niet behandelde GHD-patiënten omdat in de meeste landen groeihormoon standaard wordt voorgeschreven aan geschikte patiënten. Hierdoor kan niet aangetoond worden welke veranderingen bij patiënten met hypofyse uitval nu door (natuurlijke) veroudering komen en welke door de effecten van rGH. Daarnaast is er maar een beperkt aantal centra die hun lange-termijn resultaten hebben gerapporteerd (2) waardoor de patiënten aantallen laag zijn en dezelfde patiënten cohorten vaak opnieuw worden beschreven. De winst van rGH therapie op de lange termijn bij volwassen zal daarom een punt van aandacht moeten blijven voor verder onderzoek.

In **Hoofdstuk 5** beschrijven wij de resultaten van ons eigen cohort dat minimaal 10 jaar tot 15 jaar behandeld werd met rGH. In deze studie hebben we specifiek gekeken naar het voorkomen van het metabool syndroom bij 98 patiënten met GHD die vanaf starten van de behandeling met rGH in het LUMC onder controle zijn geweest. Cardiovasculaire risicofactoren, zoals cholesterol en lipidspectrum, glucose, bloeddruk en lengte/gewicht/middel- en heupomtrek werden jaarlijks vervolgd op de polikliniek. We zagen een duidelijk gunstig effect op het cholesterol en lipidspectrum, zelfs na 10 en 15 jaar behandeling, met een significante reductie in het LDL en verhoging van het HDL in vergelijking met het cholesterol spectrum voor aanvang van behandeling. Echter het voorkomen van het metabool syndroom van deze patiënten veranderde niet, integendeel, dit percentage steeg bij mannen verder door naar 57.1% na tien jaar behandeling. Dit was een zeer forse stijging ten opzichte van de 32.7% bij aanvang van therapie. Omdat rGH in Nederland algemeen verkrijgbaar is en alle geschikte patiënten behandeld worden met rGH is er geen controlegroep in de studie geïncludeerd. Doordat er in de jaren veel veranderd is op het gebied van statinegebruik, verbetering van hormonale suppletie en veranderde prevalentie van het metabool syndroom kan er dan ook geen

vergelijking plaatsvinden met historische patiënt cohorten. Om toch een betere indruk te krijgen van de geobserveerde veranderingen in de tijd is er een studie verricht met een controlegroep vanuit de Nederlandse Epidemiologie van Obesitas (NEO) studie. In deze studie werden gegevens verzameld van onder andere een cohort van gezonde controles van middelbare leeftijd (45-65 jaar) uit de omgeving van Leiderdorp, een plaats zeer dicht bij Leiden. **Hoofdstuk 6** geeft de resultaten weer van deze vergelijking tussen het metabole profiel van onze patiënten op stabiele rGH therapie voor minimaal 5 jaar en de bevolkingscontroles vanuit de NEO-studie. 161 GHD patiënten werden geïnccludeerd in de studie, na correctie voor leeftijd, sekse en BMI bleek dat het metabool syndroom significant vaker voorkwam in de GHD populatie, 53,4% *vs* 30,3%. Daarnaast was duidelijk te zien dat 5 jaar na rGH behandeling de prevalentie van het metabool syndroom was gestegen van 41% naar 53,4% ($p=0.007$). Ook was het risico op het metabool syndroom na 5 jaar behandeling nog steeds 1.3 maal hoger dan in de gezonde controle groep, onafhankelijk van leeftijd, geslacht en BMI (95% betrouwbaarheidsinterval 1.1-1.5, $p=0.008$). De patiënten met GHD hadden daarnaast een duidelijk ander metabool profiel dan de controle groep; Ze hadden een verhoogde kans op hypertriglyceridemie PR 2.0, (95% betrouwbaarheidsinterval 1.7-2.3) en een verlaagd HDL-cholesterol PR 1.8 (95% betrouwbaarheidsinterval 1.5-2.2), maar minder hyperglycemie PR 0.5 (95% betrouwbaarheidsinterval 0.4-0.7). Ondanks modernisering van hormonale suppletie en suppletie technieken veranderde het metabole profiel van patiënten niet heel veel ten opzichte van hun metabool profiel bij aanvang van rGH therapie terwijl het gewicht en dus de BMI wel fors toenam. De studie was echter niet ontworpen om verschillen in cardiovasculaire events te detecteren, maar het valt nog maar te bezien of het starten van rGH ten behoeve van een gunstiger lipidenprofiel en reductie van cardiovasculaire events effectiever is dan de reguliere behandeling met statines. Aan de andere kant werd er minder hyperglycemie gezien in GHD patiënten, ondanks een hogere BMI, wat mogelijk wel mede aan rGH therapie te danken is.

In **Hoofdstuk 7**, werden de effecten van langdurig rGH gebruik op het bot onderzocht. Voor deze studie werden 230 GHD patiënten geïnccludeerd. De botdichtheid verminderde in vrouwelijke patiënten in de loop der jaren zowel in de wervelkolom als in de heup, na een aanvankelijke stijging. In mannen werd echter een duidelijke toename van de botdichtheid van met name de wervelkolom gezien die gedurende 15 jaar behandeling persisteerde alsook een stabilisatie van de BMD van de heupen, dit in het licht van een verwachte natuurlijke afname in de loop der jaren. Het toevoegen van een bisfosfonaat liet geen extra verandering zien. De klinische fractuur incidentie was 20,1/1000 patiënt jaren en was niet duidelijk verhoogd als vergeleken werd met controle data uit de literatuur. De resultaten bij de vrouwen kunnen vertekend zijn doordat de vrouwen in de perimenopauze fase waren en oestrogeen deficiëntie in deze periode een bepalende rol speelt in het beloop van de veranderingen in BMD. Of therapie met rGH ook het fractuur risico van GHD patiënten verminderd is nog niet duidelijk aangezien

de studies die gedaan zijn niet ontworpen zijn om hier een verschil in te ontdekken en ook geen controle groep geïnccludeerd kon worden. Omdat in onze studie bijna iedereen calcium en vitamine D3 kreeg kan dit al een goede verklaring zijn voor het relatief lage fractuur risico in vergelijking met veel hogere getallen van oudere, niet behandelde, GHD cohorten.

In Hoofdstuk 8 tot slot worden de resultaten beschreven van een observationele studie naar het staken van GH vanwege uiteenlopende redenen bij patiënten na behandeling. Daarnaast is er ook een systematisch literatuuronderzoek gedaan naar dit onderwerp. We constateerden dat het stoppen van rGH therapie geassocieerd was met een stijging van het vetpercentage. Deze stijging bleek echter niet samen te gaan met een verslechtering van het cholesterolspectrum of verandering in nuchtere glucose concentraties. Patiënten die stoppen met groeihormoon vallen af, resulterend in een lagere BMI. Een subanalyse in patiënten ouder dan 60 jaar laat zelfs helemaal geen veranderingen zien behalve een iets lagere BMI. Mogelijk zou dit kunnen betekenen dat het stoppen van de behandeling met rGH niet per definitie nadelige effecten heeft op het cardiometabole profiel vooral op gevorderde leeftijd, echter dit dient nog zeker beter uitgezocht te worden.

CONCLUSIE

Behandeling met rGH heeft in een geselecteerde groep patiënten gunstige effecten. De studies hier beschreven betreffen relatief gezonde GHD patiënten, dus zonder maligniteit of diabetes. Echter een van de voornaamste indicaties waarvoor behandeling met rGH wordt geadviseerd bij volwassen patiënten met GHD, namelijk verbetering van het cardiovasculaire risicoprofiel, wordt door onze studies niet ondersteund. Het lipidenprofiel verbetert wel, maar het overgewicht en metabool syndroom verslechteren onder langdurige therapie. De gunstige effecten op het bot zijn enkel terug te vinden bij mannen en betreffen voornamelijk een toename van de botdichtheid van de wervelkolom. Informatie over reductie van het fractuurrisico ontbreekt tot op heden. Idealiter zullen prospectieve onderzoeken met een langdurige follow-up duur moeten plaatsvinden met een representatieve controle groep van onbehandelde patiënten met GHD. Een dergelijke studieopzet is echter niet haalbaar daar deze groep niet beschikbaar is in Europa. In deze studies werden geen bijzondere bijwerkingen gezien van langdurige rGH suppletie, dus het lijkt er op dat langdurige behandeling met rGH veilig gestart kan worden, zeker bij jongvolwassenen. Of rGH behandeling ook extra waarde heeft voor volwassenen van middelbare of gevorderde leeftijd valt nog te bezien. Nieuwe studies waarbij ook evaluatie van de kwaliteit van leven meegenomen worden moeten dit in de toekomst nog uitwijzen.

Appendix

Hypofyse-uitval na hoofd-halsbestraling Een systematische review en meta-analyse

Natasha M. Appelman-Dijkstra, Nieke E. Kokshoorn, Olaf M. Dekkers,
Karen J. Neelis, Nienke R. Biermasz, Johannes A. Romijn,
Johannes W.A. Smit en Alberto M. Pereira

DOEL

Systematische evaluatie van de gerapporteerde prevalentie van hypopituitarisme na bestraling van het hoofd-halsgebied in verband met cerebrale of nasofaryngeale tumoren en na totale lichaamsbestraling.

OPZET

Systematische review en meta-analyse.

METHODEN

In PubMed, Embase, Web of Science en de Cochrane Library werd gezocht naar artikelen over de prevalentie van hypopituitarisme bij volwassenen na hersenbestraling. In de referenties van belangrijke artikelen werd gezocht naar andere relevante artikelen. De meta-analyse werd verricht met behulp van een exacte 'likelihood'-methode met gebruik van een logistische regressie met 'random effects'-model op studieniveau.

RESULTATEN

Er werden 18 studies met in totaal 873 patiënten geïncludeerd. Dit waren 608 patiënten met een nasofarynxcarcinoom en 265 met 1 of meer hersentumoren. De totale dosis aan bestraling varieerde van 14-83 Gy en 40-97 Gy voor respectievelijk nasofarynx- en hersentumoren. De prevalentie van enige vorm van hypopituitarisme was 0,66 (95%-BI: 0,48-0,76). De prevalentie van groeihormoondeficiëntie was 0,45 (95%-BI: 0,27-0,55), 0,30 voor LH-FSH-deficiëntie (95%-BI: 0,16-0,29), 0,25 voor TSH-deficiëntie (95%-BI: 0,13-0,35) en 0,22 voor ACTH-deficiëntie (95%-BI: 0,13-0,29). De prevalentie van hyperprolactinemie was 0,34 (95%-BI: 0,11-0,38). Er waren geen verschillen in het effect van bestraling tussen de beide tumorgroepen. Er was geen verschil in prevalentie tussen studies met meer of minder risico op bias.

CONCLUSIE

Hypopituitarisme komt veel voor na hoofd-halsbestraling in verband met nasofarynxcarcinomen en hersentumoren. Daarom dienen alle patiënten die bestraald zijn op dit gebied langdurig vervolgd te worden met regelmatige controles van de hypofysefuncties.

Introductie

Patiënten met hersentumoren of tumoren in het neus- of keelgebied worden vaak behandeld met uitwendige radiotherapie. Dit kan na de behandeling op langere termijn resulteren in hypofyse-uitval, doordat de hypofyse vaak volledig in het bestralingsgebied ligt. Op de kinderleeftijd is hypofyse-insufficiëntie een bekende complicatie: 43% van de bestraalde kinderen ontwikkelt hypofyse-uitval.¹ Patiënten die kanker op de kinderleeftijd hebben overleefd, worden in de follow-up uitgebreid gescreend op hypofyse-uitval. Op volwassen leeftijd verloopt deze uitval echter vaak sluimerend met aspecifieke klachten, waardoor de disfunctie van de hypofyse gemakkelijk onopgemerkt kan blijven als er niet op gescreend wordt. Voor volwassenen is routinematige periodieke beoordeling van de hypofysefunctie niet opgenomen in richtlijnen voor de follow-up. In de laatste jaren zijn de meeste overlevingspercentages van de patiënten met maligniteiten verbeterd door introductie van nieuwe chirurgische, radiotherapeutische of chemotherapeutische behandelingen, waardoor meer patiënten risico lopen op hypofyse-uitval.

Bestraling van de hypothalamus- en hypofyseregio veroorzaakt een in de tijd karakteristiek patroon van hormonale uitval; hierbij valt de groeihormoonsecretie als eerste uit, gevolgd door uitval van de gonadotrofines, de hypofyse-bijnieras en de hypofyse-schildklieras.²⁻⁴ De mate van uitval is afhankelijk van de stralingsdosis. De incidentie van meerdere deficiënties na bestraling neemt toe in de tijd en naarmate de toegepaste stralingsdosis hoger is.⁵

De diagnostiek van hypofyse-uitval kan lastig zijn. In ieder geval moeten de basale ochtendwaarden van alle hypofysehormonen gemeten worden, aangevuld met functietesten van de hypothalamus-hypofyse-bijnieras en de groeihormoonas. De keuze van de functietest hangt ook af van de fysieke conditie van de patiënt. Zo zal een insulinetolerantietest (ITT) niet kunnen worden uitgevoerd bij patiënten met epilepsie of cardiale problemen. Omdat de evaluatie van endocriene functies varieert in verschillende studies, verschilt de prevalentie van hypofyse-uitval sterk in de diverse studies.^{1,6}

Het doel van deze review was een systematische evaluatie van de gerapporteerde prevalentie van hypofyse-uitval bij volwassenen na bestraling van de hersenen in verband met cerebrale of nasofaryngeale tumoren of na totale lichaamsbestraling bij een hematologische maligniteit.

MATERIAAL EN METHODEN

Het systematisch literatuuronderzoek betrof artikelen verschenen tot 14 augustus 2010. Er werd gezocht in de volgende databases: PubMed, Cochrane Library, Web of Science, Embase, 'Cumulative index to nursing and allied health literature' (CINAHL), Academic

Search Premier en Science Direct. De zoekstrategie was toegespitst op radiotherapie, hypofysefunctie, hersentumoren en nasofaryngeale tumoren. De complete strategie is terug te lezen in tabel 1.

Daarnaast werden de referenties van geïnccludeerde artikelen gecontroleerd op additionele artikelen. Inclusiecriteria waren: (a) bestraling van de hersenen voor een andere aandoening dan een hypofysetumor of totale lichaamsbestraling; (b) leeftijd van 18 jaar of ouder op het tijdstip van bestraling en (c) rapportage over de endocriene evaluatie. In geval van gemengde cohorten, zoals een cohort van kinderen en volwassenen, werden alleen data geëxtraheerd van patiënten ≥ 18 jaar. Indien verschillende artikelen over dezelfde patiëntengroep berichtten, bijvoorbeeld aparte artikelen over de functie van de schildklier- en de groeihormoonas, werden de resultaten gecombineerd in de analyse. Bij rapportage over hypofyse-uitval door de jaren heen werd het stuk met de langste follow-up meegenomen in de analyse. Alleen Engelstalige artikelen werden geïnccludeerd.

Het literatuuronderzoek resulteerde in 849 artikelen (301 in PubMed, 4 in Cochrane Library, 131 in Web of Science, 353 in Embase, 16 in CINAHL-database, 27 in Academic Search Premier, en 17 in Science Direct). Hiervan waren 616 artikelen uniek. Op basis van titel en abstract werden 378 publicaties werden geëxcludeerd: 157 artikelen vielen af omdat kinderen waren onderzocht en 7 artikelen waren niet beschikbaar. Van de overgebleven 74 artikelen voldeden 51 niet aan 1 of meer van de inclusiecriteria. Uiteindelijk waren 23 studies geschikt voor evaluatie. Enkele publicaties hadden overlap in het patiëntencohort; de resultaten van deze publicaties werden gecombineerd tot 1 studie.⁷⁻¹⁵ Zo bleven er 18 onderzoeken over voor meta-analyse (figuur 1).

Datareview en -analyse

De selectie van de studies op basis van titel en abstract werd gedaan door 2 onafhankelijke reviewers (N.M.A. en N.E.K.). De definitie van hypopituitarisme moest duidelijk in het artikel omschreven zijn, evenals de testen met bijbehorende afkapwaarden, de gebruikte assays en lokale referentiewaarden.

Inschatting van het risico op bias

Voor de inschatting van het risico op bias werden per studie verschillende onderdelen beoordeeld. Ten eerste werd gekeken naar bestraling. Als vermeld werd dat de hersenen bestraald waren, kreeg de studie 1 punt. Als er een bestralingsdosis op de hypofyse werd genoemd, kreeg de studie 2 punten. Ten tweede werd het inclusieproces van patiënten beoordeeld. Indien er opeenvolgende, niet geselecteerde patiënten geïnccludeerd waren, werd 1 punt gegeven. Als derde werd geëvalueerd hoe de diagnose 'hypofyse-uitval' was gesteld en of er stimulatietesten waren gebruikt. Er werd 1 punt gegeven als de hormonale evaluatie gedaan was door middel van dynamische functietesten en 1 punt

TABEL 1. Hoofd-Hals tumoren

Auteur	Aantal patiënten, leeftijd en geslacht	Tumor karakteristieken	Bestralingsdosis (Gy)	Tijd sinds RTx (jaar)	Endocriene evaluatie	Bias evaluatie	Hypofyseuitval per as (%)					
							Enige	GH	HPA	TSH	LH/FSH	PRL
Snyers A et al; 2009(38)	Total n=32 ^a n=21 endocriene evaluatie 56 (28-74) yr	Sinonasaal carcinoom	44-66	107 (11-253)	Basale ochtend waarden, als afwijkend: ITT, ACTH stim test or GHRH-arg	4	62%	24%	19%	14%	19%	10%
			Gemiddelde dosis op hypofyse: 51-56 Gemiddelde dosis op hypothalamus: 44-52									
Bhandare MS et al; 2008(20) ^b	Totaal n=312 200M/112V n= 112 endocriene evaluatie Geen leeftijd	Necus n=56 Nasopharynx n=119 Sinus frontalis n=2 Sinus etmoidalis n= 5 Sinus sphenoidalis n=34 Sinus maxillaris n=59 Overige n=37	Chemo n= 23 Grote spreiding: < 40 - >70 Chemo n= 40	63 (6-365)	Basale ochtend waarden: TSH, FT4, PRL, GH, Cortisol, LH, FSH, T	2	60%	36%	32%	70%	27%	15%
					If abnormal: ACTH-test, metyrapone, CRH-test, ITT + vasopressin, GST, GHRH-test, Arginine-test, TRH-test							
Lam KS et al; 1991(23) ^c	n=20 22M/9V M: 43.7±8.4 yr V: 36.8±9.0 yr	Nasopharynx	Total dosis: 60 geschatte dosis op hypofyse: 62	60	Basale ochtend waarden: LH, FSH, PRL, TSH, T4, T, E2	5	75%	55%	25%	15%	35%	30%
					ITT, LHRH 100 µg, TRH 200 µg							
Woo E et al; 1988(32)	n=11 8M/3V Age: 48(33-64) jr	Nasopharynx	geschatte dosis op hypofyse: 62-67	72-240	Basale ochtend waarden: LH, FSH, TSH, T4, cortisol, PRL, T, E2 -ITT, LHRH 100 µg, TRH 200 µg	4	82%	90%	18%	45%	55%	27%

TABEL 1. Hoofd-Hals tumoren

Auteur	Aantal patiënten, leeftijd en geslacht	Tumor karakteristieken	Bestralingsdosis (Gy)	Tijd sinds RTx (jaar)	Endocriene evaluatie	Bias evaluatie	Enige	Hypofyseuival per as (%)			
								HPA	TSH	LH/FSH	PRL
Samaan NA et al; 1987(26) ^d	Totaal n=166 98M/68V	Nasopharynx n=114 Paranasale sinus tumoren n=29	geschatte dosis op hypofyse: 57 (4-75)	12-312	ITT, TRH 500 µg; LHRH 100 µg Total T4, T3-uptake	5	75%	18%	20%	35%/20%	36%
	leeftijd: 47(6-80) jr	N Opticus/oog tumoren n=23	geschatte dosis op hypothalamus: 50 (11-75)								
Lam KS et al ; 1987(22) ^e	n=32 21M/11V	Nasopharynx	46-60	60-204	n=32 vragenlijsten mbt sexueel functioneren, galactorroe -Basale ochtend waarden: PRL, T4, FTI, TSH, T Als afwijkend: E2, T, ITT, LHRH-test, TRH-test	3	25%	6%	13%	16%	19%
	n=14 dynamische testen leeftijd: 27-50 jr										
Lam KS et al; 1986(21) ^f	n=8 1M/7V	Nasopharynx	46-61	>60	Basale ochtend waarden: LH, FSH, TSH, PRL, FT4, E2, T	5	100%	50%	50%	25%	88%
	leeftijd: 27-52 jr		geschatte dosis op hypofyse: 55-67								
Huang KE; 1979(34)	n=11 allen vrouw	Nasopharynx	Dosis op nasopharynx: 70	12-156	ITT (n=2), LHRH test, TRH test, GHRH-test (n=6), CRH test(n=4) LHRH 25 µg	3	82%	NR	NR	82%/64%	100%
	leeftijd: 19-40 jr		geschatte dosis op hypofyse: 60-65								
Rosenthal MB et al; 1976(35)	n=6 Allen Man	Nasopharynx	55-65	12-96	Basale ochtend waarden: T4, TSH, FTI, T3, cortisol, LH, FSH, GH	3	67%	50%	67%	NR	NR
	Leeftijd: 35-66 j	Chemo n=2			TRH test, ITT (n=2)						

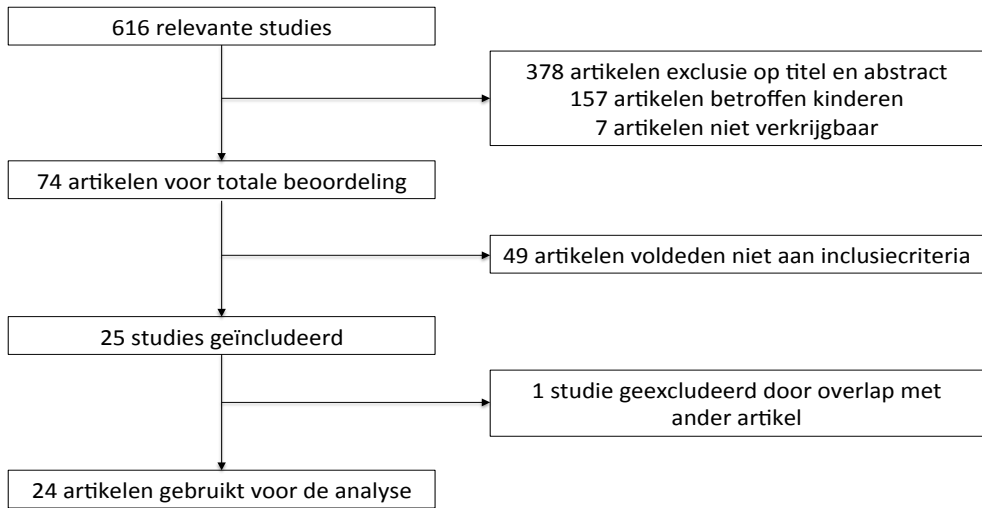
TABEL 1. Hoofd-Hals tumoren

Auteur	Aantal patiënten, leeftijd en geslacht	Tumor karakteristieken	Bestralingsdosis (Gy)	Tijd sinds RTx (jaar)	Endocriene evaluatie	Bias evaluatie	Hypofyseuitval per as (%)					
							Enige	GH	HPA	TSH	LH/FSH	PRL
Samaan SA et al; 1975(37)	n=10 7M/3V	Nasopharynx	geschatte dosis op hypofyse: 50-83	60-240	ITT, Chloorpromazine: 25 mg, TRH-test	4	100%	60%	50%	40%	30%	50%
	Leeftijd: 26-55 j leeftijd Rx: 41 (8-58 jr) ^a		geschatte dosis op schildklier 50-65 (n=12)		500 µg, LHRH 100 µg, T4, T3-uptake, 24 uurs 131-I thyroid uptake							

ACTH adrenocorticotroop hormoon; E2, estrogenen; V, vrouw; FSH, follikel stimulerend hormoon; GH₁; GHRH, groeihormoon releasing hormone; GST, glucagon stimulatie test; Gy; Gray; HPA, hypothalamus pituitary axis (hypothalamus-hypofyse bijtetas); ITT, insuline tolerantie test; LH, luteïniserend hormoon; LHRH, luteïniserend releasing hormone; N, aantal; NR, niet gerapporteerd; M, man; PRL, prolactine; Rx, radiotherapie; T, testosteron; T4, thyroxine; T3, triiodothyronine; TRH, thyreotropine releasing hormone, TSH, thyroid stimuleren hormoon;

- a Totale populatie n=168, echter slechts n=32 in langdurige follow-up en slechts n=21 endocrien geëvalueerd
- b zelfde patiënten groep als (19): klinisch hypopituitarisme: 14.1% (44/312), n=68 ondergingen dynamische testen en n=23 (33.8%) met diagnose subklinisch hypopituitarisme
- c Combinatie van resultaten met ref (45) die hetzelfde cohort beschrijft met een follow-up duur van 2 jaar
- d een deel van de resultaten werden eerder gerapporteerd in (25). Patiënten werden verdeeld in 2 groepen (> 15 jr en <15 jr). Na 4 jaar waren er geen verschillen in hypofyse uitval tussen de groepen.
- e All patiënten ontvingen vragenlijsten, alleen diegenen waarbij obv deze vragenlijsten mogelijk hypopituitarisme aanwezig was, ondergingen endocriene evaluatie (n=14)
- f All patiënten verwezen ivm symptomen passend bij hypopituitarisme

g n=5 < 16 jaar ten tijde van radiotherapie

**FIGUUR 1.**

stroomdiagram van de studieselectie

als alle patiënten in de studie getest waren. Elke studie kon zo maximaal 5 punten scoren. Studies die 0 of 1 punt scoorden, werden beschouwd als studies met een hoog risico op bias, studies met 2 of 3 punten als middelmatig risico en studies met 4 of 5 punten als laag risico op bias.

Statistiek

De belangrijkste uitkomstmaat bij deze meta-analyse was de gepoolde proportie van patiënten met hypofyse-uitval. Voor alle studies werd de proportie berekend als het aantal patiënten met hypofyse-uitval gedeeld door het aantal geteste patiënten. De meta-analyse werd uitgevoerd met een exacte 'likelihood'benadering en er werd gebruik gemaakt van een logistische regressie met een 'random effect'-model op studieniveau (dat wil zeggen dat de variantie zowel binnen als tussen de studies werd meegenomen in de analyse).¹⁶ Ook een meta-regressieanalyse werd uitgevoerd met behulp van een exacte likelihoodbenadering. Deze metaregressie werd verricht om het antwoord op de vraag te geven of de tumorlokalisatie (nasofaryngeaal versus hersenen) samenhang met de prevalentie van hypofyse-uitval. Alle analyses werden gedaan met STATA 10.0 (Stata Corp, Texas, USA).

RESULTATEN

De geanalyseerde studies werden gepubliceerd in de periode 1975-2009. Er werden in totaal 813 patiënten geïncludeerd. Van deze patiënten was 75% (608/813) behandeld voor een nasofarynxcarcinoom (tabel 2a en 2b). De overige 25% was behandeld voor hersentumoren (tabel 3a en 3b). De totale prevalentie van enige vorm van hypopituitarisme verschilde sterk tussen de studies en varieerde van 25-100%.^{9,12} Geen van de artikelen beschreef de evaluatie van patiënten die totale lichaamsbestraling hadden ondergaan in het kader van behandeling voor een hematologische maligniteit.

De meeste studies waren dwarsdoorsnede-onderzoeken waarbij de tijd tussen de bestraling en het testen varieerde van 4 maanden tot 30 jaar; ook binnen de diverse studies bestond een grote spreiding. In 2 studies werd de tijd tussen de bestraling en de endocriene evaluatie niet vermeld.^{17,18}

In 3 studies werden geen selectiecriteria genoemd.^{8,17,18} In 4 studies waren symptomatische patiënten geselecteerd door te rekruteren op een polikliniek voor complicaties na radiotherapie of door inclusie van patiënten bij wie op voorhand een vermoeden bestond op hypopituitarisme.^{9,19-21}

Inschatting van het risico op bias

Er werd 1 studie geclassificeerd als studie met een hoog risico op bias,¹⁷ 8 studies met een gemiddeld risico,^{8,9,18-20,22-24} en 9 studies met een laag risico.^{10-12,14,21,25-27}

Groeihormoon-insulineachtige-groefactor 1-as

De groeihormoon-insulineachtige-groefactor 1-as (GH-IGF-1-as) werd in 14 studies geëvalueerd; daarin waren in totaal 724 patiënten geïncludeerd. Bij 61% van de patiënten (440/724) werd deze hormoonas geëvalueerd met de bepaling van basale serumspiegels van IGF-1 of GH en een stimulatietest (een glucagonstimulatietest, een IIT, een argininetest of een gecombineerde groeihormoon-‘releasing’-hormoon(GHRH)-argininetest).^{8,9,11,12,15,18,20,21,24-26,28,29} De prevalentie van groeihormoondeficiëntie varieerde van 24-100%. De prevalentie van 100% werd gevonden in een onderzoek met 6 patiënten van wie er slechts 2 getest waren met een dynamische test, de IIT.²⁴

Hypothalamus-hypofyse-bijnieras

De hypothalamus-hypofyse-bijnieras werd geëvalueerd in 14 studies; er waren in totaal 751 patiënten geïncludeerd. Bij 61% van de patiënten (460/751) werd de as beoordeeld door bepaling van een basale cortisolconcentratie in het serum of een stimulatietest (een corticotropine-‘releasing’-hormoonstimulatietest, een IIT of een glucagon- of

TABEL 2. hersentumoren

Auteur	Aantal patienten, leeftijd en geslacht	Tumor karakteristieken	Bestralingsd osis (Gy)	Tijd sinds RTx (jaar)	Endocriene evaluatie	Bias assessment	Enige	GH	Hypofysesuitval per as (%)			
									HPA	TSH	LH/FS H	PRL
Schneider HJ et al; 2006(31)	n= 44 28 M/40 V Leeftijd: 45(20- 79) jaar	Glioom n= 40 Neuroblastoom n= 1 Meningeoma n= 18 Schanoom n= 5 Dysgerminoma n= 2 Neuroblastoom n= 1 Chondrosarcoom n= 1 Hemangioepicytroom n= 1	NR Chemo n=27	NR	Basaal serum: cortisol, 24-h urine cortisol, T3, T4, TSH, PRL, FSH, LH, test, E2 GHRH+Arg	2	37%	27%	18%	16%	29,5%	7%
Agha A et al; 2005(36)	n= 56 28 M/28 V Leeftijd: 39,3±11,9 jaar	Osteosarcoom n= 1 Glioom n= 43 Meningeoom n= 5 Pinealis tumor n= 3 Medulloblastoom n= 2 Overig n= 3	Geschatte dosis hypofyse: 54(4-97) Chemo n=7	6	Basaal serum: FSH, LH, TSH, T4, PRL, IGF-I, I, E2 ITT (n=25), Arginine stim test, ACTH stim test	5	41%	32%	21%	9%	27%	32%
Johannesen TB et al.; 2003(29)	n= 33 18M/15V n= 25 endocriene evaluatie Leeftijd Rtx: 38 (14-68) jaar	Laaggradig glioom n=27 Hooggradig glioom n=5 Anaplastisch glioom n=1	54 (45-59)	13.1 (6-25.6)	Basaal serum hormoonwaarden Indien abnormaal: TRH-test, CRH- test	3	64%	NR	4%	56%	16%	0
Popovic V et al; 2002(30)	Totaal n= 22 n= 6 >18 jaar	Medulloblastoom n= 4 Pinealis tumor n= 2	56 Geschatte dosis hypofyse: 25-30	7.6 ± 0.7 (2-13)	ITT, GHRH- GHRP6	3	67%	ITT: 67% GHRH- GHRP6: 33%	NR	NR	NR	NR
Taphoorn MJ et al; 1995(39)	n= 13 9M/4V Leeftijd:24-66 jaar	Laaggradig astrocytroom n= 7 Laaggradig oligodendroom n= 6	45-61 Geschatte dosis hypofyse 36(0-50)	3 (1-11.5)	Basaal serum hormoonspiegels TRH-test 200 µg GnRH- test 100 µg hCRV -test 100 µg GHRH 50 µg	5	77%	31%	62%	0%	15%	23%

TABEL 2. hersentumoren

Auteur	Aantal patienten, leeftijd en geslacht	Tumor karakteristieken	Bestralings- osis (Gy)	Tijd sinds RTx (jaar)	Endocriene evaluatie	Bias assessment	Hypofyseuitval per as (%)			
							Enige	GH	HPA	TSH
Constine LS et al; 1987(28) a	Totaal n=65 n=30 (>18 jaar)	Glioom n=43 Medulloblastoom n= 7	Various amounts	NR	T4 en TSH n=65 PRL n= 47 Geen dynamische testen n= 35 craniële Rtx	1	NR	NR	NR	25%
	Leeftijd: 36.5(19-65) jaar	Meningeoom n= 5 Ependymoom n= 4 Overig n= 5 Geen PA diagnose n= 2	n = 47 craniële Rtx n= 18 craniospinale Rtx							
Mechanick Jl et al; 1986(33)	n= 15 5M/10V	Astrocytoom n= 14 Medulloblastoom n= 1	40-50 gehele brein 1-22 lokale boost	2-9	PRL n=9,T4, T3, T3-resin uptake TRH 200 µg	2	60%	NR	NR	14.3%
	Leeftijd: 22-59 jaar		Chemo n=8							
Harrop JS et al; 1976(27)	Totaal n=17 n= 8 > 18 jaar	Astrocytoom: n= 2 Meningeoom: n= 3 Angiooom: n= 1 Geen PA: n= 2	40-52	6 (1-15)	ITT (n= 6), GST (n= 6), TRH (n= 8) 200 µg 250 µg Synacthen, Clomiphene stim test (n= 5), LHRH 100 µg	4	62.5%	50%	12.5%	12.5%

ACTH adrenocorticotroop hormoon; E2, oestrogeen; V, Vrouw; FSH, Follikel stimulerend hormoon; GH, groeihormoon; GHRH, groeihormoon releasing hormoon; GST, glucagon stimulatietest; Gy, Gray; HPA, hypothalamus hypofyse bijneras; ITT, insuline tolerantie test; LH, luteïniserend hormoon; LHRH, luteïniserend hormoon releasing hormoon; N:aantal; NR: niet gerapporteerd; M: man; PRL: prolactine; Rtx: radiotherapie; T: testosteron; TRH thyreotropine releasing hormoon; TSH:thyreotropine stimulerend hormoon.
a Evaluatie galactorthoe, libido, menstruele cyclus, huid en haar klachten, gewichtsverandering, koude intolerantie. Bij afwijkende vragenlijst pas testen uitgevoerd, 2 patienten wel vragenlijst ingevoerd maar niet getest.

adrenocorticotroop-hormoon(ACTH)-test). Bijnierinsufficiëntie werd gediagnosticeerd bij 0-63% van de patiënten.

Hypothalamus-hypofyse-schildklieras

De schildklierfunctie werd in 16 studies geëvalueerd bij in totaal 488 patiënten. Deze functie werd bepaald door meting van de basale concentratie thyreoïdstimulerend hormoon (TSH) en vrij thyroxine (T4) in het serum, zo nodig aangevuld met een thyreotropine-‘releasing’-hormoon(TRH)-stimulatietest. TSH-deficiëntie werd vastgesteld bij 26% van de patiënten (126/488). De prevalentie was 0-67%.

Hypothalamus-hypofyse-gonade-as

De gonadotrope as werd getest door meting van de basale concentratie LH en FSH en testosteron of oestrogeen in 14 studies met in totaal 469 patiënten. Hypogonadotroop hypogonadisme was aanwezig bij 30% van de patiënten (143/469), variërend van 30-82% in de verschillende studies.

Hyperprolactinemie

Prolactinespiegels werden gemeten in 15 studies met 502 patiënten, met hyperprolactinemie bij 144 van de 502 patiënten. In 1 studie waren geen basale prolactinespiegels bepaald.²⁹ Deze studie werd niet meegenomen in de analyse.

Meta-analyse

De gezamenlijke prevalentie van enige vorm van hypopituitarisme was 0,66 (95%-BI: 0,55-0,76). Zoals verwacht, was groeihormoondeficiëntie de meest voorkomende deficiëntie met een prevalentie van 0,45 (95%-BI: 0,33-0,57), gevolgd door luteïniserend hormoon(LH)-follikelstimulerend-hormoon(FSH)-deficiëntie (0,3; 95%-BI: 0,23-0,37) en TSH-deficiëntie (0,22; 95%-BI: 0,16-0,37). adrenocorticotroop-hormoon(ACTH)-deficiëntie had de laagste prevalentie, namelijk 0,22 (95%-BI: 0,15-0,3). De prevalentie van hyperprolactinemie was 0,34 (95%-BI: 0,15-0,6) (tabel 4). Volgens de meta-regressieanalyse had de tumorlocatie en het soort tumor geen samenhang met de prevalentie. Wij vonden geen statistisch significante associatie met de tumorlocatie en enige vorm van hypopituitarisme ($p = 0,14$), groeihormoondeficiëntie ($p = 0,36$), ACTH-deficiëntie ($p = 0,75$), TSH-deficiëntie ($p = 0,11$), LH-FSH-deficiëntie ($p = 0,21$) of hyperprolactinemie ($p = 0,44$) (tabel 5).

Gezien de wisselende kwaliteit van de studies werd een sensitiviteitsanalyse gedaan op studies waarin het risico op bias laag/intermediate was én waarin opeenvolgende, niet-

geselecteerde patiënten waren geïncludeerd.^{11,23,25} De prevalentie voor enige vorm van hypopituitarisme was in deze 4 studies 0,62 (95%-BI: 0,45-0,77), wat overeenkwam met de resultaten van de totale analyse.

Hypofyse-uitval gerelateerd aan het tijdsinterval tussen radiotherapie en evaluatie

Bij 2 studies werd gerapporteerd over de tijdsbestek tussen bestraling en het ontstaan van hypofyse-uitval; in beide studies ging het om patiënten die behandeld waren voor nasofarynxcarcinomen.^{16,27} De prevalentie van uitval was 6% na 1 jaar, 35% na 2 jaar, 56% na 3 jaar en 62% na 4 en na 5 jaar.¹¹ Over de klassieke volgorde van uitval rapporteerde 1 studie. Groeihormoondeficiëntie trad gemiddeld op na 2,6 jaar, gevolgd door uitval van de gonadotrope as en hyperprolactinemie na ongeveer 3,8 jaar, ACTH-insufficiëntie na 6,0 jaar en als laatste TSH-insufficiëntie gemiddeld na 11,0 jaar.¹⁴

BESCHOUWING

Volgens onze meta-analyse was de prevalentie van enige vorm van hypopituitarisme na craniële radiotherapie 0,66 (95% -BI: 0,55-0,76); er waren geen significantie verschillen tussen patiënten met verschillende tumoren. Beoordeling van de hypofysefunctie zou daarom geïncludeerd moeten worden in de protocollen voor evaluatie van patiënten die langere tijd overleven na bestraling van het hoofd-halsgebied.

Hypopituitarisme kan al vóórkomen binnen het eerste jaar na behandeling; sommige hypofyse-assen zullen echter pas veel later uitvallen, soms zelfs pas 11 jaar na behandeling.^{11,14} Gezien de vergaande psychische en lichamelijke gevolgen voor patiënten bepleiten we dat alle bestraalde patiënten met regelmatige intervallen, bijvoorbeeld jaarlijks, gecontroleerd worden op hypofysaire uitval.

De prevalentie van hypofyse-uitval varieerde tussen de verschillende studies, van bijna geen uitval tot volledige uitval. Deze variaties waren met name geassocieerd met het aantal geïncludeerde patiënten en de wijze van evaluatie van hypofyse-uitval.²⁴ Ook speelde het wel of niet gebruiken van dynamische testen een rol. De verschillende radiotherapeutische technieken zullen een bijdrage aan de variatie geleverd hebben, aangezien hogere cumulatieve doseringen geassocieerd zijn met een hogere incidentie van hypofyse-uitval.²⁴

De meta-regressie-analyse liet geen verschillen in effect van bestraling zien tussen hersen- en nasofarynx tumoren. De totale dosis van straling verschilde niet veel tussen beide groepen: 40-83 Gy voor patiënten met nasofarynxcarcinoom en 40-97 Gy voor patiënten met niet-hypofysaire hersentumoren. In 9 studies was het risico op bias

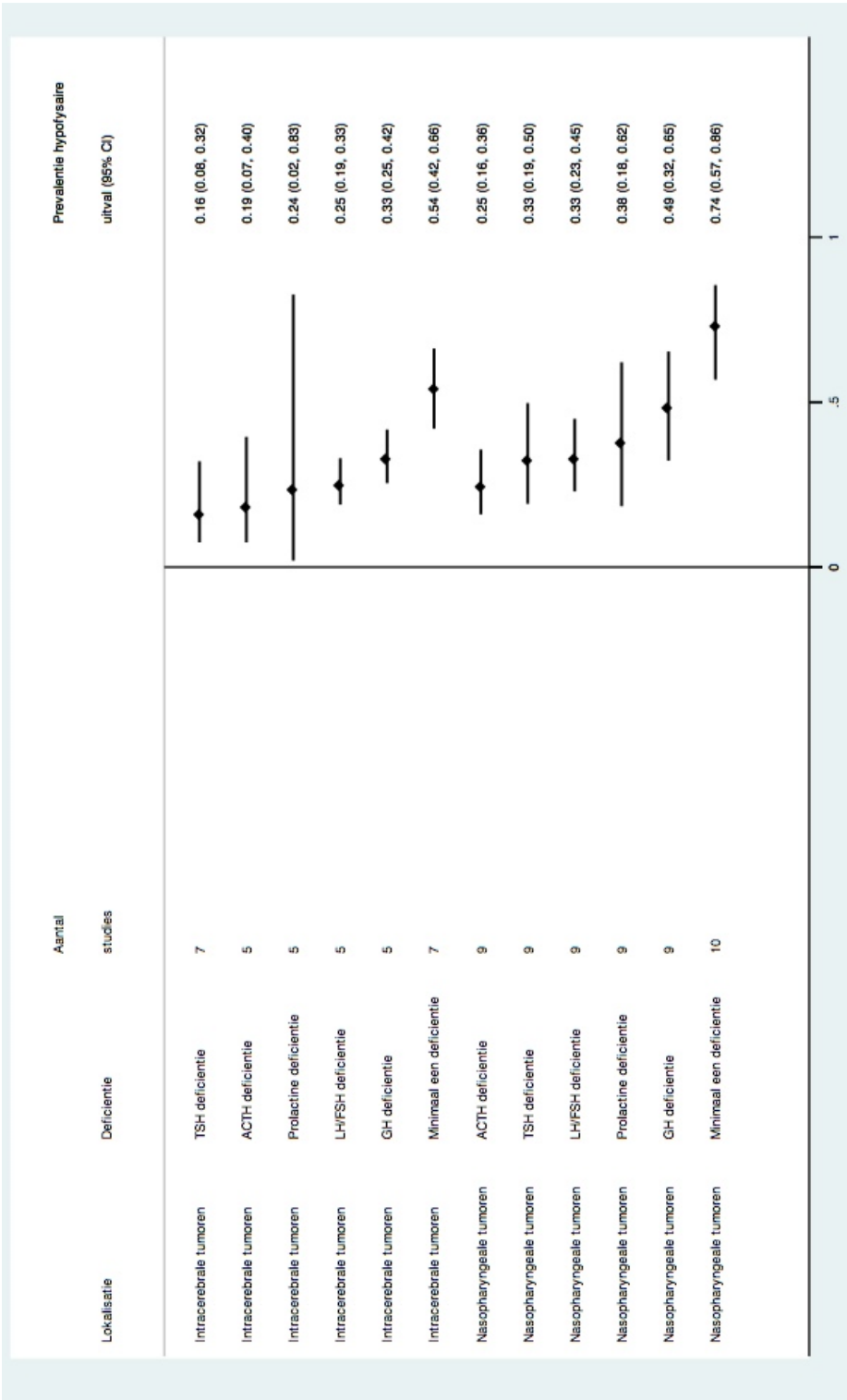
volgens onze inschatting laag. De verrichte sensitiviteitsanalyse op deze studies liet geen verschil zien met de overige studies.^{11,23,25,29}

De meerderheid van de studies betrof dwarsdoorsnede-onderzoek met grote verschillen in de tijdsduur tussen bestraling en evaluatie. Ook de patiëntenselectie varieerde sterk. Selectiecriteria werden soms niet vermeld.^{8,17,21} In andere studies hanteerden de onderzoekers een selectieprocedure die erg gevoelig was voor preselectie, bijvoorbeeld door rekrutering van patiënten vanuit een polikliniek voor complicaties van radiotherapie of door alleen patiënten met symptomen passend bij hypofyse-uitval te testen.^{9,19-21} Er waren slechts 2 prospectieve studies met in totaal 186 patiënten; de redenen voor verlies in de follow-up werden niet genoemd.^{11,14}

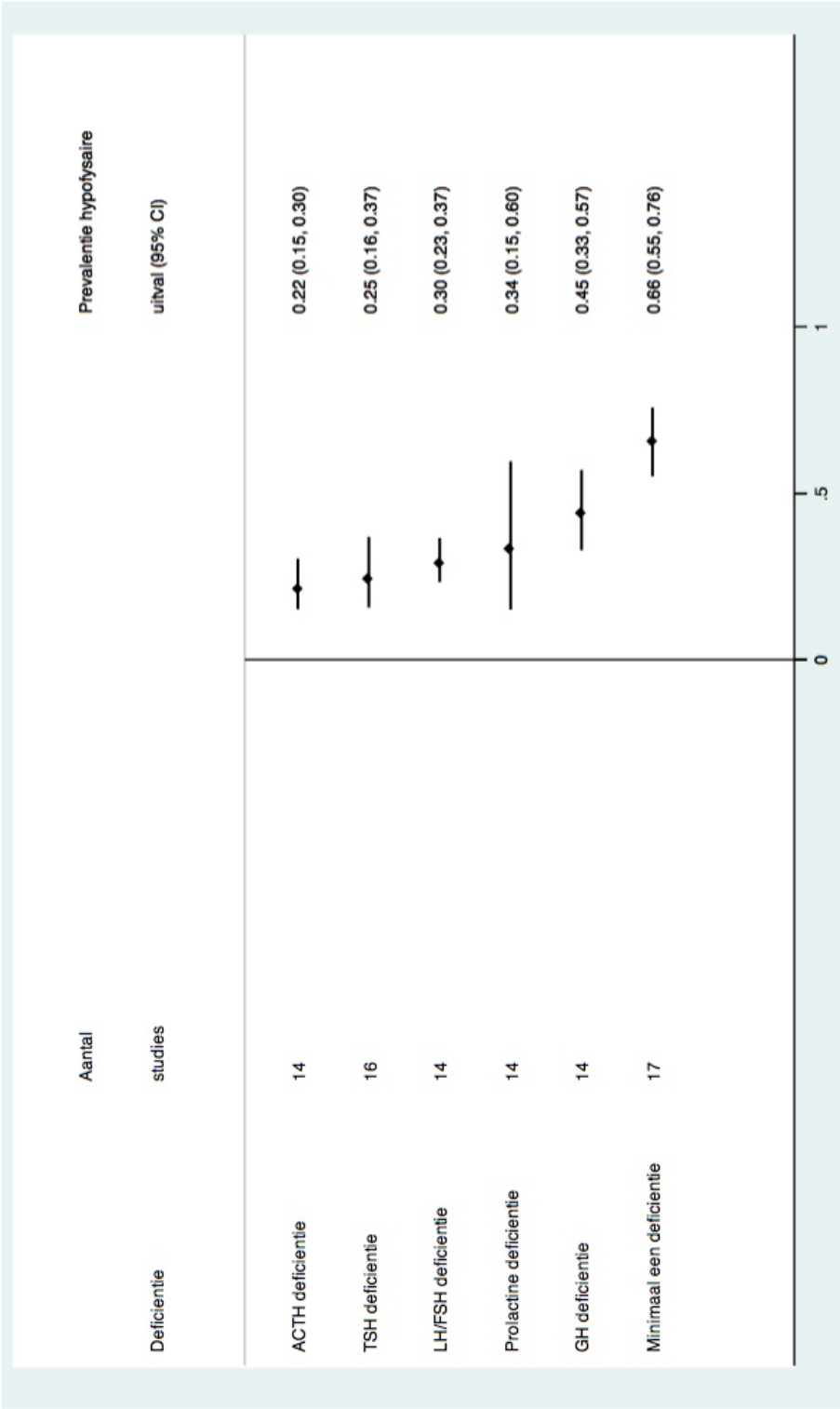
Verschillen in de evaluatie van de endocriene functie kunnen ook de vastgestelde prevalentie beïnvloeden. De meerderheid van de patiënten was niet geëvalueerd met stimulatietesten die hier optimaal voor zijn. Daardoor is het goed mogelijk dat deze uitkomsten eerder aan de lage kant zijn en dat de echte prevalentie veel hoger ligt. Daarnaast werden in de studies die wel stimulatietesten gebruikten verschillende testen en afkapwaarden gebruikt. In slechts 10 van de 18 onderzoeken werden alle hypofyseassen getest, maar binnen die studies werden niet bij alle patiënten alle assen getest. Bovendien kunnen de hypofyse-schildklieras en de hypofyse-gonade-as ook sterk beïnvloed worden door lokale bestraling van de schildklier en de gonaden zelf en door blootstelling aan chemotherapie.^{7,30,31}

Een andere uiting van hypothalamische en hypofysaire schade is hyperprolactinemie. Door verstoorde hypothalamische dopaminesecretie is de mate van hyperprolactinemie vaak erg wisselend en subklinisch. Ook kan de prolactineconcentratie na verloop van tijd normaliseren doordat de lactotrofe cellen atrofiëren bij gebrek aan hypothalamische stimulering. In geen van de studies vermeldde de onderzoekers het tijdsinterval tussen de bestraling en het ontstaan van hyperprolactinemie; er werd ook geen melding gedaan over het verdwijnen van de hyperprolactinemie.

Aanbevolen follow-up Deze resultaten en de verbeterde overleving bij de diverse ziektebeelden in acht nemend, vinden wij dat patiënten gedurende minstens 10 jaar jaarlijks gecontroleerd moeten worden op mogelijke hypofyse-uitval. Na deze 10 jaar zou ervoor gekozen kunnen worden om de intervallen te vergroten. Uiteraard dient rekening gehouden te worden met de aard van de tumor (maligne of benigne), de conditie van patiënt en medicatie bij andere morbiditeit, zoals het gebruik van dexamethason. De follow-up dient te bestaan uit een geprotocolleerde evaluatie van basale laboratoriumwaarden van de hypofyse- en eindorgaanhormonen vóór bestraling. Dit moet gevolgd worden door jaarlijks bloedonderzoek al dan niet aangevuld met stimulatietesten, tot in ieder geval 12 jaar na de initiële behandeling. Hierna zouden de intervallen verlengd kunnen worden naar 2 jaar (figuur 2). De te kiezen stimulatietesten zijn afhankelijk van de conditie van de patiënt en de klinische relevantie. Zo zal een



FIGUUR 2.
Random effects model per tumorgroep



FIGUUR 3.
Random effects model hypofysaire uitval na craniele bestraling

groeihormoondeficiëntie bij een patiënt die in het verleden behandeld is voor een maligniteit, meestal niet tot behandeling met recombinant groeihormoon leiden. Deze deficiëntie is wel een symptoom van hypothalamische en hypofysaire schade; daarom kan er bij een vitale patiënt wel gekozen worden daarop te testen als indicator van schade om al dan niet te starten met recombinant groeihormoontherapie.

CONCLUSIE

Onze meta-analyse onderstreept dat alle patiënten die bestraald zijn op de hersenen, gestructureerd gevolgd moeten worden. Omdat deze patiëntengroep zeer divers is, zal de follow-up op het individu gericht moeten zijn, waarbij de te testen assen en het soort stimulatietesten moeten worden afgewogen tegen de levensverwachting en de comorbiditeit. De follow-up kan bij de radiotherapeut of de behandelaar van de oorspronkelijke ziekte plaatsvinden, dus bij diverse specialisten. Patiënten zullen indien noodzakelijk overgedragen worden aan de endocrinoloog, die zich bewust moet zijn van de lange-termijncomplicaties van bestraling. Adviezen hierover zouden moeten worden opgenomen in de richtlijnen voor de follow-up van deze patiëntencategorieën.

LEERPUNTEN

- i. Hypofyse-uitval is een late complicatie na hoofd-halsbestraling.
- ii. Bij kinderen wordt hierop regelmatig gecontroleerd, bij volwassenen bestaan hiervoor geen richtlijnen.
- iii. De beschikbare literatuur geeft zeer uiteenlopende getallen voor de prevalentie van hypofyse-uitval.
- iv. Hypofyse-uitval na bestraling van het hoofd-halsgebied begint over het algemeen met uitval van de groeihormoonsecretie na gemiddeld 2,5 jaar, gevolgd door uitval van de gonadotrofines na bijna 4 jaar, ACTH-uitval na 6,0 jaar en TSH-uitval na gemiddeld 11,0 jaar.
- v. De prevalentie van hypofyse-uitval is 60% na bestraling van hersentumoren en nasofarynx tumoren.
- vi. Gezien de hoge prevalentie van hypofyse-uitval zijn richtlijnen nodig om de evaluatie van de hypofysefuncties van volwassen patiënten na bestraling van de hersenen geprotocolleerd te laten verlopen.

LITERATUUR

1. Gurney JG, Kadan-Lottick NS, Packer RJ, Neglia JP, Sklar CA, Punyko JA et al. Endocrine and cardiovascular late effects among adult survivors of childhood brain tumors: Childhood Cancer Survivor Study. *Cancer*. 2003;97:663-73.
2. Biermasz NR, van DH, Roelfsema F. Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. *J Clin Endocrinol Metab*. 2000;85:2476-82.
3. Little MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML. Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Q J Med*. 1989;70:145-60.
4. Toogood AA. Endocrine consequences of brain irradiation. *Growth Horm IGF Res*. 2004;14 Suppl A:S118-S24.
5. Little MD, Shalet SM, Beardwell CG, Robinson EL, Sutton ML. Radiation-induced hypopituitarism is dose-dependent. *Clin Endocrinol*. 1989;31:363-73.
6. Darzy KH, Shalet SM. Hypopituitarism following radiotherapy. *Pituitary*. 2009;12:40-50.
7. Bhandare N, Kennedy L, Malyapa RS, Morris CG, Mendenhall WM. Primary and central hypothyroidism after radiotherapy for head-and-neck tumors. *Int J Radiat Oncol Biol Phys*. 2007;68:1131-9.
8. Bhandare N, Kennedy L, Malyapa RS, Morris CG, Mendenhall WM. Hypopituitarism after radiotherapy for extracranial head and neck cancers. *Head Neck*. 2008;30:1182-92.
9. Lam KS, Wang C, Yeung RT, Ma JT, Ho JH, Tse VK et al. Hypothalamic hypopituitarism following cranial irradiation for nasopharyngeal carcinoma. *Clin Endocrinol*. 1986;24:643-51.
10. Lam KS, Ho JH, Lee AW, Tse VK, Chan PK, Wang C et al. Symptomatic hypothalamic-pituitary dysfunction in nasopharyngeal carcinoma patients following radiation therapy: a retrospective study. *Int J Radiat Oncol Biol Phys*. 1987;13:1343-50.
11. Lam KS, Tse VK, Wang C, Yeung RT, Ho JH. Effects of cranial irradiation on hypothalamic-pituitary function--a 5-year longitudinal study in patients with nasopharyngeal carcinoma. *Q J Med*. 1991;78:165-76.
12. Samaan NA, Bakdash MM, Caderao JB, Cangir A, Jesse RH, Jr., Ballantyne AJ. Hypopituitarism after external irradiation. Evidence for both hypothalamic and pituitary origin. *Ann Intern Med*. 1975;83:771-7.
13. Samaan NA, Vieto R, Schultz PN, Maor M, Meoz RT, Sampiere VA et al. Hypothalamic, pituitary and thyroid dysfunction after radiotherapy to the head and neck. *Int J Radiat Oncol Biol Phys*. 1982;8:1857-67.
14. Samaan NA, Schultz PN, Yang KP, Vassilopoulou-Sellin R, Maor MH, Cangir A et al. Endocrine complications after radiotherapy for tumors of the head and neck. *J Lab Clin Med*. 1987;109:364-72.
15. Lam KS, Tse VK, Wang C, Yeung RT, Ma JT, Ho JH. Early effects of cranial irradiation on hypothalamic-pituitary function. *J Clin Endocrinol Metab*. 1987;64:418-24.
16. Hamza TH, van Houwelingen HC, Stijnen T. The binomial distribution of meta-analysis was preferred to model within-study variability. *J Clin Epidemiol*. 2008;61:41-51.
17. Constine LS, Rubin P, Woolf PD, Doane K, Lush CM. Hyperprolactinemia and hypothyroidism following cytotoxic therapy for central nervous system malignancies. *J Clin Oncol*. 1987;5:1841-51.
18. Schneider HJ, Rovere S, Corneli G, Croce CG, Gasco V, Ruda R et al. Endocrine dysfunction in patients operated on for non-pituitary intracranial tumors. *Eur J Endocrinol*. 2006;155:559-66.
19. Mechanick JI, Hochberg FH, LaRocque A. Hypothalamic dysfunction following whole-brain irradiation. *J Neurosurg*. 1986;65:490-4.
20. Popovic V, Pekic S, Golubicic I, Doknic M, Dieguez C, Casanueva FF. The impact of cranial irradiation on GH responsiveness to GHRH plus GH-releasing peptide-6. *J Clin Endocrinol Metab*. 2002;87:2095-9.

21. Woo E, Lam K, Yu YL, Ma J, Wang C, Yeung RT. Temporal lobe and hypothalamic-pituitary dysfunctions after radiotherapy for nasopharyngeal carcinoma: a distinct clinical syndrome. *J Neurol Neurosurg Psychiatry*. 1988;51:1302-7.
22. Huang KE. Assessment of hypothalamic-pituitary function in women after external head irradiation. *J Clin Endocrinol Metab*. 1979;49:623-7.
23. Johannesen TB, Lien HH, Hole KH, Lote K. Radiological and clinical assessment of long-term brain tumour survivors after radiotherapy. *Radiotherapy and Oncology*. 2003;69:169-76.
24. Rosenthal MB, Goldfine ID. Primary and secondary hypothyroidism in nasopharyngeal carcinoma. *JAMA*. 1976;236:1591-3.
25. Agha A, Sherlock M, Brennan S, O'Connor SA, O'Sullivan E, Rogers B et al. Hypothalamic-pituitary dysfunction after irradiation of nonpituitary brain tumors in adults. *J Clin Endocrinol Metab*. 2005;90:6355-60.
26. Harrop JS, Davies TJ, Capra LG, Marks V. Hypothalamic-pituitary function following successful treatment of intracranial tumours. *Clin Endocrinol*. 1976;5:313-21.
27. Snyers A, Janssens GORJ, Twickler MB, Hermus AR, Takes RP, Kappelle AC et al. Malignant Tumors of the Nasal Cavity and Paranasal Sinuses: Long-Term Outcome and Morbidity With Emphasis on Hypothalamic-Pituitary Deficiency. *International Journal of Radiation Oncology Biology Physics*. 2009;73:1343-51.
28. Chen MS, Lin FJ, Huang MJ, Wang PW, Tang S, Leung WM et al. Prospective hormone study of hypothalamic-pituitary function in patients with nasopharyngeal carcinoma after high dose irradiation. *Jpn J Clin Oncol*. 1989;19:265-70.
29. Taphoorn MJ, Heimans JJ, van der Veen EA, Karim AB. Endocrine functions in long-term survivors of low-grade supratentorial glioma treated with radiation therapy. *J Neurooncol*. 1995;25:97-102.
30. Levine J, Canada A, Stern CJ. Fertility preservation in adolescents and young adults with cancer. *J Clin Oncol*. 2010;28:4831-41.
31. Mercado G, Adelstein DJ, Saxton JP, Secic M, Larto MA, Lavertu P. Hypothyroidism: a frequent event after radiotherapy and after radiotherapy with chemotherapy for patients with head and neck carcinoma. *Cancer*. 2001;92:2892-7.

Curriculum Vitae
Publication List
Dankwoord

CURRICULUM VITAE

Natasha Appelman-Dijkstra (geboren 13 maart 1980 te Haarlem) behaalde in 1998 haar eindexamen op het Stedelijk Gymnasium Haarlem om aansluitend te starten met de studie Geneeskunde in Leiden. Na haar arts-examen begon zij in februari 2005 als arts-assistent niet in opleiding tot specialist (ANIOS) in het Medisch Centrum Haaglanden locatie Westeinde. In januari 2006 startte zij met de opleiding tot internist in Leiden (opleiders Prof. Dr. J.A. Romijn/Prof. Dr. J.W.A. Smit). Het perifere gedeelte van de opleiding deed zij in het Medisch Centrum Haaglanden (opleider mw. dr. P.H.L.M. Geelhoed-Duijvestijn, 2006-2009) om vervolgens aansluitend de opleiding tot internist-endocrinoloog te voltooien in het Leids Universitair Medisch Centrum. Tijdens haar opleiding tot internist-endocrinoloog begon zij met promotieonderzoek bij prof. A.M. Pereira resulterend in dit proefschrift.

Natasha is vanaf haar studietijd actief geweest in het Medisch Actienetwerk van Amnesty International. In 2009 startte zij daarnaast met maken van rapportages voor asielzoekers ten behoeve van de asielpcedure via de Medische Onderzoeksgroep (MOG) van Amnesty International. Bij deze rapportages gaat men in op het verband tussen medische afwijkingen, zoals bv. littekens, en het gerapporteerde verhaal van marteling/mishandeling in het land van herkomst. In 2012 is de MOG opgegaan in het onafhankelijke instituut voor Mensenrechten en Medisch Onderzoek (iMMO) waarvoor Natasha momenteel nog steeds rapportages maakt.

Sinds het afronden van haar opleiding in april 2012 werkt Natasha als stafid op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum met als specifiek aandachtsgebied Calcium- en Botstofwisseling. In 2014 ontving zij voor haar onderzoek naar bothardheidsmetingen bij patiënten middels de nieuwe microindentatie techniek een Young Investigators award van de American Society of Bone and Mineral Research

Natasha is getrouwd met Michiel Appelman, samen hebben zij 2 kinderen, Boris (2009) en Katja (2011).

PUBLICATION LIST

1. **Appelman-Dijkstra N.M.**, Tom B., Geelhoed-Duijvestijn P.H.L.M.: Switch of Mixtard® 30 Novolet® to NovoMix® 30 Flexpen®: Clinical experiences in the Netherlands
Tijdschrift voor Diabetologie, 2006, VOL 4; NUMB 4, pages 188-191
2. **Appelman-Dijkstra N.M.**, Sonneveld M, Tom B., Geelhoed-Duijvestijn P.H.L.M.: The Switch study: Switching from BHI 30 Novolet® to BIAsp 30 Flexpen®; Clinical observations from the Netherlands;
Clinical Medicine: Endocrinology and Diabetes 2008;1:27-32
3. **Appelman-Dijkstra N.M.**, de Koning E.J.P. Gestational Diabetes and diabetes and pregnancy;
Modern Medicine April 2011
4. **Appelman-Dijkstra N.M.**, Pereira A.M., Smit V., Kapiteijn H.W. Multifocal right sided adrenal nerve tissue: Adrenal neuroanglioma;
Neth J Med. 2011 Jun;69(6):286-90
5. **Appelman-Dijkstra N.M.**, Kokshoorn N.E., Neelis K.J., Dekkers O.M., Biermasz N.R., Smit J.W.A., Romijn J.H., Pereira A.M.: Pituitary failure after cranial irradiation for non-pituitary tumors: a systematic review and metaanalysis:
J Clin Endocrinol Metab. 2011 Aug;96(8):2330-40
6. **Appelman-Dijkstra N.M.**, Kokshoorn N.E., Neelis K.J., Dekkers O.M., Biermasz N.R., Smit J.W.A., Pereira A.M.: Hypofyseuitval na bestraling:
Ned Tijdschr Geneesk. 2012;156:A4340
7. Yu Sun, Beata Bak, Nadia Schoenmakers, A.S. Paul van Trotsenburg, Wilma Oostdijk, Peter Voshol, Emma Cambridge, Jacqueline K. White, Paul le Tissier, S. Neda Mousavy Gharavy, Juan P. Martinez-Barbera, Wilhelmina H. Stokvis-Brantsma, Thomas Vulsma, Marlies J. Kempers, Luca Persani, Irene Campi, Marco Bonomi, Paolo Beck-Peccoz, Hongdong Zhu, Timothy M.E. Davis, Anita C.S. Hokken-Koelega, Daria Gorbenko Del Blanco, Jayanti J. Rangasami, Claudia A.L. Ruivenkamp, Jeroen F.J. Laros, Marjolein Kriek, Sarina G. Kant, Cathy A.J. Bosch, Nienke R. Biermasz, **Natasha M. Appelman-Dijkstra**, Eleonora P. Corssmit, Guido C.J. Hovens, Alberto M. Pereira, Johan T. den Dunnen, Michael G. Wade, Martijn H. Breuning, Raoul C. Hennekam, Krishna Chatterjee, Mehul T. Dattani, Jan M. Wit, Daniel J. Bernard: Loss-of-Function Mutations in IGSF1 Cause a Novel, X-Linked Syndrome of Central Hypothyroidism and Testicular Enlargement :
Nature Genetics 2012 Dec;44(12):1375-81
8. Claessen., K.M.J.A., **Appelman-Dijkstra N.M.**, Adoptie D., Roelfsema F, Biermasz N.R., Pereira A.M.: Metabolic profile in growth hormone deficient (GHD) adults after long-term recombinant human growth hormone (rGH) therapy.
J Clin Endocrinol Metab. 2013 Jan;98(1):352-61

9. **Appelman-Dijkstra N.M.**, Claessen K.M.J.A., Roelfsema F, Pereira A.M., Biermasz N.R.: Long-term effects of recombinant human Growth Hormone (rGH) replacement in adults with Growth Hormone Deficiency (GHD): a systematic review.
Eur J Endocrinol. 2013 May 28;169(1):1-14.
10. Purmer I, **Appelman-Dijkstra N.M.**, Dias A, Tavy D, de Lange E, Corssmit E.P.J.M.
Disappearance of epilepsy after resection of catecholamine secreting extra-adrenal paragangliomas: a case report.:
Neth J Med. 2013 Aug;71(6):313-5.
11. **Appelman-Dijkstra N.M.**: Calcium en Vitamine D, een goed begin van elke dag?
Modern Medicine Jaargang 37 nummer 12 December 2013
12. Claessen K.M.J.A., Kroon H.M., Pereira A.M., **Appelman-Dijkstra N.M.**, Verstegen M.J., Kloppenburg M., Hamdy N.A.T., Biermasz N.R.: Progression of vertebral fractures despite long-term biochemical control of acromegaly: a prospective follow-up study
J Clin Endocrinol Metab. 2013 Dec;98(12):4808-15.
13. Joustra S.D, Schoenmakers N, Persani L, Campi I, Bonomi M, Radetti G, Beck-Peccoz P, Zhu H, Davis T.M., Sun Y, Corssmit EP, **Appelman-Dijkstra N.M.**, Heinen C.A, Pereira A.M, Varewijck A.J, Janssen J.A, Endert E, Hennekam R.C, Lombardi M.P, Mannens M.M, Bak B, Bernard D.J, Breuning M.H, Chatterjee K, Dattani M.T, Oostdijk W, Biermasz N.R, Wit J.M, van Trotsenburg A.S. The IGSF1 Deficiency Syndrome: Characteristics Of Male And Female Patients.
J Clin Endocrinol Metab. 2013 Dec;98(12):4942-52
14. Claessen K.M.J.A, **Appelman-Dijkstra N.M.**, Pereira A.M, Joustra S.D, de Mutsert R, Gast K.B, den Heijer M, Smit J.W, Dekkers O.M, Biermasz N.R. Abnormal metabolic phenotype in middle-aged GH-deficient adults despite long-term recombinant human GH replacement
Eur J Endocrinol. 2013 Dec 21;170(2):263-72
15. **Appelman-Dijkstra N.M.**, Claessen K.M.J.A., Pereira A.M, Hamdy N.A.T, Biermasz N.R.: The effects of chronic recombinant human GH (rGH) treatment on the bone metabolism in adult patients with Growth Hormone Deficiency(GHD)
Clin Endocrinol (Oxf). 2014 Nov;81(5):727-35.
16. **Appelman-Dijkstra N.M.**, Malgo F, Neelis K.J., Coremans I, Biermasz N.R., Pereira A.M.: Pituitary dysfunction in adult patients after cranial irradiation for head and nasopharyngeal tumors
Radiotherapy and Oncology 2014 Oct;113(1):102-7
17. **Appelman-Dijkstra N.M.**, Papapoulos S.E.: Modern therapies in Osteoporosis
Best Pract Res Clin Rheumatol. 2013 Dec;27(6):805-820

18. Gorter E.A., Hamdy N.A., **Appelman-Dijkstra N.M.**, Schipper I.B.; The role of vitamin D in human fracture healing: a systematic review of the literature.
Bone. 2014 Jul;64:288-297
19. Malgo F, Papapoulos S.E., Hamdy N.A.T., **Appelman-Dijkstra N.M.**; Bone Material Strength as measured by microindentation in vivo is decreased independently of BMD in patients with fractures.
JBMR 2014;. p. S51 (abstract 1064), *Epub*.
20. **Appelman-Dijkstra N.M.**, Malgo F, Papapoulos S.E., Hamdy N.A.T.; Microindentation in vivo captures elements of bone fragility independently of BMD.
JBMR 2014;. p. S51 (abstract SA0290), *Epub*.
21. **Appelman-Dijkstra N.M.**, Papapoulos S.E.; Novel approaches to the treatment of osteoporosis,
Best Practice & Research Clinical Endocrinology & Metabolism Dec;28(6):843-57
22. Malgo F, Papapoulos S.E., Hamdy N.A.T., **Appelman-Dijkstra N.M.**: Bone Material Strength as measured by microindentation in vivo is decreased in patients with fragility fractures independently of Bone Mineral Density
J Clin Endocrinol Metab. 2015 Mar 13;jc20144346. [*Epub ahead of print*]
23. **Appelman-Dijkstra N.M.**, Papapoulos S.E. Modulating bone resorption and bone formation in opposite directions in the treatment of postmenopausal osteoporosis.
Drugs in press
24. Roscam Abbing R.L.P., vd Meulen A.E., Veenendaal R.A., Hamdy N.A., **Appelman-Dijkstra N.M.**. Long term effects of anti-TNF-therapy on bone mass in inflammatory bowel disease, a retrospective cohort study
Submitted

DANKWOORD

Medio 2010 begon ik aan een promotietraject naast mijn opleiding tot endocrinoloog, een bijzondere verstrengeling van 2 trajecten waar ik zeer veel van geleerd heb. Van elk traject afzonderlijk, maar met name ook van het samenspel of soms spagaat tussen kliniek en wetenschap. Hierdoor schoten soms zelfs mijn multitasking vaardigheden tekort. Het is onmogelijk om iedereen te bedanken zonder mensen te vergeten, promoveren doe je namelijk nooit alleen. Ik ben ongelooflijk gesteund door mijn collega-AIOS (Sharita Ramautar, Esther Donga, Marielle Schroijen, Nienke Niemeijer), de stafleden van de Endocrinologie, wetenschapsstudenten (Desiree, Frank, Marnick), de polikliniek (vooral Angelique en Joyce) en staf secretaresses en natuurlijk mijn dakpannen (Nieke Kokshoorn en Kim Claessen). Ik wil jullie allemaal enorm bedanken voor de ontvangen steun, humor, tips, versnaperingen op inspannende dagen en het weer aanzwengelen van mijn onderzoek als ik dreigde te verslappen.

Prof. Dr. A.M. Pereira Arias, beste Alberto, dit traject moet voor jou ook een aparte ervaring geweest zijn. Van fellow tot promovendus, van promovendus tot directe collega. Dank voor je begeleiding, je oprechte interesse in de persoon achter de dokter (of student of patiënt) en de regelmatig broodnodige adviezen.

dr. N.R. Biermasz, beste Nienke, jij was altijd degene met de meest kritische blik en scherpe nachtelijke invallen tijdens dit hele proces. Dank hiervoor en ik laten we bepaalde aspecten van ons onderzoek gezamenlijk verder uitbouwen in de groeihormoon-bot combinatie.

dr. N.A.T. Hamdy, beste Neveen, ik heb van niemand de afgelopen jaren meer geleerd over botmetabolisme, het schrijven van papers en het leven in het LUMC dan van jou. Uiteraard ben ik zeer dankbaar voor het upgraden van mijn engelse schrijf- en spreektaal. Ik beschouw het als een eer dat ik sinds 2012 als junior staff-member werkzaam in de metabole botziekten ben.

Geachte leden van de promotie/oppositiecommissie, hartelijk dank voor het beoordelen van dit proefschrift en zitting nemen in de oppositiecommissie.

Beste Kim Claessen, dakpan en paranimf. Jij combineert intelligentie en sociale vaardigheden ook nog eens met een enorme efficiëntie en snelheid. Ik ben voor het eerst iemand tegen gekomen die nog sneller op mails reageert dan ik.

Lieve Anouk Jochems, paranimf, collega maar vooral goede vriendin. Na onze eerste voorzichtige kennismaking in het Westeinde ziekenhuis zijn we beiden toch aardig verrassend terecht gekomen. Dank voor je steun en onze discussies over uiteenlopende (werk en prive) onderwerpen. Ik vind het geweldig dat je mijn paranimf bent.

Lieve papa en mama, bedankt voor het geven van een geweldige basis in het leven en jullie onvoorwaardelijke steun en liefde. Dit geldt voor alle aspecten van mijn leven, emotioneel, intellectueel maar ook praktisch ondersteunend, als ook voor dit proefschrift en mijn werk nogal eens nodig is. Zonder jullie was ik nooit hier gekomen.

Lieve Michiel, Boris en Katja. Ik heb me alleen zo kunnen ontplooiën door onze stevige, liefdevolle thuisbasis. Dank voor jullie (b)engelengeduld en onvoorwaardelijke liefde.

