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Childhood malignant disease and consequences for growth hormone secretion, intellectual function and cardiovascular risk

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Abstract

In childhood onset growth hormone deficiency (GHD) a reduction in cardiac left ventricular mass (LVMi) and impairment of cardiac systolic function, as well as in glomerular filtration rate (GFR) has been shown. In study I, we showed that a low dose of GH treatment for 10 months resulted in increased LVMi and kidney size. No significant improvement in cardiac systolic function or GFR, except for a normalisation in GFR in 3 patients, was recorded. Hypopituitary patients receiving conventional hormone treatment, but without GH replacement, have an increased mortality from cardiovascular disease and a reduced insulin sensitivity. Hypothalamic-pituitary hormone insufficiencies are well known consequences of cranial radiotherapy (CRT) and CRT has previously been part of treatment regimens in childhood acute lymphoblastic leukemia (ALL) to prevent central nervous system relapses. A decline in intellectual function has been shown 4-8.5 years after treatment with 18-24 Gy of CRT in childhood ALL patients, but information on longer follow-up is missing. In study II, we aimed to investigate cardiovascular risk factors in former ALL patients, and in study III to evaluated insulin sensitivity before and after 12 months of GH treatment. In study IV, we evaluated neuropsychological performance and self-rated mental well-being. All comparisons were made with matched population controls. Further, the impact of 12 months of GH treatment on neuropsychological test scores was evaluated.

We have shown that, at a median 17 years after treatment with CRT and chemotherapy, the former ALL patients had an increase in cardiovascular risk factors, and a marked reduction in cardiac dimensions and performance. We suggested that GH deficiency (GHD), induced by CRT is a primary cause, because 91% of the former ALL patients were GHD, and strong correlations between stimulated GH secretion and several of the cardiovascular risk factors were recorded. GH treatment for 12 months had positive effects on body composition, but no significant improvement on insulin sensitivity was recorded. There was no difference in the self reported quality of life, but significantly lower scores in neuropsychological performance was recorded in former ALL patients, where early age at treatment had a strong negative impact on test scores. GH treatment for one year, in a subset on former ALL patients, did not improve neuropsychological performance. In study V, we showed that the GHRH-arginine test can not be used to rule out GHD due to its low negative predictive value (27%), but a failed GH response accurately reflects the presence of radiation induced GHD, illustrated by a high positive predictive value (91%).

Key words

Growth hormone deficiency, cranial radiotherapy, cardiovascular risk, insulin sensitivity, neuropsychological performance

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Table of Contents

List of papers

This thesis is based upon the following papers, referred to in the text by their roman numerals.

I. Link K, Bülow B, Westman K, Salmonsson EC, Eskilsson J, Erfurth EM. Low individualized growth hormone (GH) dose increased renal and cardiac growth in young adults with childhood onset GH deficiency. Clin Endocrinol 2001(55); 741-748

II. Link K, Moëll C, Garwicz S, Cavallin-Ståhl E, Björk J, Thilén U, Ahrén B, Erfurth EM. GH deficiency predicts cardiovascular risk in young adults treated for acute lymphoblastic leukemia (ALL) in childhood.
J Clin Endocrinol Metab 2004 (89); 5003-5012

III. Bülow B, Link K, Ahrén B, Nilsson AS, Erfurth EM. Survivors of childhood acute lymphoblastic leukemia with radiation induced GH deficiency, exhibit hyperleptinemia and insulin resistance, unaffected by 12 months of GH treatment.
Clin Endocrinol. (Oxf) 2004 Dec; 61(6): 683-691

IV. Link K, Moëll C, Österberg K, Ørbaek P, Garwicz S, Cavallin-Ståhl E, Erfurth EM. Adult survivors of childhood acute lymphoblastic leukemia with GH deficiency have normal self-rated quality of life but impaired neuropsychological performance 20 years after cranial radiation.
Submitted

V. Björk J, Link K, Erfurth EM. The GHRH-Arginine test cannot be used solely for diagnosing GH deficiency in adults with childhood acute lymphoblastic leukemia.
Submitted

Abbreviations

ALL	Acute lymphoblastic leukemia
ACTH	Adrenocorticotroph hormone
A-max	Maximal velocity of the atrial contribution of the mitral inflow
BIA	Bioelectric impedance analysis
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CRT	Cranial radiotherapy
DEXA	Dual X-ray absorbtiometry
E/A ratio	Early filling E wave/atrial filling A wave
ECW	Extracellular water
EF	Ejection fraction
E-max	Maximal velocity of the early mitral inflow
FFA	Free fatty acid
FFM	Fatfree mass
FM	Fatmass
FS	Fractional shortening
FSH	Follicle stimulating hormone
GH	Growth hormone
GHBP	Growth hormone binding protein
GHD	Growth hormone deficiency
GFR	Glomerular filtration rate
GHRH	Growth hormone releasing hormone
GRS	Growth hormone research society
HDL	High density lipoprotein
IGF-I	Insulin growth factor I
IGFBP	Insulin growth factor binding protein
IMT	Intima media thickness
IS	Insulin sensitivity
ITT	Insulin tolerance test
LA area	Area of the left atrium
LDL	Low density lipoprotein
LH	Luteinising hormone
LV area	Area of the left ventricle
LVEF	Left ventricular ejection fraction
LVIDd	Left ventricular inner dimension in diastole
LVIDs	Left ventricular inner dimension in systole
LVMi	Left ventricular mass indexed for BSA
MRI	Magnetic resonance imaging
MTX	Methotrexate
RA area	Area of the right atrium
RV area	Area of the right ventricle
S/D ratio	Ratio of systolic to diastolic pulmonary venous velocity
T3	Triiodothyronine
T4	Thyroxine
TBI	Total body irradiation
TSH	Thyroid stimulating hormone
WHR	Waist hip ratio

Introduction

Pituitary tumours and other brain tumours

Pituitary deficiency, with a varying number of hormone deficiencies, is a common sequalae of tumors in the pituitary or peripituitary area.

Craniopharyngeomas are epithelial tumours of maldevelopmental origin in the sella and suprasellar regions that are histologically benign but have a malignant way in progression and morbidity. They constitute about 6-13 % of all intracranial tumours in childhood with an incidence of 1-2 new cases per million per year. The peak year incidence is at 5-10 years but there may be a second peak in the fifth to sixth decade. The choices of therapy have mainly been radical or non-radical surgery with or without radiotherapy (Sung *et al.* 1981).

Idiophatic hypopituitarism and idiophatic GHD present early in childhood and in the latter case the most appearing sign being growth failure. The etiology is not clear where MRI has shown a spectrum from normality to absence of anterior and posterior pituitary tissue.

Optic glioma is the most frequent tumour of the optic pathways in childhood (Groswasser *et al.* 1985). It is relatively common in children with neurofibromatosis type I and is most often a pilocytic astrocytoma (*Rossi et al*. 1994). In most instances, they behave like hamartomas and are symptomatic but in some cases gliomas behave malignantly by causing visual loss, endocrinopathies or hydrocephalus (*Liu et al.* 2004).

Prolactinoma is the most common type of functioning adenoma in humans and is divided into micro- and macroprolactinomas. The tumour is rare in males and cause clinical symptoms as galaktorrhoea, amenorrea, pressure symptoms depending on the size of the tumour and varying degrees of hypopituitarism. Pituitary prolactin production is under tonic inhibition of hypothalamic dopamine and treatment with a dopamine agonist leads to shrinkage of the tumour.

Growth hormone (GH) and insulin-like growth factor I (IGF-I)

GH is a 22kD polypeptide produced by somatotrophic cells in the anterior pituitary gland and regulated by growth hormone relasing hormone (GHRH) and somatostatin. GHRH is synthezised in the arcuate and ventromedial nucleus in the hypothalamus and stimulates both GH synthesis and secretion (Wehrenberg *et al.* 1982, Barinaga *et al.* 1983). Somatostatin from the peri- and paraventricular nuclei, inhibits GH release without affecting synthesis

(Lechan *et al.* 1983, Fukata *et al.*1985). Two GH binding proteins (GHBP) have been identified in humans. GH is released in pulses, postulated to be the result of the coincidence of peaks of GHRH and throughs of somatostatin by the hypothalamus (Tannenbaum *et al.* 1984). It is suggested that GHRH is required for the initiation of pulses and somastostatin modulates the amplitude of GH pulses (Wehrenberg *et al.* 1982). 24-hour serum GH concentrations are approximately 50% higher in young menstruating females than young men (Ho *et al.* 1987), increase in response to acute physical training (Chang *et al.* 1986), increase threefold during puberty and are maximal during late puberty (Martha *et al.* -89 and 92). In normal subjects, GH secretion is maximal at night with an association with slow wave sleep stages (Takahashi *et al.* 1968). Ageing is associated with decreasing levels of GH (Ho *et al.* 1987) and IGF-I (Rosenfeld et al. 1986). Obesity is associated with decreased GH secretion (Veldhuis *et al.* 1995, Vahl *et al.* 1999) and increased GH clearance (Veldhuis *et al.* 1995).

IGF-I is a single chain peptide bound to 6 carrier proteins in the circulation, IGFBP-1 – IGFBP-6 which regulates bioavailability and action (Ballard *et al.* 1989). Serum IGF-I is mainly produced by the liver (Schwander *et al.* 1983) in response to GH secretion but also produced in many other celltypes suggesting a paracrine action (D´Ercole *et al.* 1984). IGFBP- 3 is the predominant plasma binding protein and is regulated in parallel with serum GH concentrations (Blum *et al.* 1993). Serum levels of IGFBP-I are inversely correlated to the insulin levels.

Chronic malnutrition causes suppression of serum IGF-I (Isley *et al.* 1983) and low levels are also found in conditions with activation of the immune system as in sepsis, systemic inflammatory disease and malignancies (Dahn *et al.* 1988). Serum IGF-I is age-dependent with declining levels with age (Rosenfeld *et al.* 1986).

Leptin

Leptin is a 167 amino-acid peptide identified in 1994 in a rodent model (Zhang *et al.* 1994). The name leptin is derived from the Greek word leptos meaning thin. Leptin is produced mainly in adipose tissue but recent data shows that leptin is produced in many tissues as placenta (Masuzaki *et al.* 1997), ovary (Cioffi *et al.* 1997), mammary epithelial cells (Smith-Kirwin *et al.* 1998) and the anterior pituitary gland (Dieterich *et al.* 1998). Leptin is thought to regulate adiposity and energy homeostasis and acts through a feedback loop with hypothalamus, involving neuropeptide Y (Wang *et al.*1997). Aside from being a food intake inhibitor and energy control factor, leptin is thought to take part in controlling the pituitary hormones, promoting secretion of GH, prolactin, TSH β, FSHβ/LHβ, and inhibiting the

secretion of ACTH (Sone *et al.* 2001). There is a gender difference in leptin levels with higher levels in women, even when correcting for percentage body fat, suggesting an endocrine regulation (Popovic *et al.* 2001). On the other hand, Bülow *et al.* (2003) showed that higher levels of serum leptin persisted in 80 year-old women in comparison with age matched men in spite of higher estradiol levels in the males. Leptin is higher in obese subjects who seem to be resistant to the regulatory effect of circulating leptin (Popovic *et al.* 2001). A positive relationship is found between leptin and glucocorticoids (Papaspyrou *et al.* 1997), insulin (Boden *et al.* 1997a) and estrogen (Shimizu *et al.* 1997) and a negative with testosterone (Behre *et al.* 1997) and growth hormone (Miyakawa *et al.* 1998). Elevated serum levels of leptin have been described in GHD patients treated with cranial radiation (CRT) for childhood ALL (Brennan *et al.* 1999, Birkebaek *et al.* 1998). It is, however not known whether the hyperleptinemia is caused by altered regulation of leptin expression, due to GHD or by leptin insensitivity at the hypothalamus caused by irradiation. It would therefore be of interest to examine whether the hyperleptinemia is reversible on GH replacement and also to correlate serum levels of leptin to possible cardiovascular riskfactors in patients treated with CRT for childhood ALL.

Hypopituitarism and cardiovascular risk

It is now established that patients with hypopituitarism on conventional hormone treatment but with unsubstituted GHD have an increased cardiovascular mortality in comparison with the general population (Bülow *et al.* 2000, Rosen *et al.* 1990, Tomlinson *et al.* 2001) and particulary in cerebrovascular mortality (Bülow *et al.* 1997, Tomlinson *et al.* 2001). Furthermore, women are more affected than men (Bülow *et al.* 1997). In adults with hypopituitarism with untreated GHD, total- and -LDL cholesterol as well as apo B levels are reported to be increased compared with healthy controls (Cuneo *et al.* 1993, de Boer et *al.* 1994). Serum triglycerides are reported either unchanged (de Boer *et al*. 1994) or increased (Rosen *et al*. 1993c) and further either normal or low HDL cholesterol are shown (Wüster *et al.* 1991, Rosen *et al.* 1993c).

IMT of the carotid arteries are described to be increased among GHD patients (Markussis *et al.* 1992, Pfeifer *et al.* 1999) and also to be decreased with GH treatment after 3 months and maintained after 24 months (Pfeiffer *et al.* 1999). A recent study showed a positive correlation between low levels of serum IGF-I and IMT in GHD patients and also in healthy controls (Colao *et al.* 2004), which is in line with a previous study where an inverse relation between serum IGF-I and atherosclerotic plaques was found in healthy elderly subjects (Janssen *et al.*

1998). GHD patients have reduced ECW which increases with GH treatment (Johansson *et al.* 1997) and therefore could account for some of the rapid IMT changes seen with GH therapy. Serum IGF-I has high affinity binding sites to endothelial cells (Delafontaine *et al*. 1991) and increases nitrous oxide production which could contribute to changes in vascular tone.

GHD has been shown in patients treated for childhood ALL, even with low doses of CRT (Moëll *et al.* 1998, Brennan *et al.* 1998). One previous study of cardiovascular risk factors in former ALL patients showed obesity and dyslipidemia in a small subgroup treated with CRT compared with patients treated with chemotherapy only (Oeffinger *et al.* 2003). The mechanism behind the possible increase in cardiovascular risk in survivors of childhood ALL is not clarified and in study II we aimed to investigate the prevalence of cardiovascular risk factors, in a homogenous group of former childhood ALL patients, in comparison with controls matched for age, gender, smoking habits and residence. Also, possible correlations between cardiovascular risk factors and GH secretion were investigated.

Previously, GH treatment has been initiated to promote linear growth and terminated when final height was reached. There is accumulating evidence that patients with childhood onset GHD have less lean body mass, fat mass and bone mineral content compared to age matched adult onset GHD patients (Attanansio *et al.* 1997) and should be regarded as a seperate entity in comparison with adult onset patients.

It is well known that the heart in excess of GH, as in acromegaly, show specific changes as concentric hypertrophy with an increase in left ventricular mass, where the degree of hypertrophy is related to the duration of the disease and not to circulating levels of IGF-I or GH. In parallel, a lack of GH results in specific changes depending on the time of onset of GHD (Attanasio *et al.* 2004). There is evidence that GHD in early childhood have more impact on cardiac structure and development than GHD in adulthood (Maison *et al.* 2003). Patients with childhood onset GHD exhibit a decrease in left ventricular mass, impairment in systolic function with lower fractional shortening and ejection fraction and also an early diastolic dysfunction (Amato *et al.* 1993, Merola *et al.* 1993, Cittadini *et al.* 1994). Several studies have found a lower heart rate at rest (Caidahl *et al.* 1994, Johansson *et al.* 1996) and lower exercise capacity (Ter Maaten *et al.* 1999). In parallel, GFR is increased in acromegaly and decreased in hypopituitarism (Falkheden *et al.* 1965). GH treatment of GHD patients has resulted in an increase in GFR (Jörgensen *et al.* 1989, Caidahl *et al.* 1994) but whether kidney size increases in parallel is not known. The optimal GH replacement dose has not been established and high doses of GH have resulted in LVMi even above those of normal subjects (Johansson et al. 1996). Therefore, in study I, we aimed to evaluate if a low individualized

dose of GH to childhood onset GHD patients resulted in an improvement in morphological and functional parameters of the heart and the kidneys.

Hypopituitarism and glucose metabolism

Hypopituitary adults with GHD are reported to have reduced insulin sensitivity (Hew *et al.* 1996, Weaver *et al.* 1995, Johannsson *et al.* 1995). Acromegaly, with high levels of GH also exhibit insulin resistance sometimes leading to overt diabetes. Children with GHD show an increased insulin sensitivity often causing fasting hypoglycaemia (Wolfsdorf *et al.* 1983) where the explanation is suggested to be a lesser degree of lean mass and also of a low hepatic glucose production in the presence of higher glucose utilisation (Bougnère et al. 1995). GH exerts an initial insulin-like effect (Tanner *et al*. 1992) followed, after a lag time of several hours, by an insulin antagonistic effect (Davidson *et al.* 1987) with inhibited glucose uptake and increased lipolysis.

GH treatment to GHD adults have resulted in either unchanged (Khalfallah *et al*. 2001, Svensson *et al.* 2002), worsened (Fowelin *et al.* 1993, Weaver *et al.* 1995, Christopher *et al.* 1998) or, in one study, improved insulin sensitivity (Hwu *et al.* 1997). The dose of GH were in early studies based upon the body weight or body surface area unlike dose regimens today that are titrated against the response in serum IGF-I. This might explain the worsening in insulin sensitivity seen in some studies. This is further supported by a study where 2 weeks administration of supraphysiological doses of GH (8 IU/day) resulted in abnormalities in substrate metabolism and insulin sensitivity comparable with those seen in acromegaly (Møller *et al.* 1993). GH treatment causes lipolysis and release of FFA, thought to be part in the insulin resistance seen in GHD patients (Boden 1997b). GHD patients have an increase in abdominal visceral fat stores that are reduced with GH treatment (Bengtsson *et al.* 1993) and the beneficial effect of GH on body composition and physical capacity might with time balance the negative short term impact (Jørgensen *et al. 2004*). As adult hypopituitary patients on conventional treatment but without GH is reported to be insulin resistant, it would be of interest to investigate whether survivors of childhood ALL, with a more limited reduction of pituitary function, also have impaired insulin sensitivity.

The syndrome and the diagnosis of GHD

The clinical presentation of GHD are unspecific with abnormal body composition (Rosen *et al.* 1993b), abdominal obesity (Bengtsson *et al.* 1993), reduced muscle strength (Cuneo *et al.*

1990), reduced sweating (Juul *et al.* 1993), decreased psychological well being (McGauley *et al.* 1990), impaired cardiac function (Shahi *et al.*1991), dyslipidemia (Cuneo *et al.* 1993) and reduced bone mineral content (Rosen *et al.* 1993a) and does not provide a diagnosis of GHD. According to the consensus guidelines from The Growth Hormone Research Society-98 (GRS-98), an evaluation for GHD should be considered only in patients with pituitary-hypothalamic disease, in subjects who have received cranial radiation or in those patients with childhood onset GHD. A random sample of GH does not provide any clue, as GH is secreted in a pulsatile fashion. The serum IGF-I levels are affected by conditions as prolonged fasting, malnutrition and liver disease (Thissen *et al.* 1994) and are not sufficient as diagnostic test alone, but due to its specificity a single measurement of serum IGF-I can be used as a screening test (Hoffman *et al.* 1994). The gold standard is the insulin tolerance test (ITT) (Hoffman *et al.* 1994), where the hypoglycaemia causes GH release by actions at the hypothalamic level (Cordido *et al.* 1990), by hyperactivating GHRH neurons and by inhibition of somatostatin release (Muller *et al.* 1989**,** Ghigo 1992).

The ITT has been questioned due to poor reproducibility (Hoeck *et al.* 1995), and of being hazardous especially in children (Shahi *et al.* 1992) and time consuming. The test also has contraindications as in seizure disorders and in patients with a history of ischemic heart disease. Provocative tests with pyridostigmine, clonidine and glucagons are not diagnostic in adult patients as there is an overlap between GHD subjects and normal subjects. Further the GH response to pyridostigmine is age dependent (Biller *et al.* 2002).

The GRS group recommended that two tests should be used in establishing the diagnosis of isolated GHD, but only one provocative test is appropriate in subjects with one or more additional pituitary deficits. The GRS group also recommended a reconfirmation of GHD in patients with childhood onset GHD as 50% of these patients have normal GH levels when retested at final height (Johannsson *et al.* 1999).

GHRH alone has no diagnostic value due to high within subject variability, but GHRH combined with arginine has proved to be an important diagnostic tool (Ghigo *et al.* 2001, GRS-98). Arginine is thought to act at the hypothalamic level by inhibiting somatostatin release (Muller *et al.* 1989). The GHRH-arginine test has been validated profoundly in patients with presumed direct pituitary damage from a tumour and/or surgery (Aimaretti *et al.*1998). However, the majority of patients with CRT induced GHD remain responsive to GHRH analogues (Ahmed *et al.* 1984, Grossman *et al.* 1984) and recently a comparison between the GHRH-arginine test and the ITT showed that the GHRH-arginine test resulted in false negative diagnosis in the early 5 years after CRT (Darzy *et al.* 2003). In contrast, the

ITT was more accurate within the early 5 years after CRT (Darzy *et al.* 2003). In the study by Darzy, the vast majority of the patients were exposed to high doses of both focal and whole brain CRT (median 58.3 Gy) and were treated in both childhood and adulthood. The aim of study V was to investigate the sensitivity and specificity of the GHRH-arginine test using ITT as a gold standard, and also to evaluate the positive and negative predictive values of a positive GHRH-arginine test in patients treated with a lower dose of CRT (18-30 Gy).

Cranial radiotherapy and GHD

Cranial radiotherapy (CRT) is a potent cause of hypopituitarism and the severity is related to the dose, the fraction schedule and the postirradiation time interval (Littley *et al.* 1988, Lam *et al.* 1991). The hypothalamus seems to be more radiosensitive than the pituitary where the damage seems to be time dependent as frequency and severity of hormonal deficits increase with a longer time interval after radiation (Lam *et al.* 1991, Samaan *et al*. 1975). The plausible explanation to this is the lack of hypothalamic trophic factors leading to pituitary atrophy (Clayton *et al.* 1991, Shmiegelow *et al.* 2000). The first hormone deficit is often GH, followed by loss of FSH/LH, ACTH and TSH. The time of onset and the severity of GHD is dose dependent and also dependent of time since radiation where females and children exposed in a younger age seem to be more vulnerable (Shalet *et al.* 1975). Isolated GHD have been found with as low doses as 10 Gy given as total body irradiation (TBI) (Ogilvy-Stuart *et al.* 1992).

Acute lymphoblastic leukemia (ALL)

Leukemia is a common disease in childhood and accounts for 35-40% of all childhood malignancies, where 85% of these cases are acute lymphoblastic leukaemia (Krasilnikoff 1993). There is a peak incidence at the age of 4 years but the disease occurs in all ages from infancy to adolescence and is slightly more common in boys than girls (Plasschaert *et al*. 2004). Before 1965 the event free survival (EFS) was only 5 % but with improved therapy regimens the EFS is now close to 70% (Camitta *et al*. 1997) or 80% (Faderi *et al.* 2003). The disease is characterized by an uncontrolled proliferation and maturation arrest of lymphoid progenitor cells in the bone marrow resulting in an excess of malignant cells (Plasschaert *et al.* 2004). The diagnosis is confirmed with peripheral blood smears showing an abundance of blast cells, bone marrow aspirates with leukemic lymphoblasts, cytogenetic evaluation and aspirates of cerebro spinal fluid examined for leukemic cells. The clinical symptoms are non-specific like anorexia, fatigue, irritability, lethargy and 50-75% of the patients have

asymptomatic enlargement of the liver and spleen and lymphadenopathy. With progressive bone marrow failure, pallor, bleeding and fever occur. Leukemia infiltration in CNS can lead to headache and vomiting due to increased intracranial pressure but infiltration of the meninges at diagnosis without any clinical symptoms is reported. Enlargement of the testis at diagnosis or a mediastinal mass is a sign of infiltration and indicates a higher risk for relapses. Based on age at diagnosis, white blood cell count, cytogenetic classification and CNS leukemia at diagnosis, the disease is classified into riskgroups. Before 1981, into standard and high riskgroups and thereafter, into three groups: low, intermediate and high risk (Krasilnikoff 1993).

To prevent CNS relapses, profylactic CRT and in some cases spinal radiation was part of the treatment protocol for all risk groups before 1981, thereafter only to patients with high risk leukemias and in case of relapses. Cranial radiation is today replaced by intensified systemic and intrathecally given methotrexate. The chemotherapy regimen is classified into remission, consolidation and maintenance therapy and is kept during three years.

Late complications of treatment for childhood acute lymphoblastic leukemia

Psychosocial and neuropsychological function in former ALL patients

Before 1970, the life expectancy for children diagnosed with ALL was less than a year. Therapy regimens with cranial radiation and intrathecally administred methotrexate have increased survival to about 80% today (Chessells *et al.* 1995). A number of studies have addressed psychosocial, cognitive and quality of life and also educational consequences of treatment in survivors of childhood ALL and have found deficits in attention and learning (Cousens *et al.* 1988, Rodgers *et al.* 1999) and also in fine motor coordination , visuospatial ability and somatosensory functioning (Copeland *et al.* 1985 and 1988).

Studies of psychosocial function in former ALL patients have shown greater negative mood with more symptoms of depression, anger, confusion and tension compared to siblings (Zeltzer *et al.* 1997) and also poorer self images and greater psychological stress in patients receiving CRT compared to patients treated with intrathecal methotrexate (MTX) only (Hill *et al.* 1998). Survivors of ALL are also reported to have poorer interpersonal functioning and coping for love/sex relations, and friendship compared to controls (Mackie *et al.* 2000).

Several studies have reported that a significantly lower number of ALL patients enter secondary education or college and also have a greater likelihood of entering special education or learning disabled programs (Kingma *et al*. 2000, Haupt *et al*. 1994).

Neuropsychological testing in this patient group has revealed impairments in short term memory and attention (Christie *et al.* 1995) where the mechanism is suggested to be a lower strategic planning behaviour (Rodgers *et al.* 1992). Previous studies of childhood ALL have assessed neuropsychological function in childhood or during adolescence but not in adulthood. Furthermore, neuropsychological function has not previously been related to a possible decline in GH secretion in survivors of childhood ALL which was the purpose in study IV.

Anthracyclins and cardiac toxicity

Anthracyclins as adriamycin and daunorubicin are polycyclic, aromatic, red-pigmented antibiotics whose antitumour effect results from blocking of DNA synthesis and subsequent cell death (Allen 1992). Their cardiotoxic effect is divided into early and late, depending of onset (Nysom *et al.*1998). The early effect consists of supraventricular tachycardia within hours of injection or toxic myocarditis and pericarditis within days or weeks after treatment. There are also reports of a cumulative-dose dependent effect within weeks or months with myocardial cell damage causing cardiomyopathy that may culminate in congestive heart failure (Allen 1992).

The late effects are classified as late clinical and late subclinical cardiotoxicity and defined as complications that occur at least 5 years after completed therapy. The complications are dose dependent, with the highest incidence in those with cumulative doses above 550 mg/m^2. The cardiac abnormalities are in the structure and the systolic function of the left ventricle with thin left ventricular walls, elevated afterload, reduced fractional shortening and ejection fraction in response to exercise. Some studies have also revealed a diastolic dysfunction (Nysom *et al.* 1998). Younger age at therapy and female sex are suggested to be risk factors of late cardiotoxicity (Lipschultz *et al.* 1995). Late clinical cardiotoxicity occurs in 5-10% of long term survivors 5-10 years after therapy (Nysom *et al.* 1998). Doses of 200-400 mg /m^2 have previously been considered safe (Nysom *et al.* 1998), but cardiomyopathy has been reported with doses as low as 40 mg/m^2 (Allen 1992), so the individual response to antracyclines are highly variable. In study II, we wanted to correlate the dose of antracyclines given to morphological and functional parameters of the heart.

Cardiovascular risk factors in former ALL patients

As the late effects of treatment for childhood leukemia have been recognized quite recently, only a few studies on cardiovascular complications in this patient group exist. Obesity is a known complication with early and progressive onset after treatment and tends to be more pronounced in girls (Oeffinger *et al.* 2003). Some studies have correlated obesity to exposure to CRT and GHD (Nysom *et al.* 1999) and others to treatment regimens with dexamethasone (van Dongen-Melman *et al.* 1995, Sklar *et al.* 2000). We wanted to investigate whether the reported increase in obesity persisted into adulthood and also to measure a possible preponderance of abdominal obesity with waist-hip ratio.

Serum levels of leptin are reported to be increased in this patient group (Brennan *et al.* 1999, Birkebaek *et al.* 1998) and leptin insensitivity as a result of hypothalamic damage due to CRT, or an effect per se by GH has been proposed (Brennan *et al.* 1999). Leptin, like GH, plays an important role in the regulation of body composition and carbohydrate metabolism and it would therefore be of interest to investigate whether the hyperleptinemia is reversible on GH replacement.

Grossmotor function is known to be impaired in ALL survivors with reports of a decrease in balance, strength, hand grip and running speed and agility which may affect their level of physical activity (Wright *et al.* 1998) and therefore contributing to an increased cardiovascular risk. A study of ALL patients 5 years after therapy found grossmotor difficulties and dysdiadochokinesia in 33 % of the patients (Lehtinen *et al.* 2002). Physical activity and energy expenditure have been reported to be lower in ALL patients treated with CRT (Warner *et al.*1998). Reported difficulties with grossmotor function may predispose to lower physical activity and obesity which are known risk factors for cardiovascular disease.

Higher levels of insulin and blood glucose are reported in long term survivors of bone-marrow transplantation (Taskinen *et al.* 2000) and in one previous study an increased risk for the metabolic syndrome was found in those with reduced spontaneous GH secretion (Talvensaari *et al.* 1996). Dyslipidemia is also reported in this patient group with higher triglycerides and VLDL levels (Taskinen *et al.* 2000) and lower levels of HDL cholesterol (Talvensaari *et al.* 1996) which propably is a reflection of obesity and the effect of GHD on lipid levels. In study II we aimed to evaluate the degree of physical exercise in former ALL patients both during work time and spare time and to evaluate the degree of fatness with BMI, waist-hip ratio, BIA and DEXA in comparison with controls matched for sex, age, residence and smoking.

Aims

- To assess whether a low dose of GH for 10 months to young adults with childhood GHD resulted in improvement in morphological and functional parameters of the heart and the kidneys.

- To investigate cardiovascular risk factors in a group of young adults treated for childhood acute lymphoblastic leukemia with chemotherapy and cranial radiation in comparison with controls matched for age, sex, residence and smoking habits. To evaluate any correlations between GH secretion and possible cardiovascular risk factors.

- To assess whether young adults with GHD treated for childhood acute lymphoblastic leukemia have increased adiposity, changes in leptin, insulin and insulin sensitivity compared with controls matched for age, sex and BMI. To evaluate the effect of one year of GH treatment in a low dose in serum levels of leptin, insulin and insulin sensitivity.

- To evaluate the self-reported mental well-being and neuropsychological performance in a group of young adults treated for childhood acute lymphoblastic leukemia with chemotherapy and cranial radiation in comparison with controls matched for age, sex, residence and smoking habits. Furthermore, a subset of former ALL patients with GHD, were evaluated for neuropsychological performance after one year of GH treatment.

- To investigate the sensitivity and specificity of the GHRH-arginine test compared with the ITT in the diagnosis of GHD. Further, to estimate the postitive and negative predictive values of a positive GHRH-arginine test in this group of patients.

Subjects

Patients

Paper I

Eleven patients, 9 males, with childhood onset GHD were included in the study. All but one patient had been treated with GH during childhood. The median time since termination of GH therapy before the study was 7 years (range 5-10). The median age at onset of GHD was 11 years (2-18). Characteristics of the patients are shown in table 1. When required, the patients received stable replacement therapy for at least six months prior to the study.

Table 1. Clinical and endocrine characteristics of eleven patients with childhood onset growth hormone deficiency at inclusion in the study.

Patient #	Sex	Age (yrs)	Diagnosis	Pituitary deficiency	Treatment
1	m	27	Suprasellar cyst	A/T/G/GH	S
2	m	25	Craniopharygioma	A/T/G/ADH/GH	S+RT
3	f	22	Idiopathic GHD	GH	
4	f	27	Idiopathic hypopituitarism	T/G/GH	M
5	m	26	Idiopathic hypopituitarism	A/T/G/GH	M
6	m	25	Prolactinoma	T/G/GH	S+RT
7	m	22	Idiopatic GHD	GH	
8	m	22	Optic glioma	GH	S+RT
9	m	28	Craniopharyngioma	A/T/G/ADH/GH	S
10	m	27	Craniopharyngioma	A/T/G/ADH/GH	S
11	m	19	Craniopharyngioma	A/T/G/ADH/GH	S+RT

A= ACTH deficiency; T= TSH deficiency; G= Gonadotropin deficiency; ADH= deficiency of antidiuretic hormone; GH= growth hormone deficiency according to an insulin tolerance test; S=surgery; RT=radiotherapy; M= medical treatment (dopamine agonist).

Paper II and IV

Fifty-eight patients treated for childhood ALL with chemotherapy and CRT during 1971-1992 at the Department of Pediatrics, Lund University Hospital, who were at least 18 years at assessment were invited to participate. Fourteen patients had to be excluded for various reasons: 7 patients declined to participate due to lack of time or fear of more hospital visits, 2 were treated for severe epilepsy with uncontrolled seizures, one was pregnant, one was breastfeeding, one had recently been operated for a brain tumor, one was on treatment with GH and one had emigrated. The final group consisted of 23 males and 21 females with a median age of 24.8 years (19.8-31.3).

Initially, the children were treated according to the protocols of the Swedish Child Leukemia Group and since 1981 according to the common protocols in the five Nordic countries (Gustafsson). All patients had been off all kind of chemotherapy for a median 16.7 years (6.3-23.9). For patient characteristics and radiotherapy data see table 2.

Table 2. Patient characteristics and medical history in former childhood onset ALL patients.

	Males (n=23) median (range)	Females (n=21) median (range)	All (n=44) median (range)
Age at present investigation (y)	24.6 (19.1-31.3)	24.8 (22.3-32.1)	24.8 (19.8-31.3)
Age at diagnosis (y)	4.4 (1.9-17.2)	4.1 (1.1-13.2)	4.3 (1-17)
Anthracycline mg/body surface area (m^2)	120 (80-540)	120 (40-480)	120 (40-540)
Age at CRT (y)	4.9 (2-18)	4.6 (1-14)	4.7 (1-18)
Years since CRT (y)	19 (8-27)	21 (11-27)	20 (8-27)
Target dose CRT (Gy)	24 (18-30)	24 (20-25)	24 (18-30)
Gy/fraction CRT	1.7 (1-2)	1.7 (1-2)	1.7 (1-2)
Targetdose testis (Gy) (n=12)	24 (20-25)	-	-
Gy/fraction testis	1.7 (1-2)	-	-
Standard/Intermediate/ High Risk group (n)	14/1/8	15/0/6	29/1/14

Paper III

The 11 patients in study III were recruited from the 44 patients included in study II. The patients were previously treated for ALL between the years 1972-1985. One patient had received spinal irradiation (23 Gy) and one patient was treated with GH from age 12 to 14 years. Median time between maintenance therapy and the present investigation was 20.5 years (13.5-22.5). Individual clinical characteristics are shown in table 3.

Table 3. Clinical charachteristics in 11 patients treated for ALL in childhood

Patient/Sex	Age at present investigation (years)	Age at diagnosis of ALL (years)	CRT dose (Gray)	Time since CRT (years)
1/F	31	7	24	23
2/F	25	4	24	21
3/F	33	4	23	28
4/F	30	6	24	23
5/F	32	7	24	25
6/F	26	6	24	20
7/F	25	1	20	23
8/F	27	3	24	24
9/F	29	5	24	24
10/F	26	2	23	23
11/M	33	18	14	14

Paper V

The patients in study V were the same as in study II. One patient had to be excluded due to unsufficient substitution with androgens and the final study group consisted of 43 patients (22 males). All 43 patients performed both the GHRH-arginine test and the ITT but the ITT results from 6 of the patients had to be excluded because of technical problems (n=1) and inadequate hypoglycaemia (n=5), as acceptable hypoglycaemia was not accomplished in spite of a repetition of the test with a higher dose of insulin. Clinical characteristics of the 43 patients are shown in table 4. All 43 controls performed the GHRH-Arginine test only.

Table 4. Descriptive statistics for the 43 patients that performed the GHRH arginine test. Median and range are used for all numerals.

	All patients	Peak GH during GHRH arginine test	
		< 9 µg/l	≥ 9 µg/l
n	43	28	15
Females (n [%])	21 (49)	14 (50)	7 (47)
Age at CRT (yrs)	4.7 (1.2 – 18)	4.4 (2.2 – 18)	6.2 (1.2 – 14)
Age at test (yrs)	26 (20 – 33)	25 (22 – 32)	26 (20 – 33)
Years since CRT (yrs)	21 (8.9 – 28)	21 (8.9 – 27)	22 (10 – 28)
Target dose CRT (Gy)	24 (18 – 30)	24 (18 – 30)	24 (18 – 30)
Treated with methotrexate above 1 g/m^2 (n [%])	9 (21)	4 (14)	5 (33)
BMI kg/m^2	25 (19 – 35)	27 (20 – 35)	23 (19 – 26)
S-Prolactin µg/L	7 (2 – 25)	6.5 (2 – 25)	8 (4 – 24)
S-IGF-1 µg/L	140 (80 – 330)	140 (80 – 290)	160 (90 –330)
GHRH arginine test (peak GH, µg/L)	6.2 (0.85 – 43)	3.7 (0.85 – 8.5)	14 (9.2 - 43)
ITT (peak GH, µg/L)	0.85 (0.06 –6.9)	1.0 (0.06 – 4.2)	0.81 (0.06 –6.9)

Control subjects

In paper **II, IV and V** the aim was to select one control subject for each patient enrolled in the study. To obtain this, 10 potential control subjects matched for age, sex and residence were selected randomly from a computerized register of the population in the catchment area of the patients. Potential controls were contacted by telephone and were then also matched for smoking habits. The first eligible control that agreed to participate in the study was chosen. If none of the 10 selected control subjects accepted, a new set of 10 controls were selected and this process was repeated until appropriate controls for all patients were chosen. In paper **III**, the control subjects were matched for age and gender and then contacted by telephone where complementary matching was made for BMI. The first eligible control, who agreed to participate in the study, was chosen. A difference in BMI in patients and controls of ±1.0 kg/m^2 or less was allowed.

Ethical aspects

The studies were approved by the Ethics committee of Lund University and all patients and controls signed an informed consent.

Methods

Study designs

Paper I was of open design and doppler echocardiography and renal ultrasound examinations were performed before and after 10 months of GH treatment in a low dose, median 1.5 IU (1-2). Body composition and serum IGF-I were measured before and after 1.5, 3, 6 and 10 months of GH treatment. Thyroid hormones, creatinine and glomerular filtration rate (GFR) were measured before and after 10 months of GH treatment.

Paper II was a cross-sectional study of cardiovascular riskfactors in 44 young adults previously treated for ALL with cranial radiation and chemotherapy and comparison was made with 44 controls matched for age, gender, residence and smoking. Body composition was estimated with BIA and DEXA. Doppler echocardiography was performed with measurements of cardiac dimensions as well as systolic and diastolic function. In those patients with the longest follow-up (n=29), carotid artery ultrasound was performed with estimates of IMT. The degree of physical exercise was investigated with questionnaires and levels of serum insulin, blood glucose, leptin, HDL- and LDL cholesterol, Apo A1 and Apo B, triglycerides and fibrinogen were assessed.

In paper III, insulin sensitivity, serum leptin, serum FFA and serum insulin levels were investigated in 11 patients recruited from the 44 patients in study II. The investigation was made att baseline and after 12 months of GH treatment in a low dose, median 0.5 mg/day (0.4-0.6).

Insulin sensitivity was measured by euglycemic-hyperinsulinemic clamp technique and body composition was estimated with BIA. The patients were compared with 11 controls matched for age, gender and BMI.

Paper IV was a cross-sectional study of neuropsychological functions, educational level and self- rated mental well-being, social interaction and social network in the patient population from study II in comparison with 44 controls matched for age, gender, residence and smoking. A subset of 14 patients and matched controls were evaluated for neuropsychological function after 12 months of GH treatment in a low dose, median 0.4 mg/day (0.2-0.6).

In paper V we evaluated the sensitivity and specificity of the GHRH-arginine test compared with the ITT in diagnosing GHD in 43 young adults previously treated for ALL with chemotherapy and CRT. Furthermore, the positive and negative predictive values for GHD of

a positive GHRH-arginine test in this group of patients was estimated. The impact of age at treatment, time since treatment, CRT dose, gender and BMI on the GH peak in the GHRH-arginine test was also investigated. The patients were compared with the same control subjects as in paper II and IV.

Cardiovascular investigations

Doppler echocardiography

Doppler echocardiography in **study I** was performed by one investigator using a Hewlett-Packard Co. Sonos 500. Left ventricular fractional shortening (FS) and left ventricular mass (LVM_i) were calculated in the same manner as in study II.

Transthoracic echocardiography in **study II** (Hewlett Packard Sonos 5500) was performed blinded by one of three experienced echocardiographers. Cardiac dimensions, areas, volumes and mass were indexed to body surface area (BSA) which was calculated from height (in cm) and weight (in kg) by the formula:[(BSA (in m^2) = (Height+Weight-60)]:100 Left ventricular end-diastolic (LVIDd) and end-systolic (LVIDs) cavity dimension as well as interventricular septal wall thickness (IVSd), left ventricular posterior wall thickness (LVPWd) and left atrial dimensions (LA) were measured. Left ventricular fractional shortening (FS) and ejection fraction (LVEF) were used as indices of left ventricular systolic function and was calculated as: FS= [(LVIDd- LVIDs)/ LVIDd] x 100 and LVEF = [($LVIDd^3$- $LVIDs^3$)/ $LVIDd^3$] x 100. Left ventricular mass (LV-mass) was calculated as; 1.04 $(IVS+LVID+LVPW)^3$ - $LVID^3$) – 13.6. Peak early (E-wave) and peak late diastolic filling (A-wave) velocities of the mitral flow were measured, and the E/A-ratio was calculated, as well as the time required for the E velocity to decline from its peak to its baseline (deceleration time).

Carotid artery ultrasound

In **study II** a high resolution 4-duplex ultrasound system, Acuson 128 XP and Acuson Sequoia 512, with a 7.5 MHz linear ultrasound probe, was used to measure intima-media thickness and plaque of the carotid arteries of the far wall. Measurements were performed by two blinded, skilled operators. IMT was defined as the distance between the luminal surfaces of the first and second echogenic lines. Plaque was defined as a localised area of the wall thickening over 1.2 mm, lokalised thickening 0.5 mm larger than the adjacent IMT or diffuse wall thickening over 1.5 mm. The IMT of the common carotid artery was measured

1-2 cm from the carotid bifurcation and the mean of the valus was calculated. The maximum IMT of the bifurcation was also measured, as well as the IMT of the internal carotid artery 1 cm from the bifurcation. The mean of the right and left IMT and the measurements of the two operators were calculated. The distal 5 cm of the common carotid artery, the carotid bifurcation and the proximal part (at least one centimeter) of the internal carotid artery was viewed for plaque. Differences in the measurements between the two operators were less than 0.02 mm.

Blood pressure and heart rate

Blood pressure in **study I** and **II** was measured in the right arm in the supine position after 10 minutes rest. Two measurements were made and a mean value was calculated.

Heart rate in **study II** was obtained from ECG recordings with measurements of the R-R interval (heart rate = 60*1000/R-R interval in ms).

Waist hip ratio, BMI and BIA

In **study II** waist circumference was measured at the midpoint between the lower rib margin and the iliac crest and hip circumference at the level of the trochanters, enabling the calculation of the waist/hip ratio (WHR). Body mass index (BMI) in **study I-III** was calculated as bodyweight (kg) divided by height (metres) squared. Body composition in **study II** was assessed with DEXA (Lunar Expert XL, Lunar Madison, USA). Data are expressed as estimated fat, lean tissue and total body content (kg). Body composition was also measured in **study I-III** in the supine position by bioelectric impedance analysis using the BIA 101-S technique (RJL-Systems, Detroit, MI, USA). A 50-KHz, 800-uA current was applied. Data are expressed as percentage fat and fatfree mass.

Ultrasound of the kidneys

The ultrasound examination of the kidneys in **study I**, was performed with a Toshiba SSA-270-A and a convex array 3.75 MHz transducer, or a Dornier Al 3200 and a 5 MHz curved linear transducer. The same investigator performed all examinations. At least three measurements were performed to image the longest projection of each kidney and the received value was abbreviated to the closest 0.5 cm. The intra-investigator variation was estimated as 5 %.

Test procedures

GHRH–arginine test

The test was performed in all patients and controls after an overnight fast with an iv. bolus dose of GHRH (Ferring, Malmö, Sweden) 1µg/kg at time zero and thereafter an iv. infusion of Arginine-hydrochloride (0.5 g/kg) between zero and 30 minutes. Blood samples were collected every 15 minutes between -15 to + 90 min.

Insulin tolerance test

The ITT was performed after an overnight fast with 0.1-0.2 U/kg iv. soluble insulin (Actrapid, Novo Nordisk A/S, Bagsvaerd, Denmark). A blood glucose level < 2.2 mmoL/L were considered as adequate hypoglycaemia. Blood samples for analysis of serum GH were collected every 15 minutes for -15 min to +90 minutes.

Euglycemic-hyperinsulinemic clamp

Insulin sensitivity in **study III** was determined by a euglycemic-hyperinsulinemic clamp. After an overnight fast and insertion of catheters into antecubital veins in both arms, baseline samples of glucose and insulin were taken and a constant infusion started (0.28 nmol/m^2 body surface area/min). After four minutes, a variable-rate 20% glucose infusion was added to maintain the blood glucose level at 5.0 mmol/L as determined every 5 min. Samples for insulin assay were taken at 60 and 120 min. Insulin sensitivity was estimated as the glucose infusion rate during the second hour of the clamp divided by the mean 60 and 120 min insulin concentrations. Serum FFA was measured at 60 and 120 min and FFA-clamp was calculated as the mean of these two values.

Biochemical assays

Blood samples were drawn in the morning after fasting since midnight and were stored at -70°C until analysis.

GH and IGF-I

In **study I –III**, serum GH was analysed by an immunofluorometric method (Wallac Oy, Turku, Finland). The detection level for serum GH was 0.03 mIU/L. The intra and interassay CV´s were 5% or less at the level of 4mIU/L. The kit standards were in **study II** and **III**, calibrated against the International Standard 80/505 (European Pharmaco).

In **study I-III**, serum IGF-I was measured by an immunoradiometric assay (Nichols Institute of Diagnostics, San Juan Capistrano, CA, USA). The intra- and interassay CVs were 3.6% or less and 9.1% or less, respectively in **study I** and in **study II** 16 % at the level of 60 µg/L and 11 % at the level of 300 µg/L. In **study III** intra- and interassay CVs were 13.3 % or less.

The reference range for serum IGF-I in adults aged 21-28 years was 129-385 µg/L in **study I** and 122-400 µg/L in age's 19-40 years in **study II.**

Blood glucose, insulin, FFA and leptin

In **study II and III** venous blood glucose was analysed with Hemocue Blood Glucose Analyser (Hemocue AB, Ängelholm, Sweden). The instrument was controlled daily using a standard microcuvette and weekly using a hemolysate (Eutrol, Wageningen, The Netherlands) with known glucose concentration. Serum insulin in **study II and III** was measured with a competitive radioimmunoassay with intra- and interassay CVs of 7.1% or less. Free fatty acids (FFA) in **study III** were determined spectrophotometrically (Wako Chemicals, Neuss, Germany). Serum leptin in **study II and III** was analysed with a double-antibody radioimmunoassay using rabbit antihuman leptin antibodies, ^{125} I labelled human leptin tracer and human leptin as standard (Linco Res., St Charles, Mo, USA). Interassay CV is 1.9% at low levels (<5 ng/ml) and 3.2% at high levels (10-15 ng/ml). Intrassay CV is 1.5% at low levels and 3% at high levels. The limit of detection is 0.5 ng/ml.

FSH, LH, testosterone, estradiol, SHBG, thyroid hormones and prolactin

In **study II** serum FSH, LH were analysed with an electrochemiluminiscence technique (Roche, Elecsys, Mannheim, Germany). Serum testosterone, estradiol, and SHBG levels were measured by commercially available immunoassays. In **study I** and **II** serum TSH, free T4 and free T3 were analysed with an immunofluorometric technique (Auto Delfia, Wallac Oy, Tutku, Finland). Serum free T3 in **study I** was analysed by a radioimmunoassay. The reference ranges for serum free T4 and T3 were 9 to 22 and 3.1 to 7.2 pmol/L respectively, and for serum TSH the reference range was 0.33-4.7 mUI/L.

Serum prolactin in **study II and V** was analysed with Roche Prolactin Assay on the Roche Modular analytics E170. The reference interval for women was 4-27 µg/L and for men 4-24 µg/L.

Total -, LDL-, HDL cholesterol, Apo A1, Apo B, triglycerides and fibrinogen

Fasting total-, LDL- and HDL-cholesterol, Apo A , Apo B, triglycerides and fibrinogen levels in **study II** were measured by standard procedures.

GFR, creatinine and urinary samples

In **study I**, GFR was estimated with iohexol clearance in a standardised manner. The plasma concentration of iohexol was analysed by high performance liquid chromatography with reference interval 80-125 ml/min x 1.73 m^2. Urinary proteinuria was measured with 24-hour urinary samples collected for analyses of albumin, alfa-1-microglobulin, IgG and albumin/creatinine clearance ratio. Serum creatinine was analysed by a routine enzymatic method, with a reference range of 60-115 µmol/L.

Questionnaires

Physical exercise

In **study II**, the degree of physical exercise during spare time and working time was assessed by a self-rating questionnaire in which patients and controls classified their physical activity according to a four-grade scale.

SCL-90 and rating scales for mental distress, social interaction and social network

The Symptom Checklist-90 (SCL-90) comprises 90 items in nine subscales expressing psychosomatic and emotional distress. The Global Severity Index (GSI), calculated as the average score of all 90 items, represents the overall level of distress.

The Interview Schedule for Social Interaction (ISSI) is a 33 item self-report questionnaire about the quantity and quality of social support.

The 16-item Social Network questionnaire reflects the structural level, i.e. the availability of social support but not the subjective experience of its adequacy.

Neuropsychological testing

The battery comprised the following tests: SRB:1 vocabulary, a verbal knowledge or intelligence test. WAIS-R Information, a test of contemporary and historical knowledge and WAIS-R Digit Symbol test, a test of perceptual and fine motor speed. WAIS-R Block Design test, measuring spatial, logical and speed abilities and the Cronholm-Molander verbal memory test which is an associative learning task, comprising immediate and delayed recall sections. Austin Maze test with the Milner pathway, a test of executive functions,

involving spatial learning, strategy and speed. APT Two-way Reaction Time test (APT RT-2) and APT Inhibition test (APT RT-Inhibition), a test similar to APT RT-2, where the subject is required to inhibit the response. APT k-test is a test of sustained attention.

Statistics

In ***paper I-III*** data are presented as median and range. The level of significance was set at $p \leq 0.05$ in all five papers.

In ***paper II***, patients and controls were compared with the Wilcoxon signed rank test for matched pairs and in ***paper I and III*** intraindividual comparisons of data before and after GH treatment were also made with the Wilcoxon matched pair, signed rank test. Univariate correlations were assessed using Spearman's rank order correlation test.

To assess gender dissimilarities in ***paper II***, we calculated the difference within in each patient-control pair and then tested these differences using Mann-Whitney U-test with two independent groups, i.e. male and female patient-control pairs. Data stratified by gender are only presented if the Mann-Whitney U-test for gender differences resulted in $p<0.10$. In ***paper II***, ordinary linear regression was used to assess the association between peak GH response and BMI and patient/control- status. The natural logarithm of the GH response was used as dependent variable to obtain approximately normally distributed regression residuals. Ordinary linear regression was also used to assess the association between cardiovascular riskfactors as dependent variables and GH response and BMI.

A stepwise linear regression model was used to determine variables that predicted IS-clamp in the GHD patients in ***paper III***.

In ***paper IV***, the Wilcoxon´s signed rank test was used for comparison of matched patient and control pairs and also to study the neuropsychological scores at one year follow-up. For this purpose, each patient's score on each test variable in the first examination was subtracted from the follow-up score, and the Wilcoxon´s signed rank test was used to compare the difference between scores for the matched pairs. The independent effects of years since CRT treatment, age at CRT treatment, and peak GH response during the GHRH-arginine test, on neuropsychological performance among patients (total mean z-score), were evaluated with linear multiple regression based on ranked data. Mann-Whitney's U-test was used for comparisons between subsets of patients. Differences in educational level and social status were tested by the McNemar's test for correlated proportions.

In ***paper V***, the area under the receiver operating characteristics (ROC) curve was used as an overall measure of the ability of the GHRH-arginine test to discriminate between severe GHD

and at most GH insufficiency. For the classification of severe GHD using a certain cut-off level for the GHRH-arginine test, 95% confidence intervals for the sensitivity and specificity were calculated based on the binomial distribution. In addition, positive predictive value and the corresponding negative predictive value were calculated for the patient group.

Difference in GH peak after the GHRH-arginine test among patients and controls was tested with Wilcoxon signed rank test. Bivariate associations among the patients between GH peak after the GHRH-arginine test and gender, age at CRT, age at test, years since CRT, target dose of CRT, treatment with MTX above 1 g/m^2, prolactin, IGF-I, and BMI were assessed by Spearman rank correlation coefficient (continuous variables) and by Mann-Whitney U test (dichotomous variables). In the multivariate analyses, multiple linear regression with an intercept term was employed. Explanatory variables were excluded one at a time if they had p-values above 0.30, starting with the regression model with all significant variables from the bivariate analyses included. Explanatory variables with p-values between 0.05 and 0.30 were also excluded one at a time if exclusion changed the regression coefficients of the significant explanatory variables with less than 10%.

Results

Paper I

Doppler echocardiography, blood pressure and heart rate

GH treatment did not influence systolic function (FS), determined from fractional shortening but LV-mass index increased significantly after GH treatment (p=0.04), (Table 5). Heart rate at rest increased significantly (p=0.05), but no change in systolic or diastolic blood pressure could be detected.

Table 5. Echocardiograph findings, heart rate and blood pressure in eleven patients with childhood onset GHD before and after GH treatment for 10 months.

	Before GH treatment		**After GH treatment**		**P**
	Median	Range	Median	Range	
Left heart dimensions and function					
LVIDd (mm)	48	(42-63)	51	(43-66)	0.07
LVIDs (mm)	29	(25-48)	30	(25-49)	0.5
LVPWd (mm)	7	(6-10)	8	(6-10)	0.08
IVSd (mm)	8	(6-12)	10	(7-12)	0.3
LA (mm/m^2)	36	(30-44)	37	(32-43)	0.1
LV-mass index (g/m^2)	78	(58-111)	90	(61-118)	0.04
FS (%)	40	(24-42)	38	(26-45)	>0.5
Heart rate and blood pressure at rest					
Heart rate (beats/min)	68	(54-87)	72	(57-98)	0.05
Systolic blood pressure (mmHg)	120	(105-135)	120	(105-150)	0.2
Diastolic blood pressure (mmHg)	80	(60-110)	80	(65-95)	>0.5

LVIDd= left ventricular end-diastolic cavity dimension; LVIDs= left ventricular end-systolic cavity dimension; IVSd= interventricular septal wall thickness; LVPWd= left ventricular posterior wall in diastole; LA= left atrium; LV-mass index= left ventricular mass index; FS= fractional shortening.

We also found a trend for a positive correlation between the increase in serum IGF-I and the increase in LV-mass index, although it did not reach statistical significance (r=0.57, p=0.07) (Fig. 1).

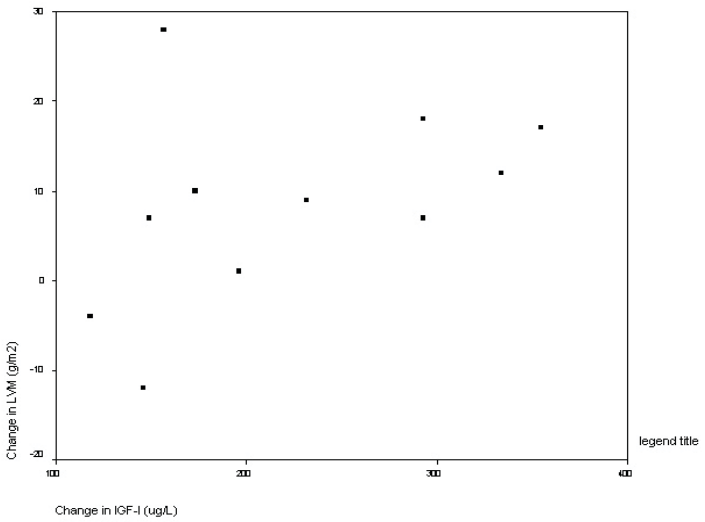

Figure 1. Correlation between the increase in left ventricular mass index and the increase in serum IGF-I in eleven patients with childhood onset growth hormone deficiency, treated with a low dose GH (median 1.5 IU/day) for 10 months (r = 0.57, P = 0.07).

Ultrasound of the kidneys and glomerular filtration rate (GFR)

Kidney length increased significantly (p=0.02) but there was no significant correlation between the increase in serum IGF-I and the increase in kidney length. No significant changes in median GFR or serum creatinine were recorded. Three patients had a subnormal GFR before GH treatment which normalised after GH treatment.

BMI, body composition and thyroid hormones

There was a significant decrease in percentage fatmass measured with BIA after GH treatment (p=0.03), but no significant change in BMI. Serum free T4 decreased after treatment (p=0.04), but no significant change in free T3 (p=0.09) was detected.

Paper II

Cardiovascular risk factors

Significantly higher plasma levels of insulin and blood glucose, and serum levels of LDL-cholesterol, Apo B, triglycerides, fibrinogen and leptin were recorded among the ALL patients compared with controls (Table 6).

Table 6. Cardiovascular risk factors and anthropological data of 44 former childhood onset ALL patients and 44 controls.

	Patients **Median (Range)**	**Controls** **Median (Range)**	**p**
Biochemical measures			
S-Insulin (mIU/L)	6.4 (1.9-34.5)	5.0 (2.3-15.7	0.002
B-Glucose (mmoL/L)	4.9 (3.0-6.2)	4.6 (3.8-5.6)	0.01
S-total cholesterol (mmol/L)	4.4 (2.6-7.7)	4.0 (2.4-6.4)	0.06
S-HDL (mmol/L)	1.2 (0.5-1.8)	1.4 (0.7-2.3)	0.03
S-LDL (mmol/L)	2.8 (1.1-4.8)	2.3 (1.2-4.9)	0.02
S-Apo A1 (mmol/L)	1.2 (0.8-1.5)	1.4 (0.9-1.8)	0.005
S-Apo B (mmol/L)	0.8 (0.5-1.4)	0.7 (0.2-1.5)	0.05
S-Triglycerides (mmol/L)	0.9 (0.4-5.6)	0.7 (0.3-3.2)	0.03
S-Fibrinogen (g/L)	2.8 (1.5-5.1)	2.3 (1.4-3.5)	0.001
S-Leptin (ng/ml)	14.5 (1.1-42.8)	4.2 (0.8-29.8)	0.000
S-Leptin/kg fatmass	0.62 (0.13-1.43)	0.32 (0.04-2.18)	0.06
Anthropological measures			
Weight (kg)	69.1 (44.0-127.2)	70.8 (51.6-108.6)	>0.5
Height (m)	1.66 (1.45-1.92)	1.74 (1.59-2.00)	<0.001
BMI (kg/m^2)	25.3 (19.2-37.4)	22.4 (18.4-34.5)	0.005
Waist circumference (m)	0.9 (0.7-1.2)	0.8 (0.6-1.1)	0.03
Hip circumference (m)	1.0 (0.6-1.2)	1.0 (0.8-1.2)	>0.5
WHR	0.9 (0.8-1.2)	0.8 (0.7-1.0)	0.001
Blood pressure			
Systolic BP (mm Hg)	120 (95-140)	112 (100-130)	0.08
Diastolic BP (mm Hg)	70 (60-86)	70 (55-85)	0.7
Body composition (BIA)			
Fat mass (%)	31.2 (13.0-44.6)	21.7 (14.1-46.8)	<0.001
Muscle mass (%)	51.5 (37-65.7)	57.8 (38.0-65.2)	<0.001
Body composition (DXA)			
Fat mass (kg)	24.0 (3.8-59.5)	15.0 (7.0-46.2)	0.002
Lean mass (kg)	42.9 (28.1-63.8)	48.0 (33.7-70.5)	<0.001

The serum levels of HDL-cholesterol and Apo A1 were significantly lower among the patients, compared with controls. The leptin levels per kg fat mass was higher among patients than controls (p=0.06), but when stratified for gender, both leptin/kg fat mass (1.04 vs 0.25; p=0.001) and serum leptin (25.2 vs 3.9 ng/mL; p<0.001) were significantly higher only among the female patients, compared with female controls.

Bodycomposition and blood pressure

Compared with controls, the patients had higher BMI, waist and WHR, but with no differences in weight, hip, or diastolic blood pressure. There was a tendency for higher systolic blood pressure among the patients (p=0.08). Body composition measured with BIA or DEXA revealed significantly higher fat mass and lower lean mass among the patients, compared with controls (Table 6).

Serum IGF-I and the GH peak to stimulation tests

Serum IGF-I levels were significantly lower in the patients than in the controls (p=0.004) and the median peak GH response to the GHRH-arginine test was 74 % lower in the patients compared with controls (6.2 vs 23.9 µg/L). Four patients had a GH peak >3ug/L to the ITT. Forty of 44 ALL patients (91%) were considered GH deficient according to the ITT and/or the GHRH-arginine test and the rest were considered to be GH insufficient. Among the patients, each unit of increase in BMI was associated with a decrease in GH response of 10.3% and when adjusted for both BMI and gender, the GH response was 67% lower among patients than controls.

Correlations between the peak GH response during the GHRH-arginine test and years since radiotherapy and cardiovascular risk factors.

In patients, the peak GH during the GHRH-arginine test was negatively correlated to several cardiovascular risk factors as total body fat (DEXA), WHR, plasma insulin and leptin and significantly positive correlations was also found to HDL cholesterol and lean mass measured with BIA. However, in a multiple regression analysis adjusting for BMI, the peak GH response was no longer correlated to any of these parameters (all p>0.3), whereas BMI was significantly associated with total body fat mass, muscle mass, plasma insulin and leptin (p<0.001 for each) and HDL-cholesterol (p=0.005), but not WHR. No significant correlation was recorded between years since radiotherapy and cardiovascular risk factors (all, p>0.20).

Estimated physical exercise

The degree of physical exercise during spare time did not differ between patients and controls (p=>0.3), but during work time the patients reported significantly more physical exercise compared with controls (p=0.04).

Doppler echocardiography and carotid artery ultrasound

Heart rate was significantly higher in the patients compared with controls (Table 7). After correction for BSA, left and right ventricular areas and right atrial area were significantly smaller in patients compared with controls. When stratified for gender, LV-mass index among the females was significantly smaller compared with controls (65 vs 78 g/m^2, p=0.03), but no such difference was recorded among the males.

Table 7. Heart rate, body surface area (BSA), echocardiographic measurements of cardiac mass, dimensions, areas and functional parameters in 43$^{\alpha}$ former childhood onset ALL patients and matched controls.

	Patients	**Controls**	**p**
	Median (range)	**Median (range)**	
Heart rate (beats/minute)	71 (57-91)	65 (48-102)	0.02
BSA (m^2)	1.73 (1.33-2.51)	1.91 (1.54-2.25)	0.002
Cardiac measurements corrected for BSA			
LV mass index (g/m^2)	79 (50-123)	86 (49-117)	0.06
LA area (cm^2/m^2)	7.2 (5.3-11.5)	7.9 (5.5-12.2)	0.08
LV area (cm^2/m^2)	14.4 (10.6-24.0)	15.6 (9.9-19.9)	0.03
LVIDd (mm/m^2)	27.2 (19.1-35.3)	25.9 (21.7-32.5)	0.07
RA area (cm^2/m^2)	7.0 (3.8-10.0)	7.6 (5.1-11.7)	0.01
RV area (cm^2/m^2)	7.3 (5.1-12.9)	8.6 (6.3-12.8)	<0.001
Left ventricular systolic function			
Fractional shortening (%)	36 (17-48)	39 (30-51)	0.01
Ejection fraction (%)	74 (43-86)	77 (66-88)	0.001
AV- plane displacement (mm/m^2 BSA)	7.8 (5.1-10.8)	8.8 (5.8-11.6)	0.01
Left ventricular diastolic function			
E/A ratio	1.69 (0.89-2.86)	1.89 (1.21-3.32)	0.004
E deceleration time (ms)	182 (125-252)	192 (128-268)	0.19

Left ventricular systolic function measured as fractional shortening, ejection fraction and AV plane displacement was significantly lower in the patients compared with controls. Also, for left ventricular diastolic function a significant reduction was recorded in the patients compared with controls when measured with E/A ratio and AV plane diastolic velocity. Of the cardiac function parameters, E/A ratio correlated to the peak GH response to the GHRH-Arginine test in the ALL patients (r=0.46, P=0.002).

Among the ALL patients there were significant negative correlations between fractional shortening and ejection fraction and the dose of antracycline (both, $r = -0.59$; $p<0.001$), but to no other heart parameter.

No differences between patients and controls were seen for the intima media thickness (n=29) except for a significantly more pronounced IMT in the right bifurcation among the patients. No plaques were detected in any of the patients.

Paper III

IGF-I and the GHRH-arginine test at baseline and after 12 months of GH treatment

GH secretion assessed by a GHRH-arginine test, showed a significantly lower peak GH response in the patients than in the controls ($p = 0.005$), but there was no significant difference in serum IGF-I levels in patients and controls. GH treatment for 12 months caused an increase in serum IGF-I in all patients ($p = 0.003$).

Anthropometric data

At baseline, the patients were significantly shorter and lighter than the controls, but there was no significant difference in the WHR. The percentage FM was higher ($p = 0.05$) and FFM lower ($p = 0.003$) in the patients compared with controls. See table 8. Twelve months of GH treatment did not change BMI or WHR, but significant changes were seen in body composition measured by BIA, with a decrease in percentage FM ($p = 0.03$) and an increase in FFM ($p = 0.02$). After GH treatment, FFM was still significantly lower in the patients than in the controls ($p = 0.003$), whereas percentage FM was similar in patients and controls. See table 8.

Table 8. Anthropometric data in 11 former ALL patients with GHD, at baseline and after 12 months of GH treatment compared with 11 sex-, -age and BMI- matched controls.

	GHD patients Baseline Median (range)	GHD patients 12 months Median (range)	Controls Median (range)	Pa	Pb
Height (cm)	158 (149-181)	158 (149-181)	167 (161-184)	0.003	
Weight (kg)	63.0 (44.0-82.0)	62.4 (45.0-73.6)	70.0 (53.5-88.0)	0.007	0.5
BMI (kg/m2)	25.0 (19.8-32.4)	24.4 (20.3-31.4)	24.1 (19.4-32.3)	0.1	0.4
Waist/hip	0.85 (0.78-1.18)	0.84 (0.79-0.85)	0.80 (0.72-0.94)	0.1	>0.5
FM (%)	35.5 (22.4-44.6)	33.8 (17.0-39.6)	31.4 (18.2-39.4)	0.05	0.03
FM (kg)	20.6 (12.8-31.6)	20.3 (10.7-27.9)	19.1 (9.6-34.6)	0.5	0.2
FFM (kg)	38.2 (28.0-62.8)	40.1 (34.3-60.7)	46.7 (40.6-65.5)	0.003	0.02

Pa = baseline versus controls, Pb= baseline versus 12 months of GH treatment

Biochemical parameters and IS-clamp

Blood glucose was similar in patients and controls, but serum insulin was significantly ($p = 0.02$) higher in the patient group (Table 9).

During the steady state of the clamp the mean blood glucose levels were similar in the patients at baseline after 12 months of GH treatment and in the controls. Median serum insulin levels during the clamp were in the patients before GH treatment 88 pmol/l (range 62.5-163.0), after GH treatment 77.5 pmol/l (range 47.5-121.0) and in the controls 68.5 pmol/l (range 44.5-139.5). There was a tendency towards a decreased IS-clamp in patients compared with controls ($p = 0.06$) (Table 9), but this tendency disappeared after correction for body composition.

Table 9. Biochemical characteristics in 11 former ALL patients with GHD, at baseline and after 12 months of GH treatment compared with 11 sex-,-age and BMI- matched controls.

	GHD patients Baseline Median (range)	GHD patients 12 months Median (range)	Controls Median (range)	Pa	Pb
IGF-I	144 (100-274)	230 (166-317)	189 (69-275)	>0.5	0.003
Glucose	4.1 (3.7-5.2)	4.4 (3.8-4.8)	4.4 (3.9-5.0)	0.4	0.4
Insulin	36 (18-60)	36 (18-60)	24 (6-36)	0.02	>0.5
IS-clamp	84.5 (44.6-163.1)	80 (45.2-146.9)	114.6 (84.7-206.3)	0.06	>0.5
IS clamp/kg FFM	2.0 (1.2-4.5)	2.2 (1.2-4.3)	2.3 (1.8-4.2)	>0.5	0.4
FFA	0.74 (0.40-1.07)	0.78 (0.54-1.32)	0.60 (0.31-1.40)	0.4	>0.5
FFA-clamp	0.18 (0.12-0.22)	0.17 (0.12-0.31)	0.15 (0.10-0.20)	0.2	0.2
Leptin	16.9 (5.8-37.0)	17.0 (1.3-30.4)	10.2 (2.5-25.3)	0.02	0.1
Leptin/kg FM	0.9 (0.3-1.4)	0.9 (0.1-1.1)	0.5 (0.2-1.1)	0.01	0.3

Pa = baseline versus controls, Pb= baseline versus 12 months of GH treatment

Serum levels of FFA and leptin and the effect of 12 months of GH treatment

Serum levels of fasting FFA, as well as FFA during clamp were not significantly different between the groups (Table 9). Serum leptin was higher in the patients ($p = 0.02$) and also when expressed per kg FM ($p = 0.01$).

GH treatment for 12 months had no significant effect on blood glucose, serum insulin, IS-clamp, serum FFA, FFA-clamp, serum leptin or serum leptin/kg FM (Table 9).

Relationships between clinical, anthropometric, biochemical parameters and IS-clamp in the GHD patients before GH treatment

In the patients, at the baseline investigation, time since CRT was significantly positively correlated to percentage FM ($r = 0.70$, $p = 0.02$) (fig.2) and serum FFA ($r = 0.67$, $p = 0.02$) and negatively correlated to IS-clamp ($r = - 0.85$, $p = 0.001$).

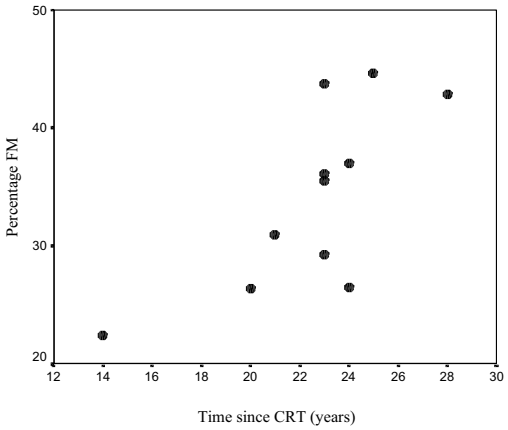

Fig 2. Relationship between time since cranial radiotherapy (CRT)and percentage fatmass (FM) in the GHD patients at the baseline investigation (r=0.70, p=0.002).

Serum FFA was negatively associated with IS-clamp (r = - 0.60, p = 0.05) (fig. 3), but there was no significant correlation between percentage FM and IS-clamp (r = - 0.45, p = 0.2).

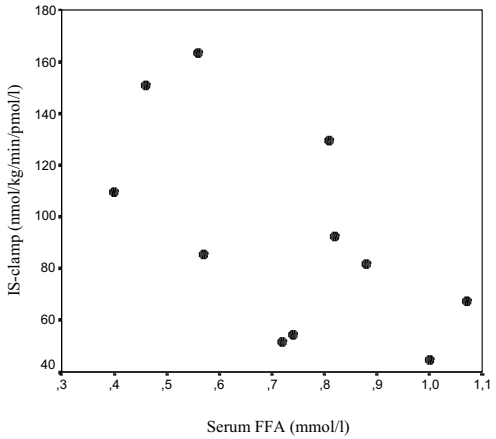

Fig 3. Relationship between serum free fatty acids (FFA) and insulin sensitivity measured by a euglycemic-hyperinsulinemic clamp (IS-clamp) in the GHD patients at the baseline investigation (r = - 0.60, p = 0.05).

In a stepwise multiple regression model including serum FFA and time since CRT as independent variables and IS-clamp as the dependent variable, serum FFA remained significantly associated with IS-clamp (p = 0.05), but the relation between time since CI and

IS-clamp was no longer statistically significant. There were no significant correlations between serum IGF-I, peak GH at the GHRH-Arginine test or the ITT and IS-clamp.

Paper IV

Mental distress, social interaction and social network

No statistically significant group differences were observed across the nine SCL-90 subscales; somatization, anxiety, interpersonal sensitivity, depression, obsession, hostility, or phobic, paranoid, and psychotic symptoms, nor for the overall distress measure GSI (all $p>0.14$). The patients showed no statistically significant difference in social interaction compared with controls (all $p>0.09$). Among the social network parameters only emotional support showed a tendency of being somewhat lower in ALL patients than among controls ($p=0.07$). Gender did not influence the results.

School education and maritial status

A significantly lower level of school education was recorded among patients, with 23 % reaching university level compared with 55 % in the control group ($p= 0.01$).

Twenty-eight of the patients compared with 17 of the controls lived alone or still with their parents, ($p=0.019$) but no difference were recorded in maritial status between the groups ($p=0.45$).

Neuropsychological tests

Compared with controls, the ALL patients had a generally lower performance in neuropsychological test, reaching statistical significance in 14 of the 20 test variables. The ALL group scored clearly lower than controls in tests of general knowledge and vocabulary ($p=0.001$), and in the test for spatial ability and speed (WAIS-R Block Design), ($p=<0.001$). In tests of short-term memory functions a slower rate of learning was observed in the Austin Maze test ($p=0.018$) and lower scores were found in Cronholm-Molander immediate and delayed recall ($p=0.009$ and $p=0.006$, respectively). Lower performance was also seen in the perceptual and fine motor speed test (WAIS-R Digit Symbol), ($p<0.001$), in the psychomotor speed tests of the reaction time type (APT RT level and variation and the RT level of the k-test), (p's ranging from <0.001 to 0.014). Gender did not influence the results.

Correlations between the summary measure of performance and the age at CRT

The summary measure of performance, the mean z-score across all tests, was lower in the patientgroup than among controls (z = -0.95; p=0.001). Patients treated with CRT before the age of six had significantly lower mean z-score across all tests (z = -1.18; n=27) than those treated at a higher age (z = -0.66; p=0.019; n=17). This was due to a subset of patients within the early treatment group having particularly low mean z-scores (figure 4). While 10 of the 27 patients treated with CRT before the age of six had a mean z-score in the range -3.4 to -1.5, none of the 17 patients treated at a higher age descended below a mean z-score of -1.3. Any relationship between mean z-score and years since CRT treatment or peak of GH during the GHRH-arginine test was not found.

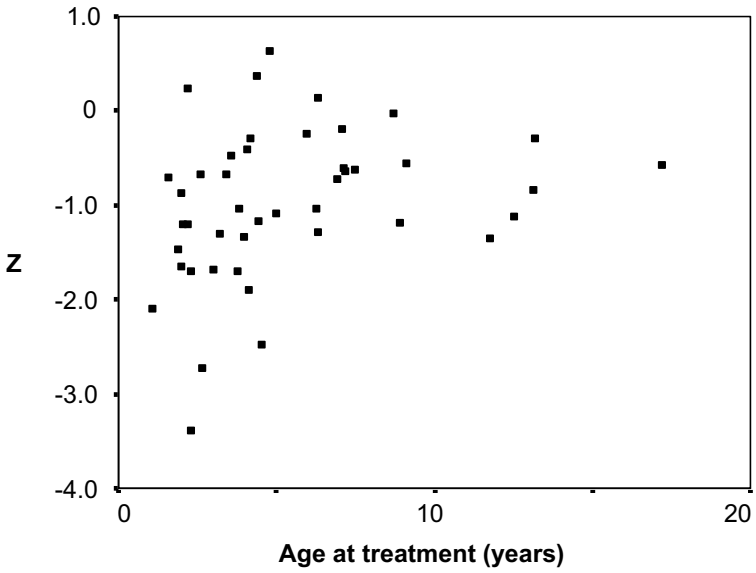

Figure 4. Mean performance level (Z-score) across all nine neuropsychological tests for the 44 ALL patients in relation to age at treatment.

The effect of one year of GH treatment on neuropsychological function in 14 former ALL patients and 14 controls

A tendency of improvement was shown across all tests in both groups for the 14 patient-control pairs after one year of GH treatment. Both patients and controls showed improvement in 4 of the 20 variables but not the same ones. However, the differences (improvements) in test scores, between the initial testing and the one year follow up, did not differ between patients and controls for any of the 20 test variables, with the exception of WAIS-R Information test scores, which improved only in the control group.

Paper V

GH response of the GHRH-arginine test among the controls was significantly higher (median 25, range 3.2 – 110) than among the patients (median 6.2, range 0.85 – 43, $p<0.001$). Using ITT as gold standard for detecting severe GHD with < 3 µg/L as cut-off level, implied high overall discrimination ability of the GHRH-arginine test (area under ROC curve 87 %, 95 % CI 79 – 95%; figure 5).

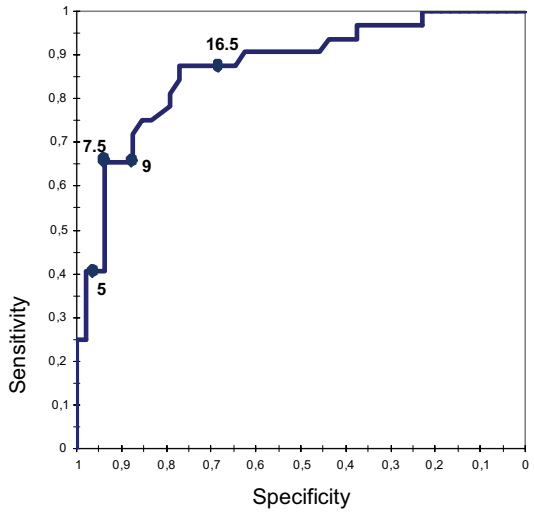

Figure 5. Receiver Operating Characteristic curve of the GHRH-arginine test for classifying severe GHD, defined as ITT below 3 µg/L. The combination of sensitivity and specificity for the peak GH cut-off level of 9 µg/L is marked with a filled circle. Note that the specificity-axis is labelled in descending order.

The sensitivity of the GHRH-arginine test using 9 µg/L as cut-off level was 66% (95% CI 47-81) which means that 21 of 32 patients with severe GHD according to the ITT were classified correctly (Table 2). Using 9 µg/L as cut-off level for the GHRH-arginine test, the probability of GHD increased to 91% if the GHRH-arginine test was positive as 21 out of 23 patients with GHRH-arginine test below 9 µg/L were truly GHD according to ITT.

Table 10. Sensitivity [a] and specificity[b] of the GHRH-arginine test for 37 patients and 43 controls using 9 µg/L as cut-off level, and using the ITT as gold standard for severe GHD.

	ITT		
GHRH-arginine test	< 3 µg/l	≥ 3 µg/L (including controls[c])	Total
< 9 µg/l	21	6	27
≥ 9 µg/l	11	42	53
Total	32	48	80

a 66% (21 of 32 subjects) with severe GHD according to the ITT were classified correctly

b 88% (42 out of 48 subjects) were classified correctly according to the ITT.

c All controls performed GHRH-arginine test only and were assumed to have normal GH levels, or being GH insufficient at most, according to the ITT.

If the GHRH-arginine test was negative, i.e. above 9 µg/L, the probability of GHD decreased to 79%, ie, the negative predictive value was 100 – 79 = 21% (3 out of 14 patients with GHRH-arginine test ≥ 9 µg/L were not GHD according to ITT).

Aiming at higher specificity of the classification by regarding the result of the GHRH-arginine test as severe GHD only if below 7.5 µg/L would increase the specificity of test to 94% (95% CI 83-99; 45 of 48 individuals without severe GHD according to the ITT were classified correctly), while leaving the sensitivity unchanged at 66%.

Regarding GHRH-arginine test as severe GHD already if below 16.5 µg/L would imply a sensitivity of 88% (28 of 32 individuals with severe GHD according to the ITT were classified correctly) and a specificity of 69% (33 of 48 individuals without severe GHD according to the ITT classified correctly). Such a broad classification of severe GHD based on the GHRH-arginine test implied positive and negative predictive values of 90% and 33%, respectively.

Table 11. Predictive values [c d] of the GHRH-arginine test among the patients, using peak GH of 9 µg/L as cut-off level, and using the ITT as gold standard for severe GHD.

	ITT		
GHRH-arginine test	< 3 µg/l	≥ 3 µg/l	Total
< 9 µg/l	21	2	23
≥ 9 µg/l	11	3	14
Total	32	5	37

[c] Positive predictive value 91% (21 out of 23 pts) were truly GHD according to the ITT.
[d] Negative predictive value 21% (3 out of 14 pts) were not GHD according to the ITT.

Correlation between the GH peak during the GHRH-arginine test, BMI, prolactin, MTX and CRT

In the correlation analyses, GH peak during the GHRH-arginine test correlated negatively with BMI ($r = -0.54$, $p<0.001$) and positively with age at CRT ($r = 0.35$, $p=0.02$) among the patients. There was a significant positive correlation between GH peak after the GHRH-arginine test and prolactin level ($r = 0.37$, $p=0.01$). Prolactin level correlated significantly with age at CRT ($r=0.37$, $p=0.01$), but not with years since CRT. Patients treated with MTX above 1 g/m^2 responded significantly higher on the GHRH-arginine test compared with others ($p =0.002$). However, patients treated with MTX above 1 g/m^2 were also treated with CRT at an older age than others (median age 13 versus 4.3 years).

In the multivariate regression analysis, only age at CRT and BMI remained significant predictors of the GH peak. Each unit of BMI above 20 can be expected to decrease GH peak by 10% (95% CI 5 – 16%). Each extra year of age before exposure to CRT can be expected to increase GH peak by 10% (95% CI 4 – 17%).

Correlations between the GH peak during the ITT and IGF- I, BMI, Prolactin, and CRT

The GH peak during the ITT among the patients showed a weak correlation with age at treatment that was close to being significant ($r=0.32$, $p=0.05$). No obvious correlation between GH peak during ITT and target dose of CRT, years since treatment, BMI, prolactin, IGF-I, or GH peak after the GHRH-arginine test was observed.

Discussion

GHD and somatic maturation

There is accumulating evidence that patients with childhood onset GHD, treated with GH until end of linear growth have not reached full somatic maturation. The age at onset of GHD seems to be of importance, as the response to GH therapy in aspects of body composition, lipids and quality of life depends on whether the GHD is of adult or childhood onset (Attanasio *et al.* 1997). Both GH and IGF-I receptors are expressed in the myocardium and are of importance for normal cardiac growth and function (Isgaard *et al.* 1999).

Several studies have shown a reduction in LVM and impairment in left ventricular systolic function in childhood onset GHD (Amato *et al.* 1993, Merola *et al.* 1993, Cittadini *et al.* 1994) and also a close relation between S-IGF-I levels and LVMi (Merola *et al*. 1993). This is in accordance with paper I where an association between the increase in IGF-I and the increase in LVMi was found. The patients in paper I were treated with a low dose of GH, at the time the lowest dose reported, titrated against the response in IGF-I and not adjusted after weight or body surface area. In spite of the low dose, there was a significant increase in LVMi without any signs of hypertrophy or rise in blood pressure that could account for the increase in LVMi. We also found a significant reduction in body fat mass and an increase in kidney size, the latter also in support of a somatic under development in patients with childhood onset GHD.

We could not show any improvement in left ventricular function measured with fractional shortening (FS) with 10 months of GH treatment. An explanation for this is that patients in studies where an effect on FS was found (Amato *et al.* 1993, Sartorio *et al.* 1997), had lower LVMi before initiation of GH treatment. It is also possible that the increase in LVMi in our patients might render an improvement in physical capacity, though not evaluated in this study.

We, as many others (Caidahl *et al.* 1994, Johansson *et al.* 1996, Ter Maaten *et al.* 1999) found an increase in heartrate after GH treatment. Plausible explanations for this phenomenon are the sodium retaining effect of GH causing an increased preload, increased conversion of T4 to T3 (not seen in our study) or an effect of GH on the release or tissue sensitivity to catecholamines (Sacca *et al.* 1994). Ten months of low dose treatment with GH also resulted in an increase in kidney size and normalization of GFR in 3 patients with subnormal levels before treatment. The previously reported increase in GFR with GH treatment (Jørgensen *et al.* 1989, Westman *et al.* 1997) is probably the result of relatively high doses of GH.

Cardiovascular risk factors in former ALL patients

There are prevoius reports of an increased cardiovascular (Rosen *et al.*1990, Bülow *et al.* 2000, Tomlinson *et al.* 2001) and cerebrovascular mortality (Bülow *et al.* 1997, Tomlinson *et al.* 2001) in patients with hypopituitarism on conventional hormone treatment but with unsubstituted GHD. Hypothalamic-pituitary hormone insufficiency is a common consequence of cranial radiation for brain tumours and GH is often the first hormone deficiency to appear (Kirk *et al.*1987).

The patients in paper II, with a confirmed diagnosis of GHD in 91%, were found to have several cardiovascular risk factors such as higher levels of insulin, blood glucose, low density lipoprotein cholesterol, ApoB, triglycerides, fibrinogen and also higher BMI and waist to hip ratio compared with controls. The strength of this study is that it is a homogenous group of patients with a long follow-up, treated for ALL in childhood with chemotherapy and CRT. The patients were compared with controls matched for sex, age, smoking and also recruited from the same catchment area as the patient which is of importance as cardiovascular risk varies geographically (Aase *et al*. 1989). The optimal control group would be patients treated for ALL in childhood with chemotherapy but without CRT and therefore not GHD. As treatment regimens have changed through the years, such a control group does not exist regarding risk classification, number of patients, chemotherapy regimens and follow-up time.

The patients in paper II were found to have dyslipidemia with significantly lower levels of Apo A and HDL cholesterol in comparison with matched controls and also higher levels of LDL cholesterol, Apo B and triglycerides. This is in line with two previous studies showing lower HDL cholesterol in ALL survivors (Talvensaari *et al.* 1996) and also hypertriglyceridemia (Oeffinger *et al.* 2001, Taskinen *et al.* 200) and higher levels of VLDL cholesterol in those patients subjected to CRT.

The levels of fibrinogen were also higher among patients than controls. There is a strong association between smoking and fibrinogen levels but that was not an explanation as patients and controls were matched for smoking. An increased level of fibrinogen together with increased WHR and dyslipidemia among patients indicates a clear risk for cardiovascular disease.

The patients in paper II were more obese compared with matched controls which is a well known complication after treatment for childhood lekemia (Sklar *et al.* 2000, Birkebaek *et al.* 1998, Oeffinger *et al.* 2003). The mechanism is unknown but reduced physical activity has been suggested as an explanation. However, the patients in our study reported the same level

of physical activity as their matched controls during sparetime but a significantly higher degree of activity during work time. This could be explained by the lower level of education amongst patients in paper IV, leading to more strenuous work. Treatment with high doses of corticosteroids known to have adverse effects on body composition has been suggested as an explanation, but the patients in our study had been off treatment since 17 years which makes steroids an unlikely cause for obesity. In paper II and III, serum levels of leptin, were significantly higher among patients than controls which has been described previously (Brennan *et al.* 1999, Birkebaek *et al.* 1998). A leptin insensitivity syndrome as a result of a hypothalamic damage caused by radiation, has been postulated (Brennan *et al.* 1999). A direct effect of GH on the production of leptin has also been suggested (Bianda *et al.* 1997, Boeni-Schnetzler *et al.* 1996). A new finding was that when serum leptin levels were stratified for gender the significance only remained for women. An explanation could be the difference in total fatmass between men and women, but the ratio leptin per kg fatmass was significantly higher only among female patients. Furthermore, male serum testosterone levels could not account for this as there was no difference in serum leptin levels between men with or without testosterone substitution. It is possible that a difference in subcutaneous and visceral fatmass could account for the higher leptin levels in women, but this was not investigated in the present study.

In childhood brain cancer survivors, an increase in the IMT of the carotid bulb has been found (Heikens *et al.* 2000), but ultrasound of carotid arteries in our patients were not different compared with controls except for a significantly increase in IMT in the bifurcation, but only on the right side. The reason for this is difficult to explain.

After controlling for body surface area, several of the echocardiographic measurements including functional parameters were lower in the patients. LVM corrected for BSA was significantly decreased in the ALL patients compared with controls, but only in women. Antracycline, is known to be cardiotoxic with a potential late onset (Allen 1992). The doses of antracyclines were similar in men and women but it is possible that females are more susceptible to this drug as female gender has been suggested as a risk factor for late onset cardiotoxicity (Lipschultz et al. 1995).

There was a significant negative association between the dose of antracycline and the systolic parameters as ejection fraction (EF) and FS, suggesting a role for antracycline in the reduced systolic function.

Patients with GHD since childhood have a reduction in LVMi and systolic function and we suggest an additive role of GHD and antracycline to the changes seen in cardiac parameters in

our patients. Supporting this is the significant correlation between peak GH during the GHRH-arginine test and the diastolic function measured with E/A ratio. On-going treatment studies with GH will enlighten this issue.

The diagnosis of GHD in former ALL patients

Serum IGF-I is known to be a poor marker for GHD in adult patients as normal levels of serum IGF-I is resorded in spite of a severe GHD in tests. The patients in paper II -V showed lower levels than their controls but individual patients had serum IGF-I levels well within the normal range. This did however not reach significance in paper III, propably due to a lower number of participants. We could not find any correlations between IGF-I levels and the peak GH response to the GHRH-arginine test which is in line with previous studies (Brennan *et al.* 1998, Darzy *et al.* 2003).

The ITT is considered the gold standard diagnostic test for the diagnosis of GHD in adulthood but the GHRH-arginine test is also proposed as it is reproducible and not age dependent (Ghigo *et al.* 2001). The ITT is thought to act at the hypothalamic level (Cordido *et al.* 1990), whereas the GHRH-arginine test also has direct stimulatory effect on the pituitary (Barinaga *et al.* 1983), where arginine is thought to inhibit hypothalamic somatostatin release (Ghigo *et al.* 2001). The hypothalamus is known to be more radiosensitive than the pituitary (Samaan *et al.* 1975, Lam *et al.* 1987) and all patients in study II-V had been subjected to CRT. A pattern of neurosecretory dysfunction has previously been described, in patients subjected to CRT, with a reduced spontaneous GH secretion in spite of a normal response to provocative testing (Blatt *et al.* 1984). In the present study we noticed a blunt GH response to the ITT also when there was a rather clear GH peak during the GHRH-arginine test. It´s therefore an attractive thought that the lack of response to the ITT seen in our patients reflects the damage to the hypothalamus caused by CRT and the lack of response seen in some of the patients in the GHRH-arginine test indicates a later damage to the pituitary. The mechanism is thought to be the lack of somatothropic factors causing atrophy and also a direct damage to the pituitary by radiation that increases with time (Clayton *et al.* 1991, Schmiegelow *et al.* 2000). This is in accordance with a study by Darzy *et al*. (2003), where patients subjected to CRT showed a more severe attenuation of the GH response to the ITT than the GHRH-arginine test, reflecting a predominace of hypothalamic dysfunction with a relatively preserved somatotrophic function.

Previously, it has been shown that obesity may influence the GH response to the GHRH-arginine test (Biller *et al.* 2002), and thus GHD due to organic hypothalamic-pituitary disease

may therefore be difficult to distinguish from the blunted response seen in obesity. This accord with the results in paper V, where a multivariate regression analysis, revealed that only age at the time of radiation and BMI, remained significant predictors of the GH peak during the GHRH-arginine test, where each unit of increase in BMI above 20 kg/m^2 can be expected to decrease the GH peak by 10%. The ITT, on the other hand showed no correlation with BMI in paper V.

In paper V we found that the GHRH-arginine test had a low negative predictive value (27%) and can not be used to rule out GHD in this patient group with a history of CRT. This is in line with the study by Darzy *et al.* (2003) who found in a mixed population of patients with brain tumours diagnosed both in childhood and adulthood and who had been subjected to CRT, a sensitivity of 50% and a specificity of 94% at a cut off level of 9 µg/L during the GHRH-arginine test. On the other hand a failed response to the GHRH-arginine test accurately reflects the presence of radiation induced GHD (Darzy *et al.* 2003), which is also indicated by the high positive predictive value (91%) of the test shown in paper V.

It has been proposed that children are more vulnerable to radiation of the hypothalamus-pituitary axis than adults and that younger children are the most sensitive of developing GHD (Shalet *et al.*1972). It has also been shown that cortical grey matter peaks at approximately 4 years of age and cortical white matter increases until age 20 (Pfefferbaum *et al.* 1994). This is supported by the results in paper V where a multivariate regression analysis showed that age at the time of radiation was significantly correlated to the peak GH at the GHRH-arginine test and each year of age above zero could be expected to increase the GH peak by 10%. Also the peak GH level during the ITT showed a correlation with age at radiation in our study which was in contrast to the study by Darzy *et al.* (2003). The patient population in the latter study consisted of a mixture of adult and childhood onset GHD and the median age at CRT was higher than in our study, 9 versus 4 years, which could be an explanation.

Hypothalamic-pituitary dysfunction after radiation is suggested to be time dependent (Lam *et al.* 1987, Littley *et al.* 1988) with an increase in the frequency and severity of hormonal deficits with a longer time interval. Darzy *et al.* (2003) showed a significant negative correlation between the peak GH during the GHRH-arginine test and time since CRT which is in contrast to our study where no such correlation could be found. The CRT in our patient group was given at an early age, median 4 years, which propably has a much stronger impact on hormone secretion that it outreaches time since CRT.

Insulin resistance in former ALL patients

Adult hypopituitary patients, with conventional hormone replacement for multiple hormone deficits but without GH are insulin resistant (Johansson *et al.* 1995, Hwu *et al.* 1997, Hew *et al.* 1996). In paper III we aimed to investigate whether this also was true in former ALL patients with a diagnosis of GHD, but without other hormone deficits. The patients had, in comparison with their sex-, age- and BMI matched controls, a significantly higher basal levels of insulin but similar levels of blood glucose which points toward insulin resistance. The results from the euglycemic-hyperinsulinemic clamp was in the same direction, with a tendency for insulin resistance ($p=0.06$) in comparison with matched controls. Insulin sensitivity and body composition are affected by other hormones as glucocorticosteroids, testosterone, prolactin and thyroid hormones. Hyperprolaktinaemia has been shown to cause hyperinsulinemia and insulin resistance (Pelkonen *et al.* 1982 , Foss *et al.* 1995), but in paper II and III the patients had significantly lower levels of prolactin compared with controls which could not have contributed to the alterations in insulin sensitivity.

The patients in paper III were not substituted for any additional hormone deficits than GH that might influence the results. The female patients were not studied during the same phase of their menstrual period, which may have minor influence as insulin sensitivity is reported to differ between follicular and luteal phases (Diamond *et al.* 1989). On the other hand a similar number of female patients and controls were treated with oral contraceptives.

Compared to BMI-matched controls the patients had higher percentage fat mass, measured with BIA, which has been reported in former ALL patients (Brennan *et al.* 1999, Nysom *et al.* 1999). Percentage fatmass was also significantly correlated with time since CRT, which may support a correlation between the increase in adiposity seen in the patient group and GHD. Patients with GHD, due to hypothalamic-pituitary disease have an increase in fat mass with predominance for abdominal obesity and a reduction in lean body mass, similar to the changes we found in former ALL patients. The tendency for insulin resistance measured with euglycemic-hyperinsulinemic clamp disappeared after correction for body composition which speaks in favour of the impaired body composition as a cause for the decreased insulin sensitivity.

FFA are known to be increased in obesity (Kelley *et al.* 2001) and thus an increased avavilability of FFA might be an explanantion for the impaired insulin sensitivity. The randle cycle was first described by Randle *et al.* (1963), where oxidation of FFA leads to an inhibition of glycolytic enzymes, which inhibits glucose uptake and results in insulin resistance. GH is a lipolytic hormone and has been reported to increase FFA (Jørgensen *et al.*

1989). Interestingly, insulin sensitivity has been reported to be restored in GH treated patients when administred together with Acipimox, a nicotinic acid derivative (Nielsen *et al.* 2001). This has been confirmed by another study in which the GH induced insulin resistance was partially prevented by coadministration of Acipimox (Segerlantz *et al.* 2001). The serum levels of FFA in our study was however, similar in patients and controls.

Insulin sensitivity has after GH treatment to adult GHD patients been reported to be worsened (Fowelin *et al.* 1993, Weaver *et al.* 1995, Christopher *et al.* 1998), unchanged (Bülow *et al.* 1999, Khalfalah *et al.* 2001), or in one previously reported study, to be improved (Hwu *et al.* 1997). In paper III the GH dose was low and titrated against the response in serum IGF-I instead of being based on body weight, which might render less negative effects on glucose metabolism. There is also evidence that the short-term effects of GH, with increased lipid oxidation and increased circulating FFA, can be balanced by the long-term beneficial effects on body composition and physical fitness (Svensson *et al.* 2003).

Quality of life and neuropsychological performance in former ALL patients

Our study shows that survivors of childhood ALL have, 20 years after CRT and 17 years after chemotherapy, impairments in neuropsychological functions with significantly lower scores than controls in vocabulary and general knowledge, spatial ability, memory and learning, and perceptual and psychomotor speed as well as lower educational level.

The patients in paper IV were GHD or in some cases GH insufficient. GHD adults are reported to experience poor body image and socialization, low energy levels, irritability, poor concentration and diminished memory (Holmes *et al.* 1995). This is in line with studies of psychosocial sequalae in survivors of ALL, showing greater negative mood disturbances and symptoms of depression, tension and anger as compared to siblings (Zeltzer *et al.* 1997) and also poorer self images and greater psychological distress in patients receiving CRT compared with treatment regimens with MTX only (Hill *et al.* 1998). The results in paper IV does not accord with this as no difference in any of the SCL-90 subscales could be found. Previously, it has been reported that patients with childhood onset GHD report less disturbances in quality of life than adult onset GHD patients where the likely explanation is that these patients does not have a memory of a GH repleted life (Christolodou *et al.* 1998) and therefore develop coping strategies and adapt to their situation (Attanasio *et al.*1997).

The patients in paper IV reported no difference in the quantity and quality of social support which also is in contrast to previous studies where ALL survivors were found to have poorer interpersonal functioning and coping for love/sex relationship and friendship. It is possible

that patients in our study may have overestimated their social interaction and support, suggested by the fact that significantly more of the patients than controls lived alone or with their parents. This is further supported by a study by Dean *et al.* (1985) showing that the percentage of patients with childhood onset GHD who were married were 30% less than expected for their age.

Impairment in short term memory and attention has previously been reported in childhood ALL survivors treated with both 18 and 24 Gy (Christie *et al.* 1995) where the mechanism were suggested to be a lower strategic planning behaviour affecting recalled memory (Rodgers *et al.* 1992). Attentional deficits have also been reported in ALL survivors treated with 18 Gy and intrathecal MTX (Rodgers *et al.* 1999). In support of this is the lower scores in tests of spatial learning and verbal memory as well as impairments in sustained attention in patients in study IV.

An assessment of educational level among patients and their matched controls revealed a lower number of ALL patients reaching university level. This is consistent with two previous studies where former ALL patients had a greater likelihood of being placed in learning disabled programs than siblings and a lower number of ALL patients entering secondary education or college (Kingma *et al.* 2000, Haupt *et al.* 1994). We could not find any correlation between educational level and early age at treatment which is in disagreement with several previous studies where early age at treatment was a risk factor for low educational level (Kingma *et al*. 2000, Haupt *et al.* 1994). On the other hand, the mean neuropsychological test scores for the patients, in study IV, correlated strongly with age at treatment.

Jancovic *et al.* (1994) studied 129 children in first remission of ALL who had received 18 Gy CRT and 74 patients treated with high dose MTX but no CRT and found a clear reduction in intellectual function in those treated with CRT after lengthening time from diagnosis. This decline appeared 4-8.5 years after completion of therapy. In paper IV, the patients were studied 8-27 years after completion of therapy and no correlation was found between time that passed since CRT treatment and scores in neuropsychological testing, indicating no further decline in neuropsychological capacity.

Individualized GH treatment for one year in 14 of the patients did not improve scores in neuropsychological testing. Explanations for the lack of effect of GH in our patient group might be an irreversible brain damage caused by CRT and/or chemotherapy in childhood or a lack of GH and IGF-I during brain development resulting in suboptimal myelinisation (D´Ercole *et al.* 1984). Binding sites for GH has been found in plexus choroideus, the

pituitary, the hypothalamus and in the hippocampus area. The latter area is considered to have an important role in learning and memory and is part of the limbic system involved in affective behaviour (Lai *et al.* 1991, Wyss *et al.* 1995).

The treatment period might also have been too short to reveal a beneficial effect of GH treatment but on the other hand GH treatment to adult onset GHD patients induced improvements after 3-12 months (Deijen *et al.* 1998, Oertel *et al.* 2004) so this is not a likely explanation.

Our results empazises the importance of identifying the specific deficits and provide adequate support at an early age as some deficits might be improved by training and also consider if GH treatment should be introduced at an early age.

Conclusion

- 10 months of treatment with a low dose GH, titrated against the response in serum IGF-I, increased LVMi and kidney length in 11 adults with childhood onset GHD. This emphazises the importance of continuation of GH treatment in childhood onset patients after final height.

- Seventeen years after treatment with chemotherapy and CRT, young adult survivors of childhood ALL, have GHD and a significant increase in cardiovascular risk factors compared with controls matched for sex, age, smoking and residence. This indicates a risk for cardiovascular morbidity and mortality and warrants intervention with advice concerning nutrition, physical activity, smoking cessation and lipid lowering therapy. Whether GH treatment will improve cardiovascular risk in this patient group remains to be elucidated.

- Young adult survivors of childhood ALL with GHD have impaired insulin sensitivity and hyperleptinemia compared with controls matched for gender, age and BMI. Twelve months of GH treatment titrated against the response in serum IGF-I, resulted in positive effects on body composition. However, hyperleptinemia, hyperinsulinemia and impaired insulin sensitivity remained unchanged which indicates that a thorough follow-up of glucose and insulin levels is necessary in this patient group after initiation of GH treatment.

- Young adult survivors of childhood ALL, treated with CRT and chemotherapy and with a confirmed diagnosis of GHD, have impaired neuropsychological performance but no difference in self-reported quality of life or social interaction compared with matched population controls. Twelve months of GH treatment in a subgroup of patients did not improve scores in neuropsychological testing. This emphazises the importance of identifying the specific deficits and provide support and training programmes at an early age.

- The GHRH arginine test cannot solely be used to diagnose GHD in survivors of childhood ALL treated with CRT and chemotherapy due to a low negative predictive value of the test. On the other hand, a failed GH response to the GHRH-arginine test accurately reflects the presence of radiation induced GHD, demonstrated by the high positive predictive value. Among patients with a high suspicion of GHD after CRT, we recommend a two-step procedure, starting with a GHRH-arginine test followed by an ITT if a GH response is observed during the GHRH-arginine test.

Populärvetenskaplig sammanfattning

Tillväxthormon (GH) insöndras stötvis från hypofysen. Insöndringen är som högst nattetid, och de högsta nivåerna ses under puberteten för att därefter avta succecivt med ökande ålder. En av de vanligaste orsakerna till brist på GH är godartade tumörer i hypofys och hypofysnära områden där även behandling med kirurgi och strålbehandling kan bidra till hypofysinsufficiens av varierande grad. GH är ofta det första hormon som försvinner vid hypofysinsufficiens följt av follikelstimulerande hormon (FSH), luteiniserande hormon (LH), binjurebarkshormon (ACTH) och thyroideastimulerande hormon (TSH). Brist på GH ger ospecifika symptom som ökad fettmassa, framförallt kring buken, minskad muskelmassa, minskad ansträngningskapacitet samt minskat välbefinnande. På 50-60-talet utvanns GH ur mänskliga hypofyser och behandling gavs nästan enbart för att öka barns längdtillväxt. I mitten på 80-talet började man framställa GH syntetiskt och indikationerna har därefter ökat och idag behandlar man även vuxna.

Avhandlingen baseras på fem delarbeten där patienterna i det första arbetet i de flesta fall behandlats för tumörer i hypofys eller närliggande områden och övriga patienter led av brist på GH eller varierande grad av hypofysär hormonbrist av idiopatisk, okänd anledning. De fyra sista delarbetena baseras på en grupp unga vuxna som i barndomen behandlats för akut lymfatisk leukemi (ALL) med cytostatika och strålbehandling mot skalle och ibland även mot ryggmärg. Strålbehandling var tidigare en del av behandlingsregimen för att förhindra återfall av leukemi i centrala nervsystemet. Strålbehandling mot skalle är sedan länge känd att orsaka, framförallt en skada på hypothalamus men med tiden även en påverkan på hypofysfunktionen. Hypothalamus är ett område i hjärnan som styr sömn–vakenhet, känsloliv och även vårt minne. Skadan är korrelerad till stråldos, ålder vid strålbehandling men även till graden av fraktionering av stråldosen.

Delarbete I

I delarbete I studerade vi 11 patienter som diagnosticerats med GH-brist som barn. Det är tidigare beskrivet i litteraturen att vuxna med GH-brist sedan barndom har en påverkan på sina hjärtan med lägre muskelmassa i vänster kammare och även en minskad pumpkraft i hjärtat. Det är också känt att patienter med akromegali, dvs med en ökad sekretion av GH har förstorat hjärta men även en ökad filtrationsgrad i njurarna (GFR) som minskar vid borttagande av hypofysen. Vårt mål var att undersöka om GH-behandling kunde öka

muskelmassan i vänster kammare, påverka dess pumpkraft och även om njurstorlek och filtrationsgrad kunde förbättras.

Vi behandlade de 11 patienterna med en låg dos GH i 10 månader och jämförde sedan en ultraljudsundersökning av njurar och hjärta före och efter GH-behandling. Vi fann att både muskelmassan i vänster hjärtkammare och njurstorleken ökade efter behandling men att filtrationsgraden i njuren inte ökat signifikant. Hos de patienter som hade låg filtrationsgrad i njuren normaliserades GFR. Resultaten i delarbete I styrker teorin att vuxna med GH-brist sedan barndom inte har uppnått full mognad av sina inre organ och att GH-behandling bör fortsätta även efter avslutad längtillväxt.

Delarbete II

I delarbete II studerade vi kardiovaskulära riskfaktorer hos 44 unga vuxna som behandlats för akut lymfatisk leukemi (ALL) i barndomen med kraniell bestrålning och cytostatika. Samtliga patienter konstaterades ha brist eller otillräckliga nivåer av GH med hjälp av två olika tester (se delarbete V för detaljer). Vi jämförde patienterna med kontrollpersoner som slumpmässigt valts ut från den allmänna befolkningen och som rekryterats från samma område som patienterna levde i. Kontrollerna matchades även avseende ålder, kön och rökvanor. Blodprover för kontroll av blodsocker, insulin, blodfetter, leptin och fibrinogen utfördes. Vi mätte även kroppsammansättning med andel fett och fettfri massa med hjälp av bioimpedansanalys (BIA) och densitometri (DEXA) och även midja-höft mått (W/H ratio) och längd i förhållande till vikt (BMI). Vi mätte även hjärtats muskelmassa och pumpkraft med ultraljud. Graden av fysisk ansträngning under arbete och under fritid utvärderades med frågeformulär. Vi fann att patienterna i jämförelse med matchade kontrollpersoner, hade stegrade kolesterolvärden med lägre nivåer av ”det goda kolesterolet” HDL och högre nivåer av ”det onda” LDL. Vi fann också en ökad fettmassa och lägre nivåer av muskelmassa samt större midjemått och högre BMI. Både insulin och blodsockernivåer var signifikant högre än hos kontroller talande för insulinresistens hos patienterna. Ultraljud av hjärta visade mindre muskelmassa i vänsterkammaren och sämre pumpförmåga än hos kontrollpersonerna. Detta kan inte förklaras av att patienterna ansträngde sig mindre då de rapporterade mer fysiskt krävande arbeten än sina kontroller och en likartad motionsnivå under fritid. Vi fann således i denna patientgrupp efter en median av 17 år efter avslutad behandling för ALL, brist på GH, ökade nivåer av blodfetter, insulinresistens, övervikt med högre fettmassa och lägre muskelmassa, minskad muskelmassa i vänster hjärtkammare och sämre pumpkraft och således en ökad risk för kardiovaskulär sjuklighet. Vi fann också samband mellan

varvarande GH-insöndring och flera av de ardiovas ulära ris fa torer vil et styr er att denna patientgrupp bör behandlas med GH. Denna patientgrupp behöver oc så s ärpt uppmär samhet och rådgivning angående ost, motion och rö stopp men även en noggrann ontroll av blodfetter och blodsoc er för att man i god tid an vidta nödvändiga åtgärder.

Delarbete III

Till delarbete III utvaldes 11 patienter av de 44 som studerades i delarbete II. Nya ontrollpersoner eftersö tes och matchades för ålder och ön men nu även för BMI. Syftet var att utvärdera insulin änslighet före och efter behandling med GH i 12 månader. Insulin änslighet utvärderades med eugly emis hyperinsulinemis clamp som innebär att man med hjälp av ett glu osdropp och ett insulindropp håller blodsoc ernivån ring 5 mmol/L genom att variera hastigheten på glu osdroppet. Vi mätte även nivåer av leptin, insulin och fria fettsyror (FFA) samt roppssammansättning med BIA. Patienterna behandlades därefter med GH under ett års tid och blodprover och insulin änslighet med clampmetodi utfördes ytterligare en gång. Vi fann då att de 11 patienterna före behandling med GH, hade i jämförelse med sina matchade ontrollpersoner en högre fettmassa och lägre mus elmassa, högre insulinnivåer och även högre leptinnivåer. Leptin är ett hormon som i huvudsa utsöndras från fettceller och agerar som en mättnadssignal till hjärnan. Leptin nivåerna var högre även utryc t per g fettmassa och unde således inte för laras av en s illnad i fettmängd mellan patienter och ontroller. Insulin änslighet med clampmetodi visade en tendens till sämre värden hos patienter än hos ontrollpersoner vil et doc försvann när insulin änsligheten orrigerades för roppssammansättning. Tolv månaders behandling med GH mins ade mängden fettmassa och ö ade mängden mus elmassa men leptinnivåer, FFA, insulin och insulin änslighet mätt med clampte ni låg oförändrat på samma nivå. Vi fann således en försämrad insulin änslighet i denna patientgrupp som inte förbättrades av 12 månaders GH behandling. Orsa en an vara förändringar i roppssammansättningen som i sin tur an bero på behandling med raniell bestrålning som påver at GH se retion eller som gett en hypothalamis s ada med störningar i leptinse retion.

Delarbete IV

I delarbete IV studerade vi samma patienter och ontroller som i delarbete II men nu undersö tes neuropsy ologis fun tion och även välbefinnande, utbildningsnivå samt social intera tion och nätver med frågeformulär. Vi fann ingen s illnad i psy osomatis t eller emotionellt välbefinnande och ej heller någon statistis t sä erställd s illnad i social

interaktion eller nätverk mellan patienter och kontroller. Patienterna hade en lägre utbildningsnivå, då 23 % i jämförelse med 55 % av kontrollerna uppnådde universitetsutbildning. Patienterna presterade sämre i neuropsykologiska tester avseende verbal intelligens, finmotoriska tester, tester för spatialt logiskt tänkande och hastighet samt i tester för associativt lärande. Om man summerade resultatet i neuropsykologiska tester till ett Z-score fann man betydligt lägre värden i patientgruppen än hos kontrollpersoner, där patienter som behandlats med strålning mot skalle före 6 års ålder hade de lägsta nivåerna. Fjorton av patienterna behandlades med GH i 12 månader och förnyad neuropsykologisk testning utfördes därefter utan någon statistiskt säkerställd förändring i resultaten vilket kan bero på en irreversibel skada i hjärnan av behandlingen eller en alltför kort behandlingsperiod med GH. Det är av vikt att på ett tidigt stadium identifiera vilka brister patienterna har och erbjuda specifik träning redan under skoltid. Det är också möjligt att GH-behandling bör sättas in på ett tidigare stadium då GH-brist under barndomen kan påverka hjärnans utveckling.

Delarbete V

I delarbete V, studerade vi GHRH-arginine testets sensitivitet (att rätt klassificera en sjuk patient som sjuk) och specificitet (att rätt klassificera en frisk patient som frisk) i jämförelse med det test (insulintolerans testet) som för närvarande är ”gold standard” för testning av GH brist. Vi ville också utvärdera huruvida man kan utesluta brist eller konstatera en brist på GH med enbart GHRH-arginine testet. Vi fann att GHRH-arginine testet har ett lågt negativt prediktivt värde, dvs man kan inte använda testet för att utesluta en brist på GH. Vi fann däremot ett högt positivt prediktivt värde, dvs. man kan med stor säkerhet fastställa en brist på GH om patienten svarar lågt på testet. Vi rekommenderar således ett förfarande i två steg, där man hos patienter som tidigare behandlats med kraniell bestrålning, med påföljande misstanke om GH-brist, först utför ett GHRH-arginine test. Om svaret ligger över gränsen för konstaterad GH-brist (9µg/L), genomförs därefter ett insulintolerans test.

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