Bone health and cardiovascular risk in hypopituitary patients on complete hormone replacement, including GH

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Akademisk avhandling

som med vederbörligt tillstånd av Medicinska fakulteten vid Lunds Universitet, för avläggande av doktorsexamen i medicinsk vetenskap, kommer att offentligen försvaras i Segerfalksalen, Wallenberg Neurocentrum, BMC, fredagen den 18 maj klockan 13.00

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Abstract

Growth hormone deficiency (GHD) is associated with decreased bone mineral density (BMD). Low BMD is correlated to increased fracture risk. There are no studies on fracture risk in GHD patients on GH therapy and no studies on BMD in adults with childhood onset (CO) craniopharyngioma (CP) on GH therapy. We have shown a doubled fracture incidence in CO GHD women and decreased incidence of fractures in adult onset (AO) GHD men. We have also shown decreased BMD in adult women with CO CP on GH therapy, in comparison to matched controls, but not in CO CP men. The cause is not known but insufficient sex steroid and GH replacements, particularly during adolescence, in CO GHD and CP women, and an adequate substitution rate of testosterone and GH in AO GHD and CO CP men are possible explanations.

GHD is also associated with increased cardiovascular mortality and morbidity. The impact of long-term GH replacement on cerebral- and cardiovascular diseases and diabetes mellitus (DM) in hypopituitary patients and the prevalence of cardiovascular morbidity and risk factors in adults with childhood onset (CO) craniopharyngioma (CP) are unknown. We have shown that the life-long incidence of non-fatal stroke was tripled in GHD women and doubled in men, but a decline was seen among both genders during the periods where most patients had GH replacement. Life-long incidence of non-fatal cardiac events was similar in the patient and control cohorts, but declined in GHD men during the periods where most patients had GH replacement. GHD women had higher prevalence of type 2 DM and lipid-lowering medication, whereas GHD men had higher prevalence of antihypertensive medication. This cardio-protective medication, together with the GH therapy may have resulted in the decline in non-fatal stroke risk, particularly noted in GHD women, and in significantly lower non-fatal cardiac risk that was seen in GHD men. We have also shown that patients with a CO CP, on GH therapy, had increased cardiovascular morbidity and particularly women were at risk. Of CP women 60% had increased risk for cardiovascular disease.

Insulin tolerance test (ITT) is the recommended test for diagnosing GHD. We have shown that GHD patients during an ITT had very low nadir blood glucose levels and few symptoms of hypoglycemia and in 31% unawareness was seen. If the ITT is still going to be recommended, we ask for more uniform recommendations.

Key words: Growth hormone deficiency, Growth hormone replacement, Fracture incidence, Bone mineral density, Cardiovascular risk, Craniopharyngioma, Insulin tolerance test

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Bone health and cardiovascular risk in hypopituitary patients on complete hormone replacement, including GH

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Department of Clinical Sciences

Lund University

2007

To Leif
Hampus
Gustaf

Tamdiu discendum est quamdiu vivas
(We should learn as long as we may live)
Seneca

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Abbreviations

ACTH	Adrenocorticotrophic hormone
ALL	Acute lymphoblastic leukemia
AO	Adult onset
ApoA1	Apolipoprotein A-1
ApoB	Apolipoprotein B
BIA	Bioelectrical impedance analysis
BMD	Bone mineral density
BMC	Bone mineral content
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CO	Childhood onset
CP	Craniopharyngioma
CRT	Cranial radiotherapy
CV	Cardiovascular
CVD	Cardiovascular disease
DXA	Dual-energy X-ray absorptiometry
DM	Diabetes mellitus
FHD	First confirmed pituitary hormone deficiency
FSH	Follicle stimulating hormone
GH	Growth hormone
GHBP	Growth hormone binding protein
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
GRS	The Growth Hormone Research Society
hs-CRP	Highly sensitive C-reactive protein
HDL	High density lipoprotein
IL-6	Interleukin-6
IGF-I	Insulin growth factor I
IGF-II	Insulin growth factor II
IGFBP 1-6	Insulin-like growth factor binding protein 1-6
IMT	Intima-media thickness
IRR	Incidence rate ratio
ITT	Insulin tolerance test
LDL	Low density lipoprotein
LH	Luteinizing hormone
MI	Myocardial infarction
OC	Oral contraceptives
PBM	Peak bone mass
pQCT	Peripheral quantitative computed tomography
PAI-I	Plasminogen activator inhibitory activity
POR	Prevalence odds ratio
PRL	Prolactin
PTCA	Percutaneous transluminal angioplasty
SMR	Standardized mortality ratio
TG	Triglycerides
tPA	Tissue plasminogen activator
TSH	Thyroid stimulating hormone

TGTV	Tumor growth into the third ventricle
T3	Triiodothyonine
T4	Thyroxine
vBMD	Volumetric bone mineral density
VMH	Ventromedial hypothalamic nucleus
WHR	Waist hip ratio
z-score	Comparison of DXA results with average age and sex matched person
11 β HSD1	11β-hydroxysteroid dehyrogenase1

Introduction

Hypopituitarism

Hypopituitarism in adulthood is often caused by pituitary tumors or tumors adjacent to the pituitary and their treatments (Regal *et al.* 2001). The prevalence of hypopituitarism in adults is approximately 30-46 patients per 100 000 inhabitants (Regal *et al.* 2001). In children idiopathic GHD and craniopharyngiomas are the most common causes of hypopituitarism (Abs *et al.* 2005). The GH axis is very vulnerable and GHD is often the first pituitary hormone deficit occurring due to pituitary and/or hypothalamic damage. The other axes subsequently follow, most often in the following order: FSH/LH, TSH and ACTH (Lindholm *et al.* 1976, Salmi 1979, Littley *et al.* 1988).

It was for long thought that life-expectancy for hypopituitary patients on conventional hormone replacement therapy except GH, was the same as for the general population (Hall 1972). Several studies (Rosén and Bengtsson 1990, Bülow *et al.* 1997, Tomlinson *et al.* 2001) have later shown that life expectancy is shortened in these patients. Rosén and Bengtsson showed an increased overall mortality. Bülow *et al*. showed tripled mortality risk in women and doubled in men. Tomlinson *et al.* confirmed previous results with 50% increased mortality risk in men and doubled in women. The increased mortality is assigned to cardiovascular disease and especially cerebrovascular disease (Bülow *et al.* 1997, and Tomlinson *et al.* 2001).

GH

GH is produced in a rhythmic pattern from the pituitary through the action of somatostatin and GHRH but also other neurotransmitters and neuropeptides (Müller *et al.* 1999). GH secretion is maximal at night (Takahashi *et al.* 1968). Exercise increases (Jenkins 1999), but obesity decreases (Scacchi *et al.* 1999) GH secretion. GH acts on several organs and tissues in the body such as brain, liver, muscle, kidney, heart and bone both directly and indirectly through production of IGF (Daughaday and Rotwein 1989). IGF-I is mainly produced in the liver and participates in the growth and function of almost every organ in the body. Hormone binding proteins (GHBP and IGFBP-I-6) carry GH and IGFs in the circulation and also affect affinity for different receptors (Ballard *et al.* 1993). GH secretion declines with age (Ho *et al.* 1987).

The need for GH in children is obvious as it is needed for linear growth (Ohlsson *et al.* 1998) and lack of GH will be unveiled as the child ceases to grow. In adults the need for GH is not as obvious and symptoms of GHD not as revealing as in children. The symptoms of GHD include abdominal obesity (Bengtsson *et al.* 1993), muscle weakness (Cuneo *et al.* 1990), general fatigue and decreased quality of life (McGauley 1989). It has also been shown changes of body composition with less muscle mass and increased fat mass (DeBoer *et al.* 1992, Rosén *et al.* 1993a), reduced sweating (Juul *et al.* 1993), decreased psychological well being (McGauley *et al.* 1990), decreased cardiac function (Shahi *et al.* 1991), dyslipidemia (Cuneo *et al.* 1993), impaired glucose tolerance (Salomon *et al.* 1991) and both equal BMD compared to controls, particular in older subjects (Toogood *et al.* 1997, Fernholm *et al.* 2000), and decreased BMD (Rosén *et al.* 1993b, Degerblad *et al.* 1995) in AO GHD and CO GHD patients (Kaufman *et al.* 1992, O'Halloran *et al.* 1993, DeBoer *et al.* 1994).

GH replacement was introduced in the 1950's (Raben 1958), but with restricted accessibility as GH was extracted from pituitaries of human cadavers why the use of GH was reserved to children (Molitch *et al.* 2006). Since 1985 GH is produced from a recombinant DNA-derived biosynthetic process and is thus available also for adults (Molitch *et al.* 2006).

With GH replacement therapy most of the adverse effects of GHD can be reversed. It has been shown improvement in body composition (Jorgensen *et al.* 1996, Chrisoulidou *et al.* 2000, Hoffman *et al.* 2004) and quality of life (Burman *at al.* 1995, Radcliffe *et al.* 2004) after GH therapy. GH treatment also exerts positive effect on the heart, increasing left ventricular mass, interventricular septum thickness, left ventricular end-diastolic diameter and stroke volume (Maison and Chanson 2003). Total cholesterol- and LDL-levels have been shown to decrease significantly after GH treatment (Maison *et al*. 2004). Improvement of glucose tolerance has also been shown (Hwu *et al.* 1997), but others have shown unchanged (Fowelin *et al.* 1993, Jorgensen *et al.* 1996, al-Shoumer *et al.* 1998, Svensson *et al.* 2002) or worsened glucose tolerance (Weaver *et al.* 1995, Roenfalch *et al.* 2000, Bramnert *et al.* 2003, Maison *et al*. 2004). The varying results are probably due to different GH dosing and duration of therapy and it has also been suggested, that GH therapy decreases insulin resistance due to a reduction of fat mass (Fowelin *et al.* 1993).

GH therapy increases bone resorption why BMD declines during the first six months of treatment (Thorén *et al.* 1993, Vandeweghe *et al.* 1993) and then returns to baseline after 12 months

(Holmes *et al*. 1995). After 18 months of treatment BMD is however increased (Kotzmann *et al.* 1998). GH acts both directly on bone formation and indirectly through IGF-I on osteoblast function and chondrocyte proliferation (Hayden *et al.* 1995). IGF-II also exerts activity on collagen synthesis and osteoblast differentiation but its effect on the skeleton is not as well known as for IGF-I (Canalis *et al.* 1991). GH stimulates the production of 1,25-vitamin D (Gray *et al*. 1985) which also is important for bone formation. In elderly persons other factors than GHD have been shown to influence IGF-I production such as sex steroids, PTH and cortisol levels (Toogood *et al.* 1997). Bone turnover is reduced in elderly GHD patients (Toogood *et al.* 1997).

In spite of these positive effects of GH therapy there are still no studies showing beneficial effect on fracture incidence or cerebrovascular events and only one showing decrease in myocardial infarction (Svensson *et al*. 2004). There are doubts about what happens to glucose tolerance during GH therapy as studies show diverging results. No study has shown beneficial effects of GH therapy on life expectancy.

Diagnosis of GHD

Severe GHD is defined by the Growth hormone Research Society as peak GH response to a provocative test of less than 3 μg/L (9 mU/L) based on the work by Hoffman *et al.* (1994). ITT is regarded as the "gold standard" (GRS 1998), but is however potentially hazardous (Shah *et al.* 1992). There is very scarce information on level of nadir and duration of nadir during an ITT and the test is not performed uniformly at different centers. That many patients have contraindications for the ITT - as previous history of seizures or cardiovascular diseases and risk factors are more prevalent in these patients than in the general population (Constine *et al.* 1993, Rosén and Bengtsson 1990, Bülow *et al.* 1997, Bülow *et al.* 2000, Tomlinson *et al.* 2001) - is also problematic. Unconsciousness and seizures have been reported in adults and death in children due to mismanagement of hypoglycemia (Jones *et al.* 1994, Shah *et al.* 1992) during an ITT. GHRH-arginine test has been recommended as alternative test, but has limitations in, for example, patients treated with CRT (Shalet *et al.* 1998), why tests may result in false, negative diagnoses.

Hypopituitarism and bone

BMD in CO GHD patients

Heritage, sex, race, nutritional status, body weight, physical activity and hormonal status are all

of importance for the skeletal development (Gilsanz *et al.* 1988, Ott 1990, Krall and Dawson-Hughes 1993, Gilsanz *et al*. 1997). In patients with CO GHD there seem to be a clear reduction in BMD (Kaufman *et al.* 1992, O'Halloran *et al.* 1993, DeBoer *et al.* 1994). CO GHD patients are shorter than controls but even after correction for height BMD is reduced (DeBoer *et al.* 1994). It has been postulated that the most important reason for the decrease in BMD is GHD, as reduced BMD has been recorded both in patients with isolated GHD and multiple pituitary deficiencies (Kaufman *et al.* 1992, DeBoer *et al.* 1994). It has also been shown that adults with CO GHD have normal trabecular density but significantly greater reduction in cortical content (Murray *et al.* 2006).

Sex steroids, GH and IGF-I stimulate the pubertal growth spurt in healthy boys and girls (van Coeverden *et al.* 2002). In women the time period between 11 and 14 years of age is critical for acquisition of BMD and in men the period between 13 and 17 years of age (Bachrach 1993, Turner *et al.* 1994). Estrogen is essential both in girls and boys as it is needed for epiphyseal closure (Bachrach *et al.* 1996). Lack of estrogens leads to delayed epiphyseal closure and decreased bone mass. In men testosterone is converted to estrogen through aromatization (Frank 2003). Testosterone acts either directly or indirectly through its effect on muscle mass, and has an effect on the overall size of the bone (Snow-Harter *et al.* 1990) also in women, though to a smaller extent than in men (Frank 2003). Androgens are secreted into the circulation from ovaries and adrenals in women (Longcope 1986). In hypopituitary women with ACTH and sex steroid deficiencies none of these androgen secretion ways work. In addition, women with hypopituitarism seldom get testosterone or DHEA substitution as studies show disparate results (Saltzman and Guay 2006). It has though been shown beneficial effect on BMD after substitution with DHEA in women (Villareal 2002, Bilger *et al.* 2005). GH therapy in CO GHD patients improves BMD, but it has been shown low BMD in spite of GH treatment during childhood (DeBoer *et al.* 1994, Degerblad *et al.* 1995, Kaufman *et al.* 1992, Koranyi *et al.* 2001). In children with hypopituitarism the induction and timing of puberty is of great importance as lack of these hormones or un-physiological dosing may have a devastating effect on skeletal development and for acquisition of PBM (Ohlsson *et al.* 1998). It is also acknowledged that less bone gain during growth and lower PBM are important determinants of fractures in the elderly (Seeman *et al.* 1989).

BMD in AO GHD patients

GH is important for bone turnover in adult skeletal tissue as well. BMD in AO hypopituitary

patients with conventional hormone replacement except GH have been shown to be both normal, particularly in older subjects (Toogood *et al.* 1997, Fernholm *et al.* 2000), and low (Rosén *et al.* 1993b, Degerblad *et al.* 1995). Similarly, as has been shown in CO GHD patients, there is no difference in BMD between AO GHD patients with isolated and multiple pituitary deficiencies (Holmes *et al.* 1994). PBM is not reached until the second decade of life (Sambrook *et al.* 1993), why GHD acquired in early adulthood may result in reduced BMD (Ohlsson *et al.* 1998). But since reduced BMD is seen also in adults acquiring their GHD after attainment of PBM, it is possible that GH is important also for maintenance of bone mass in older adults (Holmes *et al.* 1994). In healthy adults bone mass decreases continuously with age, and with accelerated speed in postmenopausal women (Looker *et al.* 1995). Aging is associated with decline in GH secretion and IGF-I concentration in serum (Nicholas *et al. 1994*) which has been suggested as a contributing factor in the age related decline in BMD (Rudman 1985)**.** In addition IGF-I has been found to be significantly lower in osteoporotic patients than in non-osteoporotic controls (Wüster *et al.* 1993)**.**

It is well documented that estrogen is essential for bone homeostasis in both men and women (Weitzmann and Pacifici 2006) although the mechanism is not fully understood. The effect of androgen on adult skeleton in men is both direct and indirect through aromatization of androgen to estrogen (Vanderschueren *et al.* 2004). There are both estrogen and androgen receptors on different bone cells in both sexes (Riggs *et al.* 2002).

Both estrogen substitution in hypogonadal adult women and androgen substitution in hypogonadal adult men increase BMD (Hammond *et al.* 1979, Katznelson *et al.* 1996). Androgen substitution in combination with estrogen increased BMD among postmenopausal women more than estrogen alone (Davis *et al.* 1995), but studies of pituitary deficient women are scarce (Miller *et al.* 2002). However, estrogen, when administered orally, impairs the GH-regulated endocrine and metabolic function of the liver, via a first-pass effect (Ho *et al.* 2006) - and perhaps on peripheral levels as well (Jorgensen *et al.* 2005) - and reduces circulating IGF-I. These effects occur in women irrespective of pituitary deficiency and are avoided by transdermal administration of estrogen or through higher GH dosing in GHD women to obtain the same IGF-I level (Mah *et al.* 2005). Testosterone replacement in men has a synergistic effect with GH, increasing the IGF-I production from the liver (Mauras *et al.* 2003).

Thyroxine substitution therapy is not known to have any effect on BMD (Fowler *et al.* 1996), not even in supra-physiological doses (Quan *et al.* 2002).

In patients with ACTH deficiency there may be a risk of over-substitution with cortisol, and as a result decreased BMD; though there are no proofs of that in the literature (Kaufman *et al.* 1992).

GHD and fracture risk

Low BMD serves as a surrogate marker for fracture risk (Melton *et al.* 1993), which is significantly increased if bone density is 1 SD below the age-predicted normal mean-value (Cummings *et al.* 1985, Melton *et al.* 1993). Patients with GHD since childhood have a greater reduction in BMD than those who develop GHD in adulthood (Attanasio *et al.* 1997). In older GHD patients no decline in BMD in comparison to age matched controls have been seen and in addition signs of decreased bone turnover has been noted (Toogood *et al*. 1997), which could reduce the risk of developing osteoporosis and even fracture risk in this group (Garnero *et al*. 1996). As substitution therapy with GH has become available for adult patients with GHD, it is important to assess BMD and fracture risk in these patients during periods with and without GH therapy. It has been shown that GH therapy for 18 or 24 months resulted in a modest increase of BMD in AO GHD men, whereas no significant changes in BMD were observed in AO GHD women (Bex *et al.* 2003). In addition, it has been shown that the treatment response in BMD after 5 years of GH therapy was more pronounced in CO than in AO GHD patients, but did not fully reach the same level as in AO GHD patients (Koranyi *et al.* 2001).

In hypopituitary patients with conventional hormone replacement without GH the fracture risk is not yet definitely settled. Both Wüster *et al.* (1991), Rosen *et al.* (1997), and Wüster *et al.* (2001) have shown increased fracture risk among GHD patients. The first study by Wüster *et al.* is a study on prevalence of osteoporosis and cardiovascular risk factors among 632 patients with pituitary insufficiency, and not on fracture incidence. In the study by Rosén *et al.* 107 AO GHD patients were compared to controls and there were qualifications for incidence calculations but controls were not completely age-matched, as they were collected from another study (WHO MONICA) and questionnaires were not distributed in the same way to patients (at the hospital) and controls (by mail); both important methodological shortcomings that may contribute to uncertain results. Finally in Wüster's next study, a mix of 2024 AO, CO, naïve (never on GH treatment) and non-naïve (not on GH treatment within 6 months prior to the study) patients from KIMS' database were compared to 392 much older controls from the EVOS study base. Only results from patients > 60 years of age could be compared to controls. The results may

thus analyse the effects of ageing itself on fracture risk rather than GHD.

There is no study on fracture incidence in GHD patients on conventional hormone replacement including GH therapy were confounders and effect modifiers also are considered. There is one study though, on fracture prevalence (Mazziotti *et al.* 2006), where vertebral fractures and BMD were assessed in GHD patients with (n=65) and without (n=42) GH replacement and were compared to controls. The prevalence of fractures was higher in GH untreated *versus* GH treated patients, and higher in GHD patients - irrespective of GH replacement - than in controls. The limitations of the study are that there is no knowledge of the relation between when the GHD diagnosis was made and when the fracture occurred, that controls were not matched, that confounders were not considered, that other fractures were not assessed, and finally that AO and CO GHD patients were mixed.

Hypopituitarism and cardiovascular risk

Several epidemiological studies have shown increased cardiovascular mortality in patients with hypopituitarism on conventional hormone treatment, but without GH therapy (Rosen & Bengtsson 1990, Bülow *et al.* 1997, Tomlinson *et al.* 2001). Bülow *et al.* (1997) and Tomlinson *et al.* (2001) showed that the greatest increase in mortality was seen for cerebrovascular disease. There was a more pronounced risk in women compared to men (Bülow *et al.* 1997, Tomlinson *et al.* 2001) but there was no gender difference in cardiac mortality (Bülow *et al.* 1997). In addition, increased incidence of non-fatal cerebral- and cardiovascular events (Svensson *et al.* 2004) was recorded among GHD patients, as well as a higher prescription of cardio protective medication in GHD women (Bülow *et al*. 2000). In none of these studies patients with a history of acromegaly or Cushing's disease were included, as these syndromes are well known to include cardiovascular morbidity and mortality (Scacchi and Cavagnini 2006).

In the study by Svensson *et al.* (2004) GH replacement therapy for a mean duration of 60 months appeared to protect from myocardial infarctions, when serious events were included but the rate of cerebrovascular events tended to increase.

Body composition

Patients with unsubstituted GHD have an increased prevalence of abdominal obesity (De Boer *et al.* 1992, Bengtsson *et al.* 1993) which has shown to benefit from GH therapy (Bengtsson *et al.* 1993, De Boer *et al*. 1996). Glucocorticoid excess is also associated with increased abdomi-

nal obesity (Johnston *et al.*1980). It has been shown that GH has an inhibitory effect on 11 β HSD1, which reduces cortisone to cortisol conversion in liver and adipose tissue (Gelding *et al*. 1998, Tiosano *et al.* 2003); thus this could contribute to adiposity in GHD patients on hydrocortisone replacement therapy. However, GH therapy to GHD subjects has shown to normalise the 11β HSD1 activity (Giavoli *et al.* 2004).

It is well known that abdominal obesity is correlated to increased cardiovascular risk. The reasons are dyslipidemia, insulin resistance, hypertension and proinflammatory abnormalities, all discussed below (Despres *et al.* 2001).

Lipoproteins

Dyslipidemia is a well known feature of GHD and in most studies increased LDL-cholesterol and ApoB levels, normal or increased TG levels, but normal or reduced HDL in comparison to healthy controls have been shown (Rosén *et al*. 1993a, Cuneo *et al.* 1993, De Boer *et al.* 1994, Hew *et al.* 1998). Also hypothyroidism is associated with dyslipidemia (Owen and Lazarus 2003), however, previous studies have shown the opposite in hypopituitary patients *i.e.* rather an over substitution of thyroxine replacement (Bülow *et al*. 2000). It has been suggested that most of the increased cardiovascular risk seen in hypopituitarism is due to dyslipidemia (Rosén *et al.* 1998, Abdu *et al.* 2001). GH replacement leads to decrease in LDL-cholesterol and ApoB levels (DeBoer *et al.* 1996, Cuneo *et al.* 1993, Caroll *et al.* 1998), and these effects have sustained for many years. In one study (Gibney *et al*. 1999) the effect on LDL-cholesterol after 10 years of GH treatment was documented, but whether this improved lipid profile result in less cardiovascular events or deaths in GHD patients is not known.

Glucose tolerance

A high prevalence of impaired glucose tolerance has been recorded in GHD patients (Johansson *et al*. 1995, Markussis *et al.* 1992) and type 2 DM (Beshyah *et al*. 1994) although hyperinsulinemia as it is seen in the metabolic syndrome seem not to be the feature (Johansson *et al.* 1995). GH substitution may further impair glucose tolerance (Sesmilo *et al*. 2000) and even lead to DM in children with GHD (Cutfield *et al*. 2000). This is likely explained by the insulin antagonistic effect of GH (Cutfield *et al.* 2000). Six months of low-dose GH replacement has shown impairment of insulin sensitivity (Weaver *et al.* 1995, Bramnert *et al.* 2003), but it has been suggested that high-dose GH replacement decreases insulin resistance due to a reduction in fat mass (Fowelin *et al.* 1993, O'Neal *et al.* 1994), which is also seen after long time replace-

ment (Svensson *et al.* 2002). The true prevalence of DM among GH treated GHD patients is not known.

Hypertension

Hypertension is an important risk factor for myocardial infarction and stroke in both women and men (Kannel 1989, MacMahon *et al.* 1990), but only one study has reported an increased prevalence of treated hypertension in unsubstituted GHD patients (Rosén *et al.* 1993a). The mechanism of hypertension in GHD patients is not known (McCallum *et al.* 2002). Of interest is, however, that GH treatment has been shown to decrease diastolic blood pressure (Caidahl *et al*. 1994, Feldt-Rassmussen *et al*. 2004, Maison *et al.* 2004),

Other cardiovascular risk markers

IMT of the carotid arteries is an independent predictor of acute myocardial infarction in men (Salonen & Salonen 1993), which has been shown to be increased in GHD patients (Markussis *et al.* 1992, Pfeifer *et al.* 1999). It has also been shown in CO GHD patients (Capaldo *et al.* 1997), but not correlated to dyslipidemia, insulin resistance or hypertension. This suggests that GHD per se is an important factor for atherosclerotic development. IMT has been shown to decrease after 6 months of GH therapy and persist after 24 months (Pfeifer *et al.* 1999).

Inflammatory markers known as cardiovascular risk markers *i.e*. fibrinogen, hs-CRP, PAI-I, tPA , IL-6 (Kannel 2005) , are increased in GHD patients (Johansson *et al.* 1994) and decrease after 18-24 months of GH therapy (Johansson *et al.* 1996, Sesmilio *et al.* 2000) which further contribute to the apprehension that GH is important for the atherosclerotic process.

Gender differences

GHD women on conventional hormone replacement except GH are at higher risk for CVD than men (Bülow *et al.* 1997, Tomlinson *et al.* 2001). In comparison with men, GHD women also have higher fat mass and leptin levels, lower lean body mass (Attanasio *et al.* 1997 and White *et al.* 2003) and higher total cholesterol, LDL cholesterol and HDL cholesterol (Attanasio *et al.* 1997, Murray *et al.* 2002). GHD women need a higher GH dose to normalize their serum IGF-I than do men (Ekman *et al.* 2002). However, men and women show similar changes in body composition and leptin (Drake *et al.* 1998, White *et al.* 2003), but improvements in the lipid profile are found to occur primarily in women (Murray *et al.* 2002) in whom greater abnormali-

ties are found at baseline. Whether there are gender differences in CVD among patients on GH therapy is still not known.

Sex steroids have also been proposed to be a reason for cardiovascular gender differences in GHD patients. Lack of estrogen in post menopausal women is correlated to increase in cardiovascular risk to the same levels as in men (Jousilahti *et al.* 1999), but in large population based studies sex steroid therapy to postmenopausal women has been related to increased cardiovascular risk (Barrett-Connor 2003, Anderson *et al.* 2004). There are currently no studies on the impact of sex steroid substitution in pituitary deficient women on cardiovascular risk. Hypopituitary women with ACTH and sex steroid deficiency are also androgen deficient (Miller *et al.* 2001). Several small studies in women receiving DHEA or testosterone substitution show diverging results for the lipid profile, but almost always decrease of HDL, and in some also decrease of LDL (Lasco *et al.* 2001, Arlt 2006). Whether androgen substitution to DHEA deficient, GHD women, affects cardiovascular morbidity or mortality is not known.

Craniopharyngioma

CP is a benign pituitary tumor with invasive growth and high recurrence rate affecting both children and adults. There are two theories on the origin of the tumor, the first claims that the tumor emerges from Ratkes pouch as a malformation during embryogenesis and the other that it develops as metaplasia within the pituitary (Oskourian *et al.* 2006). It grows invasively towards hypothalamus and the third ventricle. Men and women are equally affected, and about 1-2 persons per million and year are diagnosed. There are two peaks in age distribution, one at 5-10 years and one at 50-60 years (Bunin *et al.* 1998). Approximately 40% of all CPs are seen in patients younger than 16 years (Bunin *et al.* 1998). The treatments are operation with or without CRT, CRT only, and puncture of cyst or installation of drugs or radioactive agents into cysts (Oskourian *et al*. 2006). Due to its growth or treatment, damage to the pituitary, pituitary stalk and hypothalamus is often seen, causing panhypopituitarism and obesity in a majority of patients (De Vile *et al.* 1996, Müller *at al.* 2004).

CP and hypothalamic obesity

At least 50 % of patients with CO CP suffer from obesity after tumor extirpation (Curtis *et al.* 1994, De Vile *et al.* 1996). Obesity often develops within 6 months after surgery (Ahmet *et al.* 2006) and is related to hypothalamic lesions caused by involvement of the tumor and/or surgery

(DeVile *et al. 1996,* Müller *et al.* 2004). A relationship of VMH damage and hypothalamic obesity has been shown in animals (Bray *et al.* 1981). Typical feature of this damage is insulin hyper secretion due to reduced sympathetic nervous system activity and relative enhancement of parasympathetic activity resulting in partitioning of ingested energy substrate into adipose tissue leading to obesity (Jeanrenaud 1985, Bray *et al*. 1981, King 2006). Obese children operated for suprasellar CPs have been shown to have high fasting insulin levels (Lustig *et al.* 2003, Srinivasan *et al.* 2004) even higher than BMI matched controls (Bray and Gallagher 1975), but also high leptin levels in relation to BMI, indicating a disturbed feed-back control of leptin secretion or "leptin resistance" (Roth et al 1998). As a consequence leptin and insulin resistance due to hypothalamic damage would prevent any afferent feedback effect on caloric intake, leading to continued weight gain (Satoh *et al.* 1997). Also ghrelin, which is a brain-gut peptide with growth hormone-releasing and appetite-inducing activities, may be affected (Korbonits *et al.* 2004) as obesity is associated with low serum ghrelin levels and these levels are inhibited by insulin (Saad *et al.* 2002). Children with CP on GH therapy have excellent linear growth, but GH treatment has failed to have an ameliorative effect on weight gain in these patients (Geffner *et al*. 2004). This agrees with the finding that hypothalamic involvement, rather than endocrine deficiencies and hormonal substitution, have a major impact on obesity in patients with CO CPs (Müller *et al.* 2004).

CP and cardiovascular risk

In 1998 Bülow *et al.* found five times higher mortality rate among patients operated for craniopharyngioma than expected, and a three-fold increase in cardio- and cerebrovascular mortality. Tomlinson *et al.* (2001) confirmed this data and even showed higher rate of mortality in a British cohort. Bülow (1998), Tomlinson (2001) and later Pereira (2005), have all shown that the increased mortality is greater in females than males with craniopharyngioma. There are no studies comparing mortality rate in AO CP and CO CP, though Tomlinson *et al.* showed that the younger the patient at diagnosis of a pituitary tumor, the greater the risk of premature mortality. It has to be pointed out that all mortality studies were mostly on patients without GH therapy.

In children operated for a CP, obesity, dyslipidemia, and insulin resistance have been shown (Srinivasan *et al*. 2004). All of the 15 patients in that study were GHD but only 5 were on GH therapy. There are hitherto no studies on cardiovascular risk factors in adults with CO CP on GH therapy.

CP and bone

As previously described, adults with CO GHD, due to primarily hypothalamic-pituitary disease, have reduced BMC and BMD (Kaufman *et al.* 1992, O´Halloran *et al.* 1993, de Boer *et al.* 1994). In CP patients obesity is very common and obesity increase BMD (Felson *et al*. 1993). In addition many CO CP patients grow in spite of GHD (Phillip *et al.* 2002*)* and what impact that might have on bone formation is not known.

There are two studies investigating BMD in patients with CP, and the first one included children and adults with CO CP (Müller *et al.* 2003). In this study vBMD was quantified by pOCT and a significantly lower radial z-score was recorded, which was most obvious in lean male patients. In contrast, female gender and severe obesity seemed to be protective against low vBMD. The other study was on a mixed population of post-surgical patients with CP, pituitary adenomas and other parasellar lesions, with no information on number of GHD patients (Okinaga *et al*. 2005). Six patients had CP, and of them 2 had CO CP. All patients, but in particular CP patients, were at high risk of osteopenia. At present, there are no long term investigations of BMD and BMC in adult CO CP patients on complete hormone replacement including GH.

Aims

I. To compare the incidence of fractures retrospectively, between a large cohort of patients with confirmed GHD on replacement therapy, including GH, and a control cohort from the general population, with adjustment for possible confounders and effect modifiers.

II. To compare the incidence of non-fatal stroke and cardiac events retrospectively, from questionnaires, between a large cohort of patients with confirmed GHD on replacement therapy, including GH, and a control cohort from the general population, and to compare the prevalences of diabetes mellitus and of cardio-protective medication, at the time of distribution of questionnaires, between the cohorts, with adjustment for possible confounders and effect modifiers.

III. To assess the prevalence of cardiovascular morbidity and of cardiovascular risk factors in a group of adult patients with CO CP in comparison with controls, randomly selected from the general population, and matched for age, gender, smoking habits and residence, and, within the group of CP, evaluate the impact of disease related factors on cardiovascular risk factors.

IV. To compare BMD and BMC using DXA between a homogenous group of adults with CO CP and controls randomly selected from the general population, individually matched for sex, age, smoking habits and residence.

V. To closely monitor the blood glucose level and to register the presence of symptoms of hypoglycaemia during the ITT, in a group of patients with a high probability of GHD, consecutively recruited to this test.

Subjects

Patients

Paper I

832 patients with severe GHD by testing (peak GH < 3 µg/L) constituted the patients study base. Some patients had more than one test. The following tests had been used; insulin tolerance test (n=444), arginine and arginine-insulin tolerance test (n=219 and 5), GHRH-arginine (n=34), GHRH tests (n=29), and other (n=94) such as glucagon, clonidine, L-dopa, apomorphine tests and 24-h GH profile (n=115). At least one of the tests described above had been used among 74 patients with a 24-h GH profile, and among the remaining patients (n= 41), only 5 patients had < 2 other pituitary deficiencies (Toogood *et al.* 1994), and their 24-h GH profile showed a maximum GH ≤ 1.7 µg/L. 83% of the patients had a today recommended test (GRS 1998). The criterion for CO GHD was diagnosis <18 years, and for AO GHD diagnosis ≥ 18 years. Age at study and at confirmed GHD are shown in **Table 1**.

Table 1. Number of patients and median and 5th -95th percentiles for age at study and at confirmed growth hormone deficiency (GHD) for men and women with childhood (CO) and adult onset (AO) growth hormone deficiency (GHD).

	CO GHD		AO GHD	
	Women	Men	Women	Men
Number of patients (n)	56	44	389	343
Age at study, median (years)	28 (23-53)	27 (21-48)	57 (31-76)	59 (31-78)
Age at confirmed GHD, median (years)	11(3-15)	14 (3-17)	49 (22-70)	52 (23-72)

The major cause of pituitary deficiencies in AO GHD and CO GHD is shown in **Table 2**. In **Figure 1** pituitary hormone deficiencies and frequencies of different pituitary substitution therapies at end of study period are shown. The cumulative incidence of being, or having been, on GH substitution at the end of follow up was 100% for CO GHD women, 98% for CO GHD men, 91% for AO GHD women and 94% for AO GHD men. The CO GHD women had been on GH-therapy for, in median, 15 years (5th- 95th percentiles 5-34) at end of follow up. The corresponding figures were 12 years (5-21) for CO GHD men, 5 years (0-12) for AO GHD women, and 6 years (0-12) for AO GHD men. The median daily doses at end of study were 0.8 mg (0.4-1.4) in CO GHD women and 0.6 mg (0.2-1.0) in CO GHD men. In AO GHD women doses

were 0.4 mg/day (0.15-0.9), and in men 0.3 mg/day (0.15-0.6). All CO GHD women on sex steroid replacement had oral contraceptives except for 3 on transdermal preparations. In CO GHD women and men, sex steroid substitution was started at median 17 years of age, respectively (8-34, 13-43). In AO GHD patients, 78% of men on sex steroid replacement were on im injections of 250 mg testosterone enanthate, in most cases given every 3rd to 4th week.

Table 2. Etiology of pituitary deficiencies in 399 women and 433 men with GHD divided into CO and AO.

	Childhood onset GHD		Adult onset GHD	
	Women n (%)	Men n (%)	Women n (%)	Men n (%)
Pituitary adenoma	0 (0)	1 (2)	209 [1, 3] (61)	268 [2, 3] (69)
Craniopharyngioma	15 (27)	10 (23)	28 (8)	34 (9)
Cerebral radiotherapy [4]	9 (16)	13 (30)	21 (6)	18 (5)
Idiopathic	20 (36)	12 (27)	26 (8)	22 (6)
Trauma	1 (2)	0 (0)	2 (0)	8 (2)
Other [5]	11 (20)	8 (18)	57 (17)	39 (10)
Total	56	44	343	389

1) 57 % non-functional and 43 % hyper functioning (including prolactinomas), 2)79 % non-functional and 21 % hyper functioning (including prolactinomas), 3) 82% were operated, 49% were treated with conventional CRT, and 5% with stereotactic irradiation, 4) For other reasons than pituitary adenoma such as cerebral tumors or acute lymphatic leukemia, 5) for example Sheehan's syndrome, empty sella syndrome, meningioma, pituitary cyst and hypophysitis.

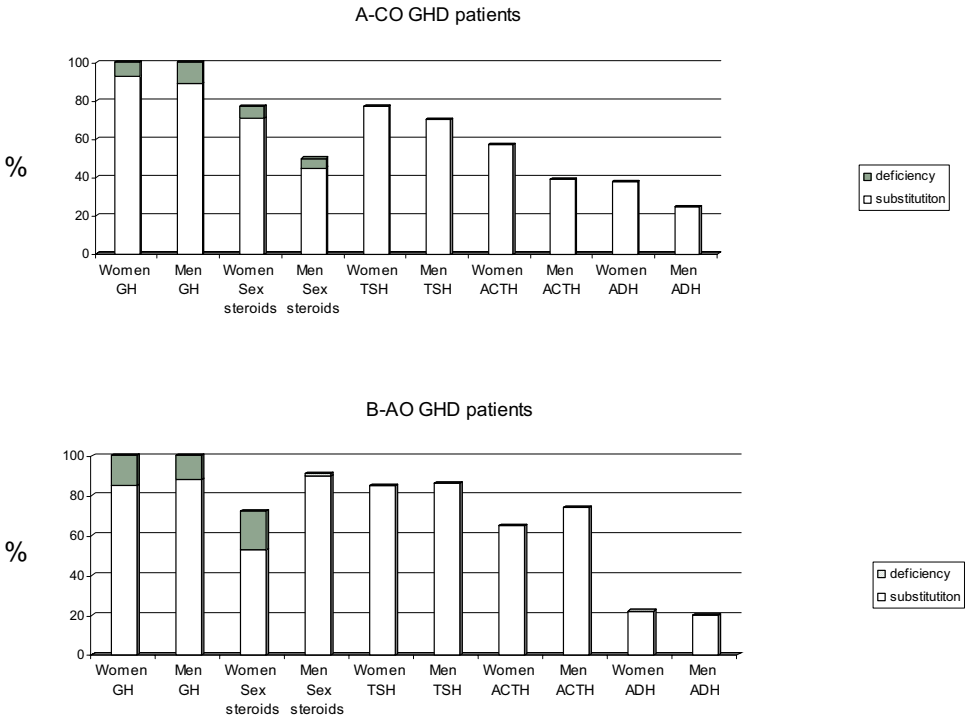


Figure 1.
A. Pituitary hormone deficiencies and substitution therapies at end of study in 56 CO GHD women and 44 CO GHD men.
B. Pituitary hormone deficiencies and substitution therapies at end of study in 343 AO GHD women and 389 AO GHD men.

Potential confounder and effect modifiers for CO GHD patients are shown in **Table 3**, and for AO GHD patients in **Table 4.**

Table 3. Median values (5^{th}-95^{th} percentiles) for characteristics of 56 CO GHD women and 44 CO GHD men and population controls (184 women and 122 men)

		Childhood onset GHD			
		Women		Men	
		Patients	Controls	Patients	Controls
Potential confounders					
Body mass index (kg/m²)		26 (19-38)	22 (18-33)	25 (20-43)	24 (20-30)
Calcium intake from dairy products (mg/day)		428 (0-920)	432 (1-1060)	400 (0-1400)	480 (0-1400)
Never smokers (%)		81	53	81	68
Physical activity at work [1] (%)	1	37	32	32	37
	2	35	24	34	18
	3	26	42	24	34
	4	2	2	10	11
Physical activity, leisure time, winter [1] (%)	1	32	14	30	21
	2	30	36	38	33
	3	37	44	28	35
	4	2	6	5	12
Physical activity, leisure time, summer [1] (%)	1	4	3	11	8
	2	56	50	52	46
	3	38	42	30	36
	4	2	5	7	10
Potential effect modifiers					
Height (cm)		161 (145-176)	167 (154-177)	174 (156-188)	161 (145-176)
Alcohol abstainers (%)		22	10	19	22
Visual problems (%)		24	2	25	24

1) Physical activity evaluated at a four graded scale at work and in leisure time during summer and winter (1: sedentary life or work; 2: light activity in leisure time or soft work; 3: regular physical activity or moderate hard work; 4: hard exercise or hard work)

Table 4. Median values (5^{th}-95^{th} percentiles) for characteristics of 343 AO GHD women and 389 AO GHD men and population controls (1089 women and 1186 men)

		Adult onset GHD			
		Women		Men	
		Patients	Controls	Patients	Controls
Potential confounders					
Body mass index (kg/m²)		26 (20-39)	25 (20-33)	27 (21-34)	26 (21-32)
Calcium intake from dairy products (mg/day)		455 (0-940)	440 (0-1000)	480 (0-1305)	480 (0-1300)
Never smokers (%)		55	51	49	43
Physical activity at work [1] (%)	1	44	32	42	33
	2	32	36	30	32
	3	23	30	23	24
	4	1	1	5	11
Physical activity, leisure time, winter [1] (%)	1	27	13	23	17
	2	46	43	56	54
	3	25	42	21	25
	4	2	2	1	4
Physical activity, leisure time, summer [1] (%)	1	6	4	7	6
	2	59	49	62	60
	3	34	45	28	30
	4	9	2	2	4
Potential effect modifiers					
Height (cm)		164 (151-174)	165 (155-175)	178 (164-191)	178 (167-189)
Alcohol abstainers (%)		21	17	15	11
Visual problems		26	10	31	10

1) Physical activity evaluated at a four graded scale at work and in leisure time during summer and winter (1: sedentary life or work; 2: light activity in leisure time or soft work; 3: regular physical activity or moderate hard work; 4: hard exercise or hard work)

Paper II

The study base constituted of 750 AO patients with severe GHD, diagnosed by testing (peak GH < 3 µg/L), of which 53 % were men and 47 % were women. The median age at the time of the study was 59 years (5^{th}-95^{th} percentile, 31-78) in men and 58 years (31-76) in women. The major cause of pituitary deficiencies was non-functional pituitary adenoma as in paper I. Of the adenomas 31% were hyperfunctioning. The ACTH secreting adenomas constituted 4% of pituitary adenomas in male patients and 15 % in female patients. Of the patients with Cushing's disease, 92 % of men and 84 % of women were judged cured at GHD. The GH secreting adenomas constituted 4 % of pituitary adenomas in men and 8 % in women. Pituitary hormone deficiencies and hormone replacement therapies at end of study period are distributed in the same way as for AO GHD patients in paper I (**Figure 2**).

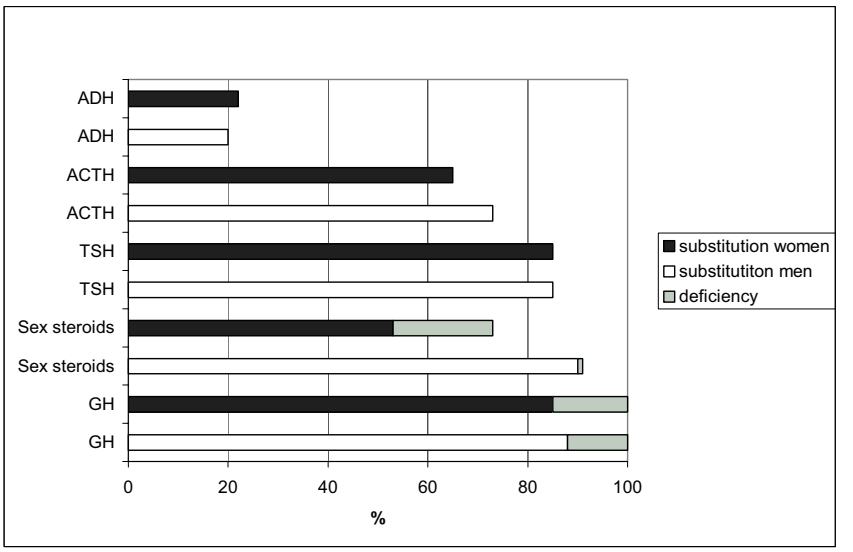


Figure 2. Pituitary hormone deficiencies and substitution therapies at end of study in 399 adult-onset (AO) growth hormone deficient (GHD) men and 351 AO GHD women.

Table 5. Median values (5^{th}-95^{th} percentiles) for characteristics of 399 men and 351 women with confirmed growth hormone deficiency (GHD) and population controls (1174 men and 1103 women).

		Adult onset GHD			
		Men		Women	
		Patients	Controls	Patients	Controls
Matching criterion					
Age at study, median (y)		59(31-78)	60 (31-78)	58 (31-76)	58 (30-75)
Potential confounders					
Body mass index (kg/m²)		27 (22-34)	26 (21-32)	26 (20-38)	25 (20-33)
Never smokers (%)		50	42	55	51
Physical activity at work [1] (%)	1	42	33	44	32
	2	30	33	32	35
	3	23	24	23	31
	4	5	10	1	2
Physical activity, leisure time, winter[1] (%)	1	23	18	26	13
	2	55	54	46	43
	3	21	25	26	42
	4	1	3	2	2
Physical activity, leisure time, summer[1] (%)	1	7	7	6	4
	2	62	59	59	49
	3	29	31	34	45
	4	2	3	9	2
Potential effect modifiers					
High[2] educational level (%)		42	39	43	39
Living alone (%)		24	19	35	26
Alcohol abstainers (%)		15	11	21	17

1) Four graded scale: 1: lowest grade of activity; 4: highest grade of activity. 2) Secondary school (Eng)/High school (Am) or University level.

The cumulative incidence of being, or having been, on GH during GHD was 92 % for women and 94% for men. At the end of follow up women and men had been on GH-therapy for in median 6 years each (5^{th}-95^{th} percentile 1-12 years in women and 1-13 years in men). Information on dosing of GH at end of study was available in 96 % of the patients on GH therapy. The median doses were 0.4 mg/day in women (0.15-0.9) and 0.3 mg/day in men (0.13-0.6) at end of study. Characteristics for the patients are shown in **Table 5**.

Papers III and IV

Forty-two patients (20 women) operated for a CP in childhood (< 20 years) during 1958 to 2000 and at least 18 years at the time of the investigation were included in the study. Three men were excluded due to weight exceeding the upper limit (≥ 135 Kg) for DXA measurements in paper IV (nr 9, 14 and 17 in **Table 6)**.

Table 6. Characteristics and treatment modalities in 22 men operated in childhood for a craniopharyngioma

#	Gender	Age at investigation	Age at op	Tumor growth	Radicality 1:st/2:nd/3:rd op, yes/no	Treatment	(CRT) Gy	Op #	Hormone substitutions
1	M	57	17	3	No	S+CRT	45/In/In	1	GH/G/T/C
2	M	56	18	3	Yes	S		1	GH/G/T/C
3	M	46	18	3	Yes	S+CRT	56	1	GH/G/T/C
4	M	42	9	3	Yes	S		1	GH/G/T/C/ADH
5	M	38	12	3	Yes	S		1	GH/G/T/C/ADH
6	M	36	5	2	No/Yes	S		2	GH/G/T/C/ADH
7	M	36	15	2	Yes	S		1	GH/G/T/C/ADH
8	M	35	8	2	Yes	S		1	GH/G/T/C/ADH
9	M	34	11	3	Yes	S+CRT	50	1	GH/G/T/C/ADH
10	M	33	9	3	No/No	S+CRT	40	2	G/T/C/ADH
11	M	30	17	2	No	S		1	ADH
12	M	28	16	3	Yes	S+CRT	55	1	GH/G/T/C/ADH
13	M	28	3	2	Yes	S		1	GH/G/T/C/ADH
14	M	28	8	3	Yes/No	S+CRT	52	2	T/C/ADH
15	M	26	14	2	No	S		1	GH/G/T/C/ADH
16	M	26	14	2	Yes	S		1	GH/G/T/ADH
17	M	25	16	3	Yes	S+CRT	54	1	GH/G/T/C/ADH
18	M	25	6	3	Yes/No	S+CRT	In/40/50.4	2	GH/G/T/C/ADH
19	M	23	22	3	No	S+CRT	54	1	GH/G/T/C/
20	M	19	6	3	No/No/No	S+CRT	50.6	3	GH/G/T/C/ADH
21	M	17	7	3	No	S+CRT	50	1	GH/G/T/C/ADH
22	M	17	4	2	Yes	S+CRT	54	1	GH/G/T/C/ADH

M: male; 1: Intrasellar growth; 2: Suprasellar growth; 3: Suprasellar growth towards the third ventricle; S: surgery; CRT: cranial radiotherapy; In: installation (Yttrium); GH:growth hormone; G: sex steroids; T: levothyroxine; C: Cortisone; ADH: antidiuretic hormone.

Number of patients on different hormone substitutions is shown in **Table 7**. All but 3 women and 3 men were on GH treatment (**Tables 6, 7** and **8**), of which two men had previously both been on GH treatment for six years, but not since 1, respective 8 years, back in time. The remaining 4 were not GHD at testing. CP women had had GH therapy for in median 10 years (0-19) and CP men for 12 years (0-33). The daily GH doses were 0.8 mg (0.4-1.6) in women and 0.5 mg (0.2-1.0) in men. Time since diagnosis of first pituitary deficiency was in median 18.5 years (3-40) in women and 21 years (0-40) in men. Seventeen women were on sex steroid replacement since 13 years (6-46) of age, mostly as combination therapies (estrogens and gestagens, n=14). Twenty men were on testosterone replacement since 13 years (3-45) of age, as intramuscular injection every 3rd to 4th week (17), transdermal (2) or oral (1) therapy.

Table 7. Hormone substitution in 20 CP women and 22 CP men at time of investigation.

	Women n (%)	Men n (%)
GH substitution	17 (85)	19 (86)
Sex steroid substitution	17 (85)	20 (91)
Thyroid substitution	18 (90)	21 (95)
Cortisol substitution	16 (80)	20 (91)
Vasopressin substitution	17 (85)	17 (77)

Table 8. Characteristics and treatment modalities in 20 women operated in childhood for a craniopharyngioma.

#	Gender	Age at invest-igation	Age at op	Tumor growth	Radicality 1:st/2:nd/3:rd op, yes/no	Treatment	(CRT) Gy	Op #	Hormone substitutions
23	F	57	18	3	Yes/Yes/No	S+CRT	55	3	GH/G/T/C/ADH
24	F	46	7	3	No/No/Yes	S		3	GH/G/T/C/ADH
25	F	46	7	3	Yes/No	S+CRT	35.0	2	GH/G/T/C
26	F	41	12	2	Yes	S		1	C/ADH
27	F	40	3	3	Yes	S		1	GH/G/T/C/ADH
28	F	37	12	2	Yes	S		1	T/ADH
29	F	32	12	3	Yes	S		1	GH/G/T/ADH
30	F	31	11	1	Yes	S		1	GH/T/C/ADH
31	F	28	20	2	No	S+CRT	54	1	GH/G/T/C
32	F	28	9	2	Yes	S		1	GH/G/T/C/ADH
33	F	29	20	3	Yes	S		1	G/T
34	F	27	14	1	Yes	S		1	GH/G/T/C/ADH
35	F	24	15	2	Yes	S		1	GH/G/ADH
36	F	23	3	3	Yes/No	S+CRT	In/50	2	GH/G/T/C/ADH
37	F	23	6	3	No/No	S+CRT	58.6	2	GH/G/T/C/ADH
38	F	22	15	3	No	S+CRT	55	1	GH/G/T/C/ADH
39	F	22	10	2	Yes	S		1	GH/G/T/C/ADH
40	F	20	17	1	Yes	S		1	GH/G/T/C/ADH
41	F	18	13	3	Yes	S		1	GH/G/T/C/ADH
42	F	19	4	3	Yes/No/Yes	S+CRT	50	3	GH/G/T/C/ADH

F: female; 1: Intrasellar, 2: Suprasellar and 3: Suprasellar growth towards the third ventricle; S: surgery, CRT: cranial radiotherapy; In: installation (Yttrium) GH: growth hormone, G: sex steroids, T: levothyroxine; C: Cortisone, ADH: antidiuretic hormone

Paper V

Sixteen patients (9 men and 7 women) were consecutively recruited due to a high probability of GHD (**Table 9**). The median age of the examined group was 39 years (range 22-59). Detailed information on background diagnosis and pituitary deficiencies are given in **Table 9**.

Table 9. Detailed information on 16 patients with GHD (GH < 3 µg/l) investigated with an ITT.

Pt no.	Gender	Age (years)	BMI (kg/m2)	Diagnosis	Radio- therapy	Other pituitary deficiency at investigation
1	M	25	20·0	Epipharynx cancer	Yes	TSH
2	M	54	24·3	Nonfunctioning pituitary adenoma	Yes	Panhypopituitary
3	F	51	28·7	Craniopharyngioma	Yes	Panhypopituitary
4	M	39	25·4	Idiopathic	No	Panhypopituitary
5	F	21	22·2	Nonfunctioning pituitary adenoma	No	Panhypopituitary
6	F	53	28·0	Prolactinoma	Yes	Panhypopituitary
7	M	53	23·8	Nonfunctioning pituitary adenoma	Yes	FSH, LH
8	M	59	25·8	Nonfunctioning pituitary adenoma	Yes	TSH, ACTH
9	M	47	27·6	Empty sella	No	No
10	F	22	25·6	ALL-irradiation therapy	Yes	No
11	M	25	25·7	ALL-irradiation therapy	Yes	No
12	M	22	20·1	Idiopathic	No	No
13	F	39	28·2	Nonfunctioning pituitary adenoma	No	TSH
14	F	30	33·7	Nonfunctioning pituitary adenoma	Yes	TSH, FSH, LH
15	M	45	24·7	Nonfunctioning pituitary adenoma	No	Panhypopituitary
16	F	51	24·0	Acromegaly (cured)	Yes	Panhypopituitary

F, female; M, male; BMI, body mass index; ALL, acute lymphoblastic leukemia

Controls

Paper I and II

Statistics Sweden (Sweden's national statistics agency) selected four controls each from the Swedish population register for all patients. The controls were successfully matched for age, gender, county of current residence, and country of birth, but else they were randomly selected. Seventy eight percents were willing to participate. In **paper I** the final control cohort constituted of 2581 subjects. The participation rate was somewhat higher among women (n= 1273, 77%) than among men (n=1308, 73%). The median age was 58 years (5^{th} -95^{th} percentile 26-77) in males and 55 years (25-74) in females. **In paper II** the final cohort constituted of 2314 subjects.

Also here the participation rate was somewhat higher among women (n=1104, 79 %) than among men (n=1210, 76 %). The median age was 60 years (5^{th} -95^{th} percentile 31-78) for men and 58 years (30-75) for women. Potential confounders and effect modifiers for controls in **paper I** are shown in **Tables 3** and **4**. The characteristics for controls in **paper II** are shown in **Table 5**.

Paper III and IV

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, gender and residence (rural/non rural) were selected randomly from a computerized register of the population in the catchments area of the patients (Southern Swedish Medical Region). Potential controls were contacted by telephone and were then also matched for smoking. 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.

Ethical aspects

The studies were approved by the Ethics committee of Lund University and in study I and II also by the Ethics committees of the universities of Göteborg, Stockholm, Uppsala, Linköping and Umeå.

Methods

Paper I

Identical questionnaires were sent by mail to all patients and controls. The questions addressed background information, such as intake of dairy products, consumption of alcohol, smoking history, physical activity, country of birth, body-weight and height. The key question concerned history of fractures, specified for age, fracture site and whether it was caused by a trauma (traffic or ski-accident) or not. Three follow-up periods were defined (**Figure 3 A and B**); 1) from calendar-year of confirmed GHD until calendar-year of first fracture thereafter, or end of follow up at December 31, 2002 (GHD-period); 2) from start of first confirmed pituitary hormone deficiency (FHD) until calendar-year of confirmed GHD (FHD-GHD-period); 3) from FHD to first fracture or end of follow up at December 31, 2002 (FHD-period). The same follow-up periods were applied for the matched population controls and incidences for first fracture were calculated. The FHD-GHD period was very short (median 0 year) in CO GHD patients and the person year under risk to small to allow incidence calculations.

A

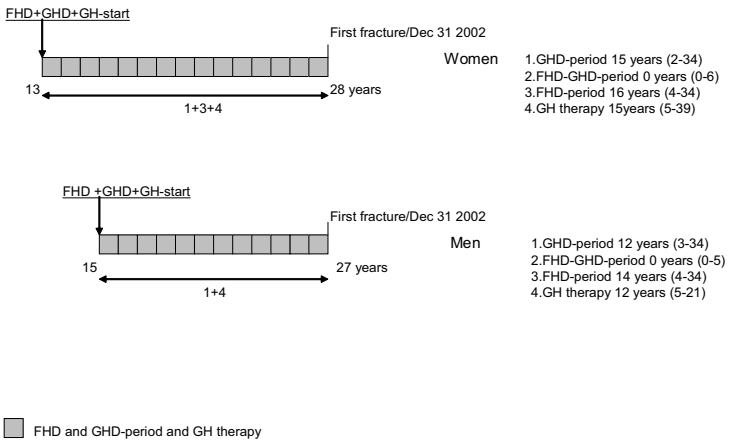


Figure 3A The median length of the different follow-up periods and GH therapy in CO GHD women and men, 5th-95th percentiles within brackets.

B

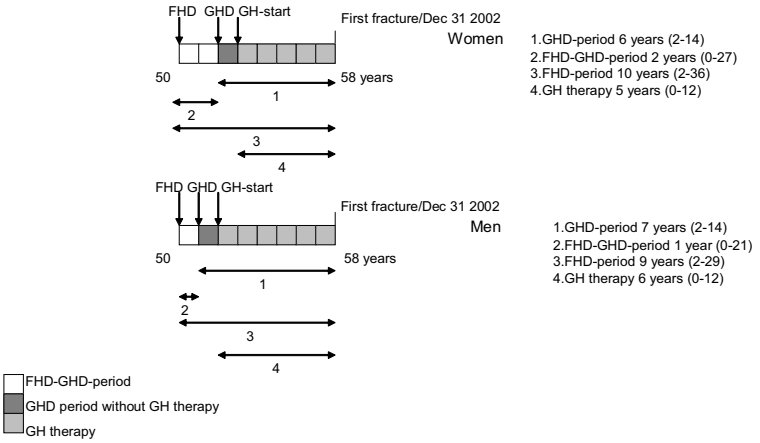


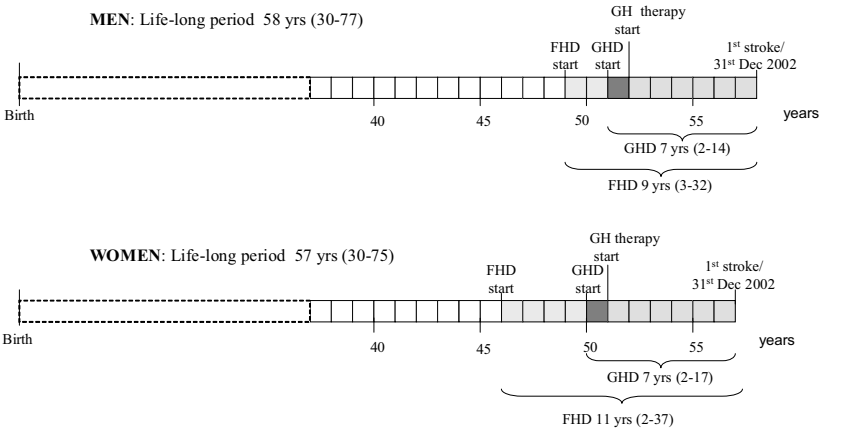
Figure 3B. The median length of the different follow-up periods and GH therapy in AO GHD women and men, 5th-95th percentiles within brackets.

Paper II

Also here the same questionnaire was used for both the patient and the control cohorts. The questions addressed background information such as current consumption of alcohol, smoking history, country of birth, educational level and residency, body-weight and height. Present diabetes (treated with diet, oral medication or insulin) and use of antihypertensive, lipid lowering or anti-thrombotic drugs were asked for. The subjects were also asked whether they had had any cardiovascular event or procedure (stroke, myocardial infarction [MI], percutaneous transluminal coronary angioplasty [PTCA], or by-pass surgery), and if so, which calendar-year it had occurred. For incidence calculations two outcome measures were used 1) *non-fatal stroke*, and 2) *non-fatal cardiac disease* (MI, PTCA or by-pass surgery). Three follow-up periods were defined (**Figure4**) for the incidence studies 1) life-long follow up from birth until calendar-year of first cardiovascular event or until end of follow up at December 31, 2002 (Life-long period); 2) from calendar-year of first confirmed pituitary hormone deficiency (FHD) to first cardiovascular

event or end of study (FHD-period); 3) from calendar-year of confirmed GHD until calendar-year of first cardiovascular event or end of follow up at December 31, 2002 (GHD-period). Independent analyses were made for stroke and cardiac disease. The same follow-up periods were applied for the matched population control cohort. Incidences of first non-fatal stroke and non-fatal cardiac disease were estimated retrospectively for all follow-up periods. Prevalences of diabetes, antihypertensive, lipid lowering and anti-thrombotic medication at the time of distribution of questionnaires were also calculated.

Figure 4



Definitions of the follow-up periods:
Life-long:-period: From birth to 1st stroke/31st Dec 2002
FHD- period: From year of first confirmed pituitary deficiency to 1st stroke/31st Dec 2002
GHD-period: From year of confirmed GH deficiency to 1st stroke/31st Dec 2002

Figure 4 Time table illustrating the length (years) of the follow-up periods for stroke (lifelong, FHD and GHD) for median man and median woman. The figures within brackets are the 5th and 95th percentiles for the whole groups. Corresponding analyses were performed for cardiac events.

Paper III and IV

Paper III

The prevalence of cardiovascular morbidity and of cardiovascular risk factors in patients with CO CP in comparison to matched controls was assessed. Each patient and control was investigated during one day.

Anthropometric measurements were performed, blood pressure taken and blood samples for biochemical assays drawn. Degree of physical activity was evaluated.

Paper IV

BMD and BMC, assessed with dual-energy X-ray absorptiometry, DXA (Lunar Expert XL and Lunar Prodigy, Lunar Madison, USA), of femoral neck, L2-L4 and total body were compared between patients and controls. Biochemical assays were also compared and anthropometric measurements performed. Degree of physical activity was evaluated.

Anthropometric measurement in study III and IV

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) in **study III**. Body weight was measured after overnight fast and body height was measured barefoot. Body mass index (BMI) was calculated as body weight (kg) divided by height (meters) squared. Scinfold (Harpenden Skin fold Caliper) was measured in the abdominal subcutaneous fat lateral to the umbilicus, used in **study III**. The mean value of 4 measurements was used. Body composition was measured in the supine position by bioelectric impedance analysis using the BIA 101-S technique (RJL-Systems, Detroit, MI, USA), with a 50-KHz, 800-uA current. Data are expressed as kg of fat and muscle, respectively.

Criteria for metabolic syndrome, and cardiovascular risk prediction in study III

The International Diabetes federation definition of the metabolic syndrome (Alberti *et al*. 2006), consider central obesity as a key issue, together with at least 2 of 4 possible risk factors (raised TG, reduced HDL-cholesterol, raised blood pressure, raised fasting plasma glucose) was used in study III. Risk prediction of cardiovascular disease (CVD) with hs-CRP and LDL-cholesterol were also used in study III (de Ferranti *et al.* 2007).

Biochemical assays in study III and IV

All blood samples were drawn in the morning, after fasting since midnight.

Hormones

Serum IGF-I was measured by and immunoradiometric assay (Nichols Institute of Diagnostics, San Juan Capistrano, CA, USA). The intra-assay CVs were 16% and 11% at 60 μg/L and 300 μg/L, respectively.

Plasma FSH and LH were analysed with an electro-chemiluminescence technique (Roche, Elecsys, Mannheim, Germany). Serum testosterone, plasma estradiol and serum SHBG levels were measured by commercially available immunoassays. Serum DHEAS levels were determined by a commercially available competitive immunoassay (DPC, Diagnostic Products Corp., CA, USA)

Plasma TSH, free T4, free T3 and cortisol were analysed with an immunofluorometric technique (Auto Delfia, Wallac, Oy, Tutku, Finland). The intra-assay CVs for plasma free T4 and plasma free T3 were <4.1% and 8.0%, respectively, and inter-assay CVs were 4.1% and 8%, respectively.

Plasma PRL was analysed using a reagent from Roche (Roche Modular Analytics E170). The reference range for plasma PRL in women was 4-27 μg/L and for men 4-24 μg/L. Interassay CVs were 3.2 % at 10 μg/L and 3.1% at 46 μg/L.

Blood-glucose, insulin, c-peptide, leptin and lipids

Venous plasma glucose was calculated in **study III** using a Hemocue Blood Glucose Analyser (Hemocue AB, Ängelholm, Sweden). According to the manufacturer, the standard deviation between the cuvettes is <0.3 mmol/L. Serum insulin was measured with a competitive radioimmunoassay, with intra-assay CVs, at low, medium and high levels, of less than 5.2%. Serum C-peptide in **study III** was analysed with an immunoluminometric method using monoclonal mouse antibodies (Liaison C-Peptide, DiaSorin, Saluggia, Italy). The intra-assay CVs were 4.7 % at 0.69 and 2.5 % at 2.5 nmol/L. Serum Leptin in **study III** was measured by RIA (Linco, St Charles, USA) with the limit of detection at 0.5 ng/ml. Within-assay CV is between 4.6 and 6%. Interasssay variations are 6.8-9.5%. All Leptin assays were run in duplicate. Fasting plasma total

cholesterol, LDL- and HDL-cholesterol, ApoA-1, ApoB, hs-CRP, TGs and plasma fibrinogen levels in **study III** were measured by standard procedures. ApoB/ApoA-1 ratio was calculated.

Blood pressure in study III

Blood pressure was measured in the right arm in the supine position after 10 minutes rest. The mean value of two measurements was used. Electrocardiogram was performed in resting position in the morning.

Physical activity in study III and IV

Physical activity was assessed in three different ways:

1. The degree of physical exercise during leisure time and working time was assessed by a self-rating questionnaire and classified into a four-grade scale (Wilhelmsen *et al.* 1976). The students graded their activity at school as activity at work. Patient-control-pairs, where one or both did not work, were not participating in the estimation of physical activity at work.

2. Patients and controls were interviewed by a dietician, and asked to describe their daily activity pattern. Activities were classified as "active" (standing, walking, house keeping, sports) or "inactive" (sleeping, lying down, reading, sitting, watching TV), and summarized into 15 minutes intervals (i.e. a total of 96 intervals occurred during 24 hours).

3. All participants wore a pedometer for three days, and this is a method to evaluate average physical activity (Tudor-Locke et al 2005) and it has been shown that 3 days of registration is enough to evaluate average physical activity. Patients and controls wore their pedometer on the same weekdays, and the sum of all steps was recorded.

Paper V

An ITT was performed in all patients as a 90-minutes-test and serum GH was analyzed at -15, 0, 15, 30, 45, 60, 75 and 90 min. At 0 min insulin (Actrapid; Novo Nordisk, Gentofte, Denmark) was administered as an iv bolus of 0.1 IU/kg. The dose was often adjusted (reduced by 10-40 %) if concomitant pituitary deficiency (especially ACTH deficiency) was present (**Table 21**). Blood glucose was analyzed from the forearm vein and was monitored at –15, 0, 10, 15, 20, 25, 30 and 35 min and then every 5 to 10 min up to 90 min. At a blood glucose level around 2.2 mmol/l more frequent blood glucose testing was performed (every other minute).

During this period the patient was interviewed for symptoms of warmness, dizziness, tiredness, palpitations, and whether blurred vision appeared. Furthermore, their pulse, tendency of sweating and paleness was registered during the hypoglycemic phase and when the blood glucose level was below 2.0 mmol/l oral fructose or juice was given. At blood glucose levels around 1.3 mmol/l, and if the hypoglycaemia was prolonged, an iv bolus infusion of glucose (30%) was administered.

Venous blood glucose was analysed with Hemocue Blood Glucose Analyser (Hemocue AB, Ängelholm, Sweden). According to the manufacturer, the standard deviation between the cuvettes is < 0.3 mmol/L. Serum GH was analysed by an immunofluoromethric method, DELFIA hGH (Wallac Oy, Turku, Finland). The detection level for serum GH was 0.01 µg/L and the intra- and inter-assay CV was 5 % and 3 %, respectively, at a level of 1.5 µg/l and at a level of 7.7 µg/l the intra- inter-assay CVs were 3 % and 5 %, respectively. The recommendations for cut-off values for the ITT are based on the results obtained with polyclonal competitive RIAs calibrated against the pituitary derived preparation International Reference Preparation (IRP) 80/505 (1 mg = 2.6 µg/l) (Growth research Society, 1998).

Statistics

Paper I and II

For comparisons between the GHD patients and the population controls (cohort affiliation), the IRR and 95% CI for first fracture, first stroke or first cardiac disease (MI or PTCA or by-pass surgery) were estimated retrospectively with Poisson regression models using EGRET software (Statistics and Epidemiology Research Corporation, Seattle, WA, USA). A lower limit of the 95% CI above unity is interpreted as a significantly increased incidence. Likewise, an upper limit of the 95% CI below unity is interpreted as a significantly decreased incidence. In order to assess for possible effect modification separate analyses were performed for men and women and in **paper I** also for patients with AO GHD or CO GHD. In **paper II** the prevalences of diabetes mellitus, use of antihypertensive, lipid lowering or anti-thrombotic drugs were also compared between the patient and the control cohorts calculating POR. Smoking habits (ever/never), BMI (<20, 20-30 and >30 kg/m^2), physical activity (low or high) and in **paper I** calcium intake (<250, 250-1000 and >1000 mg/day based on intake of dairy products) were considered as potential confounders. They were included in the model, one at a time, together with cohort affiliation, and were kept in the model, if they changed the crude effect estimate with more than 15%. In a second estimation of the above described incidence and prevalence calculations in **paper II**, patients with acromegaly and Cushing's disease were excluded.

Paper III and IV

Data were presented as median and range. Patients and controls were compared with the Wilcoxon signed rank test for matched pairs. Bivariate correlations were assessed using the Spearman rank correlation coefficient. Comparison of binary outturns between patients and controls were calculated using McNemar's test. In order to assess differences due to tumor growth in **study III** we used Mann-Whitney U-test with two independent groups. Multiple linear regressions were calculated; in **study III**, with BMI included in the model for insulin, ldl, hdl and apoB/apoA1 ratio and in **study IV** with fat mass included in the model, for BMD, BMC, and *z*-scores. We regarded $p<0.05$ as statistically significant.

Paper V
Median and ranges were expressed.

Results

Paper I

A non-significant overall risk increase for fractures was seen during the GHD-period in women, based on 62 fractures, but the risk was confined to CO GHD women, who had a more than doubled risk (IRR 2.29, 95 % CI 1.23-4.28), whereas no risk increase (IRR 1.08) was observed among AO GHD women (**Table 10**). Also the FHD period was associated with increased fracture risk among CO GHD women (IRR 2.23 95% CI 1.20-4.14), whereas it was not meaningful to evaluate the risk for the FHD-GHD period, due to too few person years under risk. For AO GHD women there was no risk increase for the FHD or the FHD-GHD period (IRR 1.11 and 1.45 respectively, 95% CI 0.84-1.47 and 0.96-2.20 respectively) (**Table 10**). None of the potential confounders qualified for being included in the final models.

Table 10. Fracture incidence among 399 women with growth hormone deficiency (GHD) in comparison with matched population controls and with respect to childhood (< 18years) or adulthood onset of (≥ 18 years), and with different observation periods.

	GHD patients		**Population**			
	Cases	PY [1]	Cases	PY [1]	IRR	95% CI
GHD period						
All	62	3 282	163	11 032	1.28	(0.95, 1.71)
CO GHD	16	913	26	3 403	2.29	(1.23, 4.28)
AO GHD	46	2 369	137	7 629	1.08	(0.77, 1.51)
FHD-GHD period						
AO GHD	32	2 205	73	7 309	1.45	(0.96, 2.20)
FHD period						
All	81	5 282	221	17 966	1.24	(0.97, 1.61)
CO GHD	16	948	27	3 566	2.23	(1.20, 4.14)
AO GHD	65	4 334	194	14 400	1.11	(0.84, 1.47)

1) Person year under risk

Male GHD patients showed a totally different pattern (**Table 11**). Totally 29 fractures were observed among the male patients during the GHD-period, corresponding to a significantly decreased fracture incidence as compared with the matched population controls (IRR 0.56, 95 % CI 0.38-0.83; **Table 11**). The IRR point estimates were similar for CO GHD and AO GHD men, but the incidence was significantly decreased in AO GHD men only. Considering the maximum length observation period (FHD-period), the pattern was similar and the risk was again significantly lower for the AO GHD men but not for CO GHD men. For the time period between year of start of FHD-period and year of start of GHD-period (FHD-GHD period), there was a pattern of decreased fracture risk for the AO GHD male patients, but for the CO GHD, the low number

did not allow a meaningful evaluation (**Table 11**). None of the potential confounders qualified for being included in the final models.

Table 11. Fracture incidence among 433 men with growth hormone deficiency (GHD) in comparison with matched population controls and with respect to childhood (< 18years) or adulthood onset of (≥ 18 years), and with different observation periods.

	GHD patients		Population			
	Cases	PY [1]	Cases	PY [1]	IRR	95% CI
GHD period						
All	29	3 547	151	10 348	0.56	(0.38, 0.83)
CO GHD	8	694	35	1 865	0.61	(0.28, 1.32)
AO GHD	21	2 853	116	8 483	0.54	(0.34, 0.86)
FHD-GHD period						
AO GHD	16	1 859	58	5 188	0.77	(0.44, 1.34)
FHD period						
All	45	5 248	201	15 062	0.64	(0.47, 0.89)
CO GHD	9	711	35	1 964	0.71	(0.34, 1.48)
AO GHD	36	4 537	166	13 098	0.63	(0.44, 0.90)

1) Person year under risk

The fraction of traumatic (traffic or ski-accident) fractures did not differ significantly between GHD patients and population controls, irrespective of gender and age of GHD onset (all p-values >0.3). There were no differences in percentage osteoporotic fractures (vertebra, wrist, upper arm, and hip) neither between CO GHD women and controls, nor between AO GHD men and controls (all p-values >0.3) (**Table 12**).

Table 12. Distribution of type of first fractures in childhood onset (CO) and adult onset (AO) growth hormone deficient (GHD) patients and in men and women from the comparable control population and trauma as cause of fractures.

	CO GHD women		CO GHD men		AO GHD women		AO GHD men	
	Patients n (%)	Control population n (%)	Patients n (%)	Control population n (%)	Patients n (%)	Control population n (%)	Patients n (%)	Control population n (%)
Total number of patients	56	184	44	122	343	1089	389	1186
Vertebra	1 (6)	1 (4)	0	0	1 (2)	6 (4)	0	7 (6)
Wrist	4 (25)	7 (27)	1 (12.5)	7 (20)	8 (17.5)	44 (32)	4 (19)	22 (19)
Upper arm	1 (6)	2 (7.5)	0	0	2 (4.5)	12 (9)	2 (9.5)	5 (4)
Hip	0	0	0	0	2 (4.5)	6 (4)	1 (5)	2 (2)
Tibia	1 (6)	2 (7.5)	0	1 (3)	1 (2)	4 (3)	0	6 (5)
Foot	0 (0)	4 (15)	0	6 (17)	10 (22)	24 (18)	6 (28.5)	10 (9)
Rib	4 (25)	2 (8)	0	5 (14)	7 (15)	15 (11)	3 (14)	44 (38)
Clavicle	0	3 (12)	1 (12.5)	4 (11.5)	2 (4.5)	2 (1)	0	3 (2.5)
Others	5 (31)	5 (19)	6 (75)	12 (34.5)	13 (28)	24 (18)	5 (24)	17 (14.5)
Total number of fractures	16 (100)	26 (100)	8 (100)	35 (100)	46 ((100)	137 (100)	21 (100)	116 (100)
Osteoporotic fractures [1]	6 (37)	10 (39)	1 (12.5)	7 (20)	13 (28)	68 (50)	7 (33.5)	36 (31)
Traumatic [2]	5 (31)	12 (46)	2 (25)	18 (51)	11 (24)	33 (24)	4 (19)	27 (23)

1) Vertebra, wrist, upper arm, and hip, 2) Traffic or ski-accident as cause of fracture.

Paper II

The incidence of non-fatal stroke and cardiac events

The retrospective life-long incidence of non-fatal stroke was three times higher in female patients as compared to controls, whereas it was doubled in men compared to controls (**Table 13a**). Near doubled IRRs were observed for non-fatal stroke among both genders during the FHD-period, but the IRRs did not differ significantly from unity. For the GHD-period a non-significant increase of non-fatal stroke was observed for the male, but not for female patients. However, the latter analysis was hampered by the very few cases.

Table 13a. IRR calculated from Poisson regression models for non-fatal stroke among AO GHD patients as compared with age and gender matched control cohort subjects.

	AO-GHD patients		**Control cohort**		**AO-GHD *vs.* Control cohort**	
Disease/Follow up period/Gender	Cases	PY[a]	Cases	PY[a]	IRR	95% CI
Stroke						
Life-long incidence						
Men	29	21949	51	69918	1.81	1.15-2.86
Women	22	19036	24	61116	2.94	1.65-5.25
FHD-period[b]						
Men	18	4727	33	14693	1.70	0.95-3.01
Women	11	5001	18	16185	1.98	0.93-4.19
GHD-period[c]						
Men	13	2857	26	9034	1.58	0.81-3.08
Women	4	2630	13	8331	0.97	0.32-2.99

a) Person-years under observation, b) From year of first hormone deficiency to first stroke/ or dec 31, 2002, c) From year of diagnosis of growth hormone deficiency to first stroke or dec 31, 2002.

The incidence of non-fatal cardiac event was slightly below unity for both male and female patients (**Table 13b**). During the FHD-period, the incidence in male patients was significantly reduced to about half of the incidence in controls, whereas the IRR in women did not differ from unity. The same pattern was seen during the GHD-period. However, the analyses for females were hampered by the very few observed and expected cases.

When patients with acromegaly and Cushing's disease were excluded the results did not change.

Table 13b. IRR calculated from Poisson regression models for cardiac events among AO GHD patients as compared with age and gender matched control cohort subjects

	AO-GHD patients		Control cohort		AO-GHD *vs.* Control cohort	
Disease/Follow up period/Gender	Cases	PY[a]	Cases	PY[a]	IRR	95% CI
Cardiac events[d]						
Life-long incidence						
Men	25	21931	96	68171	0.81	0.52-1.26
Women	7	19323	24	60722	0.92	0.39-2.13
FHD-period[b]						
Men	11	4826	69	14366	0.47	0.25-0.90
Women	6	5063	19	16132	1.01	0.40-2.52
GHD-period[c]						
Men	9	2873	59	8771	0.47	0.23-0.90
Women	4	2638	15	8274	0.83	0.28-2.52

a) Person-years under observation, b) From year of first hormone deficiency to first cardiac disease or dec 31, 2002, c) From year of diagnosis of growth hormone deficiency to first cardiac disease or dec 31, 2002, d) Comprises myocardial infarction, by-pass surgery and PTCA.

The prevalence of DM and use of cardio protective drugs

Even after confounder adjustment the prevalence for DM among GHD women was twice that of the population controls, but no increased prevalence was seen in GHD men (**Table 14**). GHD women and controls had diet (25% *vs*. 27%), oral medication (21% *vs*. 19%), combination of oral medication and insulin (18% *vs.* 16%), or insulin treatment (36% *vs.* 38%) for their DM to the same extent, which also GHD men had. When patients with acromegaly and Cushing's disease were excluded the prevalence for DM among female patients was higher than in controls (POR 2.02 (1.17-3.49), P=0.01), but after confounder adjustment the POR was no longer statistically significant (POR 1.57 (0.87-2.84), P=0.13).

GHD women had twice as often lipid lowering drugs as the population controls, but there was no difference in men (**Table 14**). Among the male patients, but not the female patients, 28% increased use of antihypertensive drugs was observed. When smoking was included in the model the increase was statistically significant (POR 1.33, 95% CI 1.01-1.75, P=0.04, not in Table). The use of antithrombotic drugs was not increased among the female patients, whereas a borderline significant increase was seen in the male patients. When patients with acromegaly and Cushing's disease were excluded, only minor changes of the prevalences occurred.

Table 14. Crude and confounder adjusted Prevalence Odds Ratios (POR) for diabetes mellitus, the use of antihypertensive drugs, lipid lowering drugs and antithrombotic drugs among men and women with AO GHD as compared with the control cohort.

	Prevalences		Crude analyses		Confounder adjusted analyses		
	AO GHD (Yes/No)	Control cohort (Yes/No)	POR 95% CI	P	POR 95% CI	P	Confounders included in the model
Women							
Diabetes mellitus	30/319	39/1048	2.53 1.54-4.13	<0.001	1.95 1.15-3.32	0.01	BMI, Physical activity, Smoking
Antihypertensive drugs	80/267	217/882	1.22 0.91-1.63	0.18	1.05 0.77-1.42	>0.5	BMI
Lipid lowering drugs	49/296	79/1005	2.11 1.44-3.08	<0.001		-	
Antithromobotic drugs	24/327	71/1033	1.07 0.66-1.72	>0.5		-	
Men							
Diabetes mellitus	27/366	77/1115	1.07 0.68-1.68	>0.5		-	
Antihypertensive drugs	98/300	243/954	1.28 0.98-1.68	0.07			
Lipid lowering drugs	51/334	166/1021	0.94 0.67-1.32	>0.5		-	
Antithromobotic drugs	64/335	150/1,060	1.35 0.98-1.85	0.06		-	

Paper III

Prevalence of cardiovascular morbidity or the metabolic syndrome

6 patients (1 woman and 5 men) were on treatment for CVD, of them 1 man had DM (insulin treated), 4 patients had anti-hypertensive drugs (1 woman and 3 men) and 3 men had lipid lowering drugs. The corresponding morbidity among controls was 1 man on lipid lowering drugs and 1 on antihypertensive drugs. 8 patients and 3 controls fulfilled the criteria for metabolic syndrome according to IDF. There were more patients on treatment for CVD and/or with manifestations of the metabolic syndrome compared to controls (11 *vs.* 3, P<0.001).

Cardiovascular risk factors, risk prediction of CVD, and anthropometric measurements

Compared to controls, both CP men and women had significantly higher serum insulin levels, but without significant differences in plasma glucose levels (Table 2). Only women, but not men with CP, had significantly higher plasma levels of LDL-cholesterol, apo B/apoA-I ratio, TG, fibrinogen and hs-CRP as compared to controls. No significant differences were recorded in the plasma levels of HDL-cholesterol in either CP women or CP men, as compared to controls. There was no change in plasma lipid levels when patients and controls on lipid-lowering drugs were excluded.

Twelve CP women and 7 CP men had high cardiovascular risk using hs-CRP and LDL-cholesterol levels (at least intermediate level of both risk indicators). Corresponding figures for controls were 2 women and 10 men.

Twelve CP women and 10 CP men had increased cardiovascular risk (medium or high) according their apo B/apoA-I ratio (> 0.6 in women and >0.7 in men). Corresponding figures for controls were 4 women and 10 men.

Compared with controls both CP men and women had significantly higher serum leptin and leptin per kg fat mass.

After linear regression, and with adjustment for BMI, insulin, LDL-, HDL-cholesterol, and apoB/apoA-I ratio did not differ significantly between CP women ($P \geq 0.08$, all variables), or CP men, as compared to controls ($P > 0.3$). Serum leptin levels were significantly higher in both CP men and women, even when BMI was included in the model ($P = 0.03$ in men, and $P = 0.002$ in women).

No significant differences in systolic or diastolic blood pressure or in heart rate were recorded in men or women with CP, as compared with their controls (Table 2). Whether patients with antihypertensive medication were excluded made no difference.

No significant difference in height was observed in men or women with CP as compared to controls. Significantly higher weight, BMI, skin fold measurement of lateral of umbilicus, and waist and hip measurements were recorded in both CP men and women, as compared to controls, but only CP women had significantly increased WHR.

Body composition measured with BIA showed significantly higher kg fat mass in both men and women with CP, as compared to controls. Muscle mass was significantly higher in female pa-

tients compared to controls, but for men there was no significant difference between patients and controls.

Hormone assessments

Serum IGF-I levels and plasma PRL did not differ in CP patients compared to controls (**Table 16**). Plasma levels of TSH and cortisol were significantly lower and plasma free T4 was significantly higher in both CP women and men, but plasma free T3 was significantly lower in CP men only. CP women had significantly lower serum DHEA and testosterone levels as compared to controls. There was no difference in serum testosterone for CP men compared to controls and no differences in serum SHBG or estradiol levels, neither for men nor for women with CP, as compared to controls. Serum FSH- and LH-levels were significantly lower in both CP women and men.

Table 15. Cardiovascular risk factors, blood pressure, anthropometric measurements and body composition measured with BIA in 42 CO CP patients and 42 matched controls.

	Women		Men		P-value	
	Patients (n=20) Median (Range)	Controls (n=20) Median (Range)	Patients (n=22) Median (Range)	Controls (n=22) Median (Range)	Women	Men
Biochemical measures						
S-insulin (mIU/L)	6.0 (2.0-16.0)	3.0 (2.0-16.0)	5.5 (1.0-38.0)	4.0 (1.0-7.0)	0.004	0.02
Insulin/ kg fat mass (BIA)	0.13 (0.07-0.35)	0.10 (0.03-0.28)	0.11 (0.02-0.51)	0.09 (0.02-0.33)	0.14	0.41
S-c-peptide (nmol/L)	0.8 (0.2-1.6)	0.5 (0.3-1.2)	0.67 (0.13-1.9)	0.54 (0.22-1.2)	0.04	0.16
P-glucose (mmol/L)	4.4 (3.6-5.8)	4.9 (3.7-5.4)	4.5 (3.7-11.1)	4.6 (3.7-5.9)	0,08	>0.3
P-cholesterol (mmol/L)	4.8 (2.4-6.7)	4.4 (2.4-5.6)	4.2 (1.3-6.9)	5.0 (3.0-6.9)	0.13	0.18
P-HDL (mmol/L)	1.49 (0.9-2.4)	1.8 (1.3-2.2)	1.2 (0.8-2.4)	1.3 (0.8-2.2)	0.09	0.19
P-LDL (mmol/L)	3.4 (2.2-4.6)	2.7 (1.2-4.0)	2.9 (0.5-5.3)	3.2 (1.6-5.4)	0.01	>0.3
ApoB-ApoA ratio	0.67 (0.35-1.08)	0.46 (0.23-0.71)	0.68 (0.37-1.40)	0.64 (0.29-2.00)	0.01	>0.3
P-TG (mmol/L)	1.2 (0.4-2.4)	0.8 (0.3-1.8)	1.2 (0.6-8.5)	1.2 (0.3-3.7)	0.03	>0.3
P-fibrinogen (g/L)	3.7 (2.4-4.0)	3.1 (2.3-4.1)	2.7 (2.0-4.6)	3.0 (2.0-3.9)	0.004	>0.3
P-hs-CRP (mg/L)	3.6 (0.4-23.0)	0.4 (0.4-13.0)	0.7 (0.4-17)	0.4 (0.4-9.6)	0.02	>0.3
S-Leptin (ng/ml)	33.8 (16.0-214)	10.6 (3.3-36.9)	15.3 (2.7-57.0)	4.7 (1.7-22.3)	<0.001	0.007
S-Leptin/kg fat mass (BIA)	0.95 (0.56-4.48)	0.36 (0.12-1.6)	0.34 (0.07-0.82)	0.15 (0.04-0.68)	<0.001	0.03
Blood Pressure						
Systolic BP (mmHg)	112 (102-155)	108 (90-140)	120 (107-140)	116 (100-148)	0.14	>0.3
Diastolic BP (mmHg)	71 (55-85)	70 (60-82)	80 (60-87)	76 (55-85)	>0.3	>0.3
Heart rate (ECG)	64 (50-81)	60(50-81)	63 (45-84)	57 (44-75)	>0.3	>0.3
Anthrometric measurements						
Height	172 (155-177)	168 (160-1759)	180 (163-193)	180 (170-193)	>0.3	>0.3
Weight (kg)	93 (53-122)	60 (49-108)	92 (60-149)	82 (58-111)	<0.001	0.006
BMI (kg/m²)	31 (19-41)	21 (18-36)	28 (21-41)	24 (20-34)	<0.001	0.006
Waist (cm)	95 (72-126)	72 (64-114)	100 (77-131)	90 (73-108)	<0.001	0.006
Hip(cm)	108 (87-120)	90 (64-114)	104 (86-129)	96 (70-116)	0.001	0.005
Waist: hip ratio	0.9 (0.8-1.0)	0.8 (0.7-1.0)	0.9 (0.45-1.4)	1.0 (0.4-1.6)	0.003	0.15
Umbilical skin fold (cm)	2.8 (1.7-4.5)	1.6 (0.7-3.2)	2.8 (1.4-12.9)	1.8 (0.8-3.7)	<0.001	0.002
Body composition (BIA)						
Fat mass (kg)	44.8 (22.7-60.9)	30.0 (11.1-46.8)	45.6 (30.0-74.6)	39.0 (9.4-86.3)	<0.001	0.005
Musclemass (kg)	32.0 (27.0-41.5)	30.8 (25.0-41.0)	48.8 (28.5-59.5)	46.2 (35.0-55.0)	0.02	>0.3

Table 16. Serum and plasma hormone levels in 42 CO CP patients and 42 matched controls.

	Women		Men		P-value	
	Patients (n=20) median (range)	Controls (n=20) median (range)	Patients (n=22) median (range)	Controls (n=22) median (range)	Women	Men
S-IGF-I (µg/L)	205 (70-328)	201 (125-385)	182 (35-536)	203 (125-315)	>0.3	>0.3
P-Prolactin (µg/L)	14 (1-55)	15 (5-33)	8 (1-50)	11 (5-25)	>0.3	>0.3
P-Cortisol (nmol/L)	104 (10-635)	402 (125-1445)	21 (10-380)	348 (201-501)	0.001	<0.001
P-TSH (mU/L)	0.01 (0.00-3.2)	1.95 (0.7-3.9)	0.1 (0.1-0.74)	1.6 (1.0-6.5)	<0.001	<0.001
P-freeT4 (pmol/L)	19 (13-26)	15 (13-21)	18 (15-39)	17 (14-21)	0.02	0.01
P-freeT3 (pmol/L)	5.1 (2.8-6.9)	5.4 (4.2-7.8)	4.6 (2.9-13.0)	6.0 (4.6-6.9)	0.11	0.001
P-Estradiol (pmol/L)	177 (40-777)	180 (40-1452)	121 (40-661)	113 (81-185)	>0.3	>0.3
S-DHEA (µmol/L)	1.8 (0.8-7.4)	3.7 (2.1-9.7)	-	-	0.006	-
S-testosterone (nmol/L)	0.4 (0.4-1.8)	1.2 (0.5-2.4)	15.6 (0.4-34.5)	15.1 (6.8-28.3)	0.003	>0.3
S-SHBG (nmol/L)	57 (13-164)	61 (19-242)	24 (8-82)	23 (11-57)	>0.3	>0.3
P-FSH (U/L)	0.2 (0.2-6.2)	6.0 (0.2-63.0)	0,2 (0.2-11.0)	3.3 (1.0-9.7)	0.002	0.001
P-LH (U/L)	0.2 (0.2-7.1)	4.7 (0.2-22.0)	0.2 (0.2-4.4)	3.1 (1.7-10.0)	0.003	<0.001

Evaluation of physical exercise

There were significantly lower degrees of physical activity during spare time in winter and summer for CP men, but for CP women there was only significant difference in spare time during winter as compared to controls (**Table 17**). There were no significant differences for physical activity at work for CP men or CP women compared to controls. The estimated inactive time was significantly higher in CP men in comparison to controls, but there was no significant difference for women. Number of steps during three days, calculated by use of a pedometer was significantly lower for CP men, but no significant difference was recorded for women with CP.

Table17. Physical activity evaluated by the participants at a four graded scale at work and in leisure time during summer and winter (1=sedentary life or work, 2=light activity in spare time or soft work, 3=regular physical activity or moderate hard work, 4=hard exercise or hard work), and as estimated active and inactive time during an ordinary day and by counting steps for three days with a pedometer.

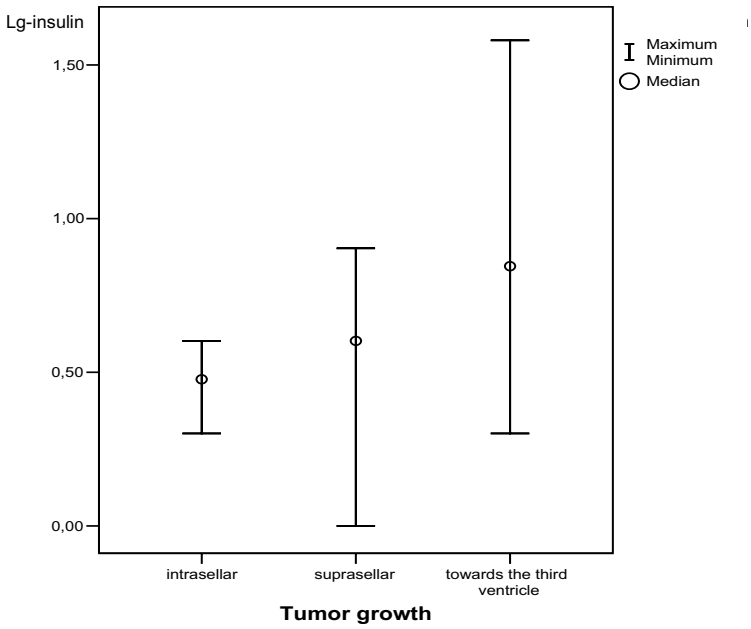
		Women		**Men**		**p-value**	
		Patients n (%)	Controls n (%)	Patients n (%)	Controls n (%)	Women	Men
Physical Activity at work[1]	1	5 (42)	5 (42)	10 (67)	6 (40)	>0.3	0.11
	2	4 (33)	7(58)	2 (13)	3 (20)		
	3	3 (25)	0 (0)	3 (20)	4 (27)		
	4	0 (0)	0 (0)	0 (0)	2 (13)		
Physical activity leisure time summer	1	0 (0)	0 (0)	4 (18)	1 (5)	0.19	0.04
	2	12 (60)	9 (45)	11 (50)	9 (41)		
	3	8 (40)	9 (45)	7 (32)	9 (41)		
	4	0 (0)	2 (9)	0 (0)	3 (14)		
Physical activity leisure time winter	1	3 (15)	2 (10)	7 (32)	1 (5)	0.046	0.01
	2	10 (50)	6 (30)	9 (41)	10 (45)		
	3	7 (35)	7 (35)	6 (27)	9 (41)		
	4	0 (0)	5 (25)	0 (0)	2 (9)		
Estimated active time (15 min), median (range)		20 (9-63)	28 (14-49)	16 (3-45)	26 (9-54)	0.21	0.02
Estimated inactive time (15 min), median (range)		76 (33-87)	68 (47-82)	80 (51-93)	70 (42-87)	0.21	0.02
Pedometer[2] (steps) median (range)		23870 (6081-48800)	32422 (18684-31154)	21395 (9758-51993)	30382 (2062-64043)	0.12	0.02

[1]7 pairs of men and 8 pairs of women were excluded from the analysis as they were not "working", [2] 4 pairs of women did not participate, due to technical problems with the pedometer.

Correlations between disease-related factors and cardiovascular risk factors

Years since first operation was correlated to waist (r= 0.33, p=0.034), but no other cardiovascular risk factors (P-cholesterol, P-LDL, P-HDL, P-TG, ApoB/ApoA-1 ratio, P-glucose, S-insulin, S-leptin, WHR and blood pressure ≥ 135/85). Age at first operation was negatively correlated with waist (r=-0.32, p=0.04), but no other cardiovascular risk factors. Tumor extension correlated with plasma insulin (r=0.57, P<0.001, **Figure 5**), waist (r=0.49, P=0.001), WHR (r=0.39, p=0.01) and blood pressure ≥ 135/85 (r=0.45, P=0.003).

Figure 5. Correlation between tumour growth and the logarithm for s-insulin in CO CP patients (r=0.57, p<0.001).



Tumor characteristics', cardiovascular risk factors, hormone assessments, in patients with and without TGTV

Patients with TGTV (n= 25) had significantly more operations (≥ 2 operations, 10 *vs*. 1 patient) and were more often treated with CRT (17 *vs*. 2 patients) than patients without TGTV (n=17). Otherwise no significant differences were recorded for physical activity (any modality), gender (14 men with TGTV and 8 without TGTV), age or time since 1:st operation, dose of CRT, number of pituitary deficiencies, time since first pituitary deficiency, age at or time since start of GH therapy, or number of patients who had severe visual disturbances (data not shown). Number of patients without GH therapy and, with ACTH and thyroxine deficiency was the same in both groups, as were the dosing of hydrocortisone and levothyroxine (data not shown).

CP patients with TGTV had significantly higher weight, BMI, waist, fat mass and muscle mass, but no significant difference in physical activity (any modality), compared to CP pa-

tients without TGTV (**Table 18a**).

There were significantly higher levels of serum insulin and insulin per kg fat mass, but no significant difference in plasma glucose, leptin or leptin/kg fat mass, among patients with compared to patients without TGTV (**Table 18b**).

Ten out of 11 patients (8 men and 2 women) on treatment for cardiovascular diseases and/or with manifestation of the metabolic syndrome had TGTV, though no significant differences were recorded for the levels of LDL-, HDL-, and total cholesterol, TG, apoB/apoA-I ratio, fibrinogen, or hs-CRP between groups.

Serum levels of IGF-I and free T3 were not significantly different, but serum free T4 was significantly higher and serum TSH, significantly lower in the TGTV group.

Table 18a. Anthropometric measurements and physical activity in patients with and without tumor growth into the third ventricle (TGTV).

	Patients with tumor growth into the third ventricle (n=25)	Patients without tumor growth into the third ventricle (n=17)	
Anthropometric measurements and body composition (BIA)	Median (range)	Median (range)	***P***
Weight (kg)	105 (77-131)	80 (53-110)	0.001
BMI (kg/m^2)	32 (21-41)	25 (19-35)	0.001
Waist (cm)	105 (77-131)	89 (72-109)	0.002
Hip (cm)	114 (86-129)	99 (87-116)	0.003
Waist:hip ratio	0.94 (0.83-1.05)	0.88 (0.76-0.99)	0.02
Fat mass (kg)	48 (30-75)	40 (23-56)	0.001
Muscle mass (kg)	42 (30-60)	37 (27-50)	0.03
Physical activity* n (%)			
Physical activity at work	1, 2, 3, 4 10(59), 4 (24), 3(18), 0	1, 2, 3, 4 8(57), 3 (21), 3(21), 0	>0.3
Physical activity leisure time/summer	1, 2, 3, 4 2 (8), 13(52), 1(40), 0	1, 2, 3. 4 2(12), 10(59), 5(29), 0	>0.3
Physical activity leisure time/winter	1, 2, 3, 4 5(20), 13(52), 7(28), 0	1, 2, 3, 4 5(29), 6(35), 6(35), 0	>0.3
Estimated active time (15 min), median (range)	17 (3-63)	19 (6-51)	>0.3
Estimated inactive time (15 min), median (range)	79 (33-93)	77 (45-90)	>0.3
Steps with pedometer, median (range)	20122 (6081-42299)	27607 (8142-51993)	0.06

* 1=sedentary life or work, 2=light activity in spare time or soft work, 3=regular physical activity or moderate hard work, 4=hard exercise or hard work

Table 18b. Cardiovascular risk factors and serum and plasma hormone levels in patients with and without tumor growth into the third ventricle (TGTV).

	Patients with tumor growth into the third ventricle (n=25)	Patients without tumor growth into the third ventricle (n=17)	
Cardiovascular risk factors and serum and plasma hormone levels	Median (range)	Median (range)	***P***
P-Leptin (ng/ml)	28 (4-215)	16 (3-67)	0.07
Leptin/kg fat mass (BIA)	0.53 (0.12-4.48)	0.56 (0.007-1.43)	>0.3
S-insulin (mIU/L)	7 (2-38)	4 (1-8)	<0.001
Insulin/ kg fat mass (BIA)	0.16 (0.04-0-51)	0.10 (0.02-0.35)	0.02
P-glucose (mmo/L)	4.6 (3.6-11.0)	4.4 (3.7-5,9)	>0.3
P-Cholesterol (mmol/L)	4.4 (2.9-6.9)	4.7 (1.3-6.5)	>0.3
P-LDL (mmol/L)	2.9 (0.5-5.4)	3.2 (2.2-4.7)	0.23
P-HDL (mmol/L)	1.2 (0.7-8.5)	1.3 (0.9-2.4)	0.23
P-TG (mmol/L)	1.2 (0.7-8.5)	1.2 (0.4-2.5)	>0.3
ApoB/ApoA-I ratio	0.68 (0.35-1.40)	0.66 (0.37-1.09)	>0.3
P-Fibrinogen (g/L)	3 (2-5)	3 (2-4)	>0.3
P-hs-CRP (mg/L)	2.8 (0.4-23)	0.4 (0.4-16)	0.07
Systolic Blood Pressure (mmHg)	115 (102-155)	120 (102-130)	>0.3
Diastolic Blood Pressure (mmHg)	78 (55-88)	72 (60-80)	0.25
P-IGF-I (μg/L)	182 (34-526)	196 (94-328)	>0.3
P-TSH (mU/L)	0.01 (0.0-0.37)	0.02 (0.1-3.2)	0.002
P-free T3 (pmol/L)	4.8 (2.8-6.2)	4.8 (2.9-3.7)	>0.3
P-free T4 (pmol/L)	20 (16-39)	17 (13-31)	0.010

Paper IV

Anthropometric measurements, BMD, BMC, and calcium intake

No significant difference in height was recorded between CP patients and controls. Weight and BMI were significantly higher in CP women, but not in men, in comparison to controls. Fat mass measured with BIA was significantly higher among CP women and men (**Table 19**). CP women, but not men, had significantly higher muscle mass, compared to controls.

Table 19. Anthropometric measurement, body composition (BIA), bone mineral density (BMD), bone mineral content (BMC) and z-score in 20 women and 19 men with a craniopharyngioma and in matched controls

	Women		Men		P	P
	Patients (n=20) median (range)	Controls (n=20) median (range)	Patients (n=19) median (range)	Controls (n=19) median (range)	Women	Men
Age at investigation	28 (18-57)	28 (18-57)	30 (17-57)	30 (17-57)	>0.3	>0.3
Anthropometric measurements						
Weight (kg)	93 (53-122)	60 (49-108)	88 (60-134)	84 (58-111)	<0.001	0.16
Height (cm)	172 (155-177)	168 (160-175)	179 (163-193)	180 (170-193)	>0.3	>0.3
BMI (kg/m²)	31 (19-41)	21 (18-36)	27 (21-38)	25 (20-34)	<0.001	0.10
BIA						
Fatmass (kg)	44.8 (22.7-60.9)	30.0 (11.1-46.8)	44.0 (30.0-67.0)	39.4 (9.4-55.4)	<0.001	0.03
Muscle mass (kg)	32.0 (27.0-41.5)	30.8 (25.0-41.0)	47.0 (28.5-53.0)	46.3 (35.0-55.0)	0.02	>0.3
DXA						
BMD femoral neck (g/cm²)	1.01 (0.68-1.15)	1.07 (0.84-1.27)	1.05 (0.72-1.34)	1.12 (0.71-1.38)	0.07	>0.3
BMC femoral neck (g)	4.9 (3.5-6.0)	4.8 (3.8-6.3)	6.1 (3.5-8.2)	6.4 (4.2-7.9)	>0.3	>0.3
Z-score femoral neck	-0.35(-1.4-0.5)	0.7 (-1.4-2.8)	-0.15 (-2,3-1.4)	0.4 (-2.2-2.0)	0.004	>0.3
BMD L2-L4 (g/cm²)	1.09 (0.83-1.42)	1.24 (0.96-1.60)	1.30 (0.72-1.47)	1.20 (0.83-1.50)	0.03	>0.3
BMC L2-L4 (g)	50.5 (21.5-69.3)	53.4 (39.7-69.0)	66.0 (31.1-80.6)	64.5 (43.1-82.2)	0.07	>0.3
z-score L2-L4	-1.6 (-3.3-2.2)	0.65 (-1.0-3.6)	-0.05 (-2.3-1.4)	0.0 (-3.3-1.4)	0.004	>0.3
BMD total body (g/cm²)	1.09 (1.01-1.22)	1.18 (1.04-1.34)	1.19 (0.94-1.44)	1.22 (1.10-1.40)	0.006	0.16
BMC total body (g)	2421 (2160-3232)	2426 (2030-3070)	3037 273-4102)	3048 (2556-4125)	>0.3	0.30
Biochemical assays						
p-TSH mU/L	0.01 (0.00-3.2)	1.95 (0.7-3.9)	0.01 (0.01-0.74)	1.6 (1.0-6.5)	<0.001	<0.001
p-Free T4 pmol/L	19 (13-26)	15 (13-21)	18 (15-39)	17 (14-21)	0.02	0.02
p-Free T3 pmol/L	5.1 (2.8-6.9)	5.4 (4.2-7.8)	4.6 (2.9-13.0)	5.9 (4.6-6.9)	0.11	0.003
p-Cortisol nmol/L	104 (10-635)	402 (125-1445)	21 (10-380)	335 (201-501)	0.001	<0.001
p-Estradiol pmol/L	177 (40-777)	180 (40-1452)	127 (48-661)	114 (82-185)	>0.3	>0.3
s-DHEA µmol/L	1.8 (0.8-7.4)	3.7 (2.1-9.7)	-	-	0.006	-
s-testosterone nmol/L	0.4 (0.4-1.8)	1.2 (0.5-2.4)	17.9 (0.4-34.5)	14.5 (6.8-28.3)	0.003	>0.3
s-IGF-I µg/L	205 (70-328)	201 (125-385)	187 (73-526)	207 (124-315)	>0.3	>0.3
s-Insulin (mIU/L)	6 (2-16)	3 (2-16)	5 (1-25)	4 (1-7)	0.004	0.12

BMD, BMC and z-score did not differ between CP men and controls at any site (**Table 19**). After linear regression, and with adjustment for BMI, BMD, BMC and z-scores did not differ sig-

nificantly between CP men and controls (P > 0.1). In contrast, CP women had significantly lower BMD at total body, L2-L4, BMC at L2-L4 and z-scores at femoral neck and L2-L4 compared to controls.

In CP men there were significant correlations between BMC of L2-L4 (r=0.5, P=0.048), BMD and BMC of femoral neck (r=0.49, P=0.035 and r=0.65, P=0.003), BMC of total body (r= 0.53, P=0.02) and muscle mass. Only BMC of femoral neck correlated with fat mass (r=0.57, P=0.011).

In CP women there were significant correlations between BMC of the femoral neck (r=0.53 and P=0.016), total BMC (r=0.59 and P=0,006) and muscle mass. BMC of the femoral neck (r=0.60, P=0.005) and BMC of the total body (r=0.655, P=0.002) correlated with fat mass.

Calcium intake was significantly lower in CP men compared to controls [median 300mg (range 0-1200mg) *vs*. 600mg (0-2400mg), P=0.008], but there were no differences in women [590 mg (0-1400mg) *vs*. 415 mg (0-1380mg), P=0.35]

Biochemical assays

CP women had significantly lower serum DHEA and testosterone levels compared to controls, but no difference was recorded in serum testosterone levels for men (**Table 19**). Serum IGF-I levels did not differ between patients and controls of either gender. Serum insulin was significantly higher in CP women than controls but no difference was seen in men. Plasma TSH, free T4, and cortisol were significantly lower in both CP men and women than in controls

Evaluation of physical exercise

CP men and women had significantly lower degree of physical activity during spare time in winter as compared to controls (**Table 20**) and estimated inactive time was significantly higher in CP men in comparison to controls. The numbers of steps did not differ between CP women or men, as compared to controls.

Table 20. Physical activity evaluated by the participants at a four graded scale at work and in spare time during summer and winter (1:sedentary life or work; 2:light activity in spare time or soft work; 3:regular physical activity or moderate hard work; 4:hard exercise or hard work), and as estimated active and inactive time during an ordinary day and by counting steps for three days with a pedometer

	Women		**Men**		**P-value**	
	Patients (n=20) median (range)	Controls (n=20) median (range)	Patients (n=19) Median (range)	Controls (n=19) median (range)	Women	Men
Physical Activity at work (15 men, 12 women)	2 (1-3)	2 (1-2)	1 (1-3)	2 (1-4)	>0.3	0.1
Physical activity spare time/summer	2 (2-3)	3 (2-4)	2 (1-3)	3 (1-4)	0.19	0.06
Physical activity spare time winter	2 (1-3)	3 (1-4)	2 (1-3)	2.5 (1-4)	0.046	0.02
Estimated active time (15 min)	20 (9-63)	28 (14-49)	16 (3-45)	26 (9-54)	0.21	0.007
Estimated inactive time (15 min)	76 (33-87)	68 (47-82)	80 (51-93)	70 (42-87)	0.21	0.007
Pedometer three days (n) (22 men, 16 women)	23870 (6081- 48800)	32422 (18684- 31154)	21395 (9758- 51993)	30382 (2062- 64043)	0.12	0.05

Paper V

All patients were GHD confirmed by a peak GH < 3 μg/l to the ITT (**table 21**). The median insulin dose used was 0.07 IU/kg (0.06-0.1). The median nadir blood glucose level for the whole group was 1.3 mmol/l (1.0-1.9), and was reached at a median 25 min (20-33) after the insulin infusion. The median duration of hypoglycaemia (blood glucose < 2.2 mmol/l) was, for the whole group, 25 min (15-33). All symptoms recorded during the ITT are shown in **Table 21**. In five (31%) of the patients neither the doctor, nor the patient recorded any signs or symptoms during the hypoglycaemic phase and their median nadir blood glucose level was 1.4 mmol/l (range 1.1-1.9) and the duration of blood glucose < 2.2 mmol/l was 25 min (range 20-33). Of these five patients with unawareness, four had panhypopituitarism and one had isolated GHD. The remaining 11 patients were symptomatic, and tiredness (n=6) and dizziness (n=3) were the most frequent symptoms. In these patients with symptoms, the median nadir blood glucose level was 1.3 mmol/l (range 1.0-1.6), and the duration of blood glucose < 2.2 mmol/l was 25 min (range 15-30).

Table 21. Detailed information on doses of insulin, b-glucose levels, duration of nadir and symptoms during an ITT (peak GH< 3 µg/l) in 16 patients.

Pt #	Gender F/M	Insulin U/kg	B-glucose at –15 min mmol/l	Nadir B-glucose mmol/l	Nadir B-glucose min after time 0	Duration of B-glucose ≤ 2.2 mmol/l	Peak GH µg/l	Symptoms	Iv glucose during the test
1	M	<0.1	4,1	1.2	30	15	0.27	Anxiety, tachycardia	No
2	M	0.09	4,4	1.4	20	20	0.15	None	No
3	F	0.08	4,8	1.3	27	27	0.27	Tired, warm	Yes
4	M	0.07	4,9	1.1	30	30	<0.03	None	Yes
5	F	0.1	4,2	1.0	30	30	<0.03	Dizzy, tired, pale	Yes
6	F	0.07	4,5	1.5	22	22	0.12	None	No
7	M	0.08	4,6	1.2	25	25	0.22	Blurred vision	Yes
8	M	0.08	4,1	1.2	25	25	0.22	None	Yes
9	M	0.07	4,4	1.9	33	33	0.14	None	No
10	F	0.07	4,7	1.2	25	25	0.81	Dizzy, pale, tired, perspiring	Yes
11	M	0.08	4,1	1.6	25	25	2.4	Dizzy, pale, tired, perspiring	No
12	M	0.07	4,2	1.5	25	25	0.58	Perspiring, warm	No
13	F	0.06	4,4	1.3	25	25	0.22	Shaky	Yes
14	F	0.07	4,6	1.4	30	30	0.05	Warm	No
15	M	0.06	4,0	1.3	25	25	0.42	Warm, tired	Yes
16	F	0.07	4,6	1.2	25	25	0.1	Tired, sleepy	Yes

Discussion

Bone health in hypopituitary patients on replacement therapy

It is well known that low BMD is related to increased fracture risk (Melton *et al.* 1993), why it is particularly interesting that the results in paper IV are consistent with the results in paper I. We found decreased BMD in CO CP women in paper IV and increased fracture risk in CO GHD women in paper I. We also found that there was no difference in BMD between CO CP men and controls in paper IV and there was no difference in fracture incidence in CO GHD men compared to controls in paper I.

Fracture risk

The women in paper I had an overall risk increase for fractures, reaching significances in CO GHD women only, who had a more than doubled risk. The GHD men, on the contrary, had a generally decreased incidence of fractures, reaching significance in AO GHD men only.

The increased fracture risk in CO GHD women was not confined to the traditional osteoporotic fracture sites. Reasons for the increase in fracture incidence among CO GHD women could have been discontinuation of GH at completion of growth which may limit the attainment of PBM (Drake *et al.* 2003). It has been shown decreased BMD despite treatment with GH during childhood in patients with CO GHD (Kaufman *et al*. 1992, DeBoer *et al.* 1994, Degerblad *et al.* 1995, Koranyi *et al*. 2001). However, the finding that CO GHD men did not have increased fracture risk speaks against GHD as the main cause of the increased fracture risk seen in CO GHD women. During most of the observation period the patients had been on GH substitution, and the GH doses seemed adequate with higher doses in CO than in AO patients and in women compared to men (Janssen *et al.* 1997).

Cortisone or thyroxine substitution could not explain the increased fracture risk as there were no significant gender differences with respect to these hormones. CO GHD women were to a large extent sex steroid deficient, which is well known to correlate to ovarian androgen deficiency (Rivera-Woll *et al.* 2004) and many of these women also had adrenal androgen deficiency, as 57 % were ACTH deficient. Even if CO GHD women had estrogen supplementation during puberty, they did not have testosterone or DHEA treatment, and both estrogens and testosterone

are necessary for bone formation (Frank 2003). Age at start of sex steroid medication was in median 17 years for both men and women with CO GHD, and late onset of puberty is related to low PBM in both genders (Gordon *et al.* 1991, Finckelstein *et al.* 1992), but it has been shown that a smaller proportion of total bone mineral content achieved during puberty may be gender dependent in men compared to women (Gordon *et al.* 1991), why hypogonadism is more deleterious to the female than to the male skeleton. In addition, oral estrogens have been shown to desensitize the effect of GH on IGF-I activity in the liver (Mah *et al.* 2005), which leads to decreased IGF-I effect on the bone tissue as well (Matthews *et al.* 1988). Thus, a more likely explanation of the increased fracture risk confined to CO GHD women, than GHD itself is an interaction between GHD and estrogen and androgen deficiencies during particularly puberty when bone develops and matures.

CO GHD men had no difference in fracture risk compared to the control cohort, probably explained by sufficient testosterone and GH replacement. Estrogen is needed for the development of male bone mass also, but as testosterone is converted to estrogen, testosterone replacement is sufficient to provide both hormones (Frank 2003).

The median age was 59 years for AO GHD men and 57 years for AO GHD women at the time of distribution of questionnaires, which means that they had not yet reached the age when osteoporotic fractures are common. One study of GHD patients who were 60 years or older showed no difference in BMD compared to controls (Murray *et al.* 2004). It has also been shown that AO GHD women do not have the same positive effect of GH therapy on BMD as men have (Bex and Bouillon 2003), which is in concordance with our findings with lower fracture incidence in AO GHD men, but no difference in AO GHD women, compared to population controls. In addition, as fractures in men are known to occur during sporting activities and at work (Garraway *et al*. 1979), a more cautious lifestyle in GHD patients, due to visual problems together with a higher degree of disability retirement and sick leave (Jonsson and Nilsson 2000, Ehrnborg *et al.* 2000) could be one speculative explanation. However, in the current study, visual problems were more frequent in GHD patients, irrespective of gender and age of onset, as compared with the control subjects, and can thus not explain the observed gender difference in fractures.

In spite of a higher rate of cortisol deficiency and substitution in AO GHD men compared to women, and with no difference in the thyroxine substitution, there was no increased fracture risk

among men. Thus, substitution for these hormones did not seem to constitute a risk factor for fractures. It can be speculated that the high substitution rate of testosterone (90%) and GH (94%) have resulted in lower fracture risk in AO GHD men (Anderson *et al.* 1997).

The strengths of the study are the matching of population controls to patients, the use of equal questionnaires distributed in the same manner to patients and controls, a sufficient number of patients and population controls for enough statistical power, an almost as high rate of responders to questionnaires among patients (82 %) as among population controls (78 %) and the considerations of confounders. The present patient cohort seemed comparable to previous cohorts estimating fracture risk (Wüster *et al.* 1991, Rosén *et al.* 1997, Regal *et al.* 2001, Abs *et al.* 2005) as the majority of the AO GHD patients (74%) had a confirmed GHD due to a pituitary tumor or its treatment and CO GHD patients had craniopharyngioma and idiopathic GHD. The shortcomings in this study are that the fractures are self-reported, and not verified with x-ray, but there are no evidence for over or under reporting, as both higher and lower fracture risk was recorded in patients, compared to the population controls. Another disadvantage is that we have not been able to compare the results in patients on GH replacement with those without, since the person-years under risk were too few to allow a meaningful interpretation of GHD specific fracture risk in patients without GH replacement. Also the present patient cohort was based on a cross section of survivors, and we cannot exclude that deceased patients not included in the cohort might have differed from the participating patients and caused a limited selection bias, but there is no evidence for selective participation among patients alive at the time of inclusion in the study.

BMD and BMC in adults with CO CP

The results in paper IV showed that compared to matched population controls, CP women, but not men had significantly reduced BMD. This is to our knowledge the first time that BMD has been studied in adult CO CP patients.

It is well known that BMD in CO GHD patients is reduced (Kaufman *et al.* 1992, O'Halloran *et al.* 1993, DeBoer *et al*. 1994), which may illustrate the potential role of GH in the acquisition of PBM (DeBoer *et al*. 1994). CO CP patients are not necessarily representative of any CO GHD patient, as they often have hypothalamic involvement and obesity (Srinivasan et al 2004). In this study CO CP women had significantly higher BMI than controls, but not CO CP men. As obesity is protective against low BMD (Felson *et al.* 1993) the outcome in CO CP women could be

quite different than in other CO GHD women. It is noteworthy that CO CP men in this study did not differ in BMD in comparison to controls. Other studies have shown decreased BMD in CO GHD men in comparison to controls (Kaufman *et al.* 1992, DeBoer *et al.* 1994), but an important difference is that these men stopped GH therapy when they had reached their final height, while the CO CP men in this study had continuous or only short discontinuation of GH therapy.

Pituitary deficiencies and substitution therapies were the same in CP women and men, and GH therapy and other hormone substitutions were given since approximately the same age. Sex steroids were introduced at approximately the same age in CO CP patients (at 16 years in women and 15 in men) in paper IV as in CO GHD patients (at 17 years in both men and women) in paper I, with probably the same negative effect on bone mass in women as described above (page 57). Both CP men and women had significantly higher serum free T4 and suppressed TSH levels, compared to controls, but suppressive thyroxine replacement does not seem to affect BMD (Quan *et al.* 2002), and the different outcome in CO CP women and men speaks against an effect of T4. Glucocorticoid treatment is known to induce osteoporosis (Saag 2003), but not replacement therapy (Mora *et al.* 1996) and in this study gender difference in BMD can not be explained by current glucocorticoid replacement, as both CP men and women had the same hydrocortisone doses. Thus insufficient sex steroids or GH replacement during adolescence are possible explanations to the decreased BMD seen in CO CP women.

Cardiovascular risk in hypopituitary patients

Both in paper II and III CV risk in hypopituitary patients was studied, but in different ways and in different cohorts. Still, the results seem to correspond, as GHD women always seem to be at higher CV risk than GHD men, irrespective of AO or CO. In paper II only AO GHD patients were studied, while in study III a specific group of CO GHD patients with CP was studied. Previous studies showing increased CV risk in women (Rosén and Bengtsson 1990, Bülow *et al.* 1997, Tomlinson *et al.* 2001) have studied patients without GH replacement, but in the present studies most of the patients were on GH replacement therapy.

Non-fatal stroke and cardiac disease

In paper II GHD women had tripled and GHD men doubled life-long incidence of non-fatal stroke, but a decline was seen among both genders during the periods after diagnosis of first pituitary hormone deficiency (FHD) and GH deficiency (GHD), during which most patients had

GH replacement. Still GH replacement may not be the only reason to the decline as there are other factors of importance, such as fatal strokes, were not included in the study. Also the higher prescription of antihypertensive medication in GHD men, and anti-lipid medication in GHD women, could have contributed to the decline in stroke incidence.

Subclinical hypothyroidism or glucocorticoid overdose could increase CV risk (Owen and Lazarus 2003, Beshyah *et al.* 1999), but it has previously been shown that these patients have too high thyroxine doses (Bülow *et al.* 2000); thyroxine and glucocorticoid doses and the percentage on replacement were similar in GHD men and women in this study, why differences between genders cannot be explained by these replacements.

Sex steroid therapy for post-menopausal women has been shown to relate to increased cardiovascular risk (Barrett-Connor *et al.* 2003, Anderson *et al.* 2004), but it is not known whether early replacement contributes to increased incidence of stroke in GHD women. In comparison to population controls, GHD women in this study used sex steroid replacement more frequently before age 50, and in comparison to population controls there was no difference in the use of the different estrogen- and gestagen compounds, or evidence that GHD women, who suffered a non-fatal stroke, used sex steroid replacement more often than those who did not.

In addition, GHD patients visit their doctor more often than the general population, and as endocrinologists are nowadays aware of the increased cardiovascular risk among GHD patients, higher prescriptions of cardio-protective drugs are probable. This cannot, however, explain the difference between men and women.

Life-long incidence of non-fatal cardiac events was similar in the patient and control cohorts, but declined significantly in GHD men during the FHD- and GHD-periods. It has previously been shown a decline in myocardial infarction (fatal and non-fatal) after 5 years of GH therapy (Svensson *et al*. 2004). But without difference when fatal cardiac events were excluded, which may suggest that GH replacement is protective against serious myocardial infarction. Low serum IGF-I may be involved in the pathogenesis of ischemic heart disease (Juul *et al.* 2002) which is in accordance with the above described findings. Another explanation is the significant increase in antihypertensive medication in GHD men, and that these men did not smoke.

Diabetes prevalence and cardio-protective drugs

GHD women had significantly higher prevalence of type 2 DM and lipid-lowering medication,

whereas GHD men had significantly higher prevalence of antihypertensive medication.

The increase in DM was seen even when patients with acromegaly and Cushing's disease were excluded, but as POR for type 2 DM was no longer significant when confounders were included in the model, the increase was partly attributed to higher BMI and lower physical activity. Thus it seems that DM could be avoided if BMI and physical activity are carefully supervised. Previously shorter GH treatment periods have shown increase in blood glucose and insulin levels (Maison *et al.* 2004, Verhelst *et al.* 2005), with a more deleterious effect in GHD women (Maison *et al.* 2004), but provided that fat mass was reduced and lean mass increased during longer GH therapy, unchanged insulin sensitivity has been recorded (Svensson *et al.* 2002). Unfortunately, we have no information on the incidence of DM in the present study, and cannot state whether there was a decline or an increase in DM after 6 years of GH therapy.

The increased prescriptions of lipid-lowering drugs in GHD women could possibly be explained by the increased prevalence of DM, shown in our GHD women, as guidelines for DM treatment recommend lipid-lowering drugs at rather low levels of total cholesterol (ADA 2002).

The significant increase in antihypertensive drug prescription recorded in GHD men, even when smoking was included in the model, is not easy to explain. An observational bias is possible, as patients on GH therapy attend to their doctor more often than the general population, and that endocrinologists are aware of the increased cardiovascular risk among GHD. This can not, however, explain the difference between men and women. On the contrary, as target blood pressure levels in diabetics are more narrow (<130/80) (ADA 2002), a higher prescription of antihypertensive drugs would be expected among the GHD women.

Cardiovascular risk in adults with CO CP

In corroboration with a previous mortality study (Bülow *et al.* 1998), the adult CO CP women in paper III had higher cardiovascular risk than controls, while CO CP men did not. Still both CO CP men and women had significantly increased insulin and leptin levels, and more fat mass. In addition, CO CP patients with TGTV had significantly higher levels of insulin, more fat mass and significantly higher levels of insulin per kg fat mass compared with CP patients without TGTV, and this was not explained by differences in hormone substitutions, patients' background characteristics, or in physical activity. Ten out of 11 patients on treatment for cardiovascular diseases, or with the manifestations of the metabolic syndrome had TGTV.

Of importance in this study is that the majority of the CP patients were since long treated with GH, and with no significant difference in the serum IGF-I levels between patients and controls. CP women had significantly more, and CP men equal, muscle mass, which may speak in favour of sufficient GH substitution (Molitch *et al.* 2004). Still CP women had signs of dyslipidemia in comparison to controls, which is probably not caused by GHD alone and would probably have been even worse if GH replacement was not provided (Svensson *et al.* 2002).

BMI in CP patients was significantly higher than in controls and, as we regarded it as an outcome, patients and controls were not matched for BMI. Some of the differences between patients and controls could thus be due to that difference, and in CP children associations between tumor growth into the hypothalamus and obesity have been reported (De Vile *et al.* 1996, Müller *et al.* 2004). In order to evaluate the impact of BMI multiple linear regressions were calculated, with BMI included in the model for insulin, LDL-, HDL-cholesterol, and apoB/apoA1 ratio. Hyperinsulinemia and hyperleptinemia has been shown to be associated with hypothalamic involvement of the tumor (Roth *et al.* 1998, Lustig *et al.* 2003, Srinivasan *et al.* 2005). This is in accordance with the present study, showing an increased basal insulin level, and a significantly positive correlation between tumor growth and basal insulin levels, in both CP men and women, as well as showing significantly higher levels of plasma insulin and insulin/fat mass ratios in the TGTV patients. In addition, increased levels of leptin, and leptin/kg fat mass were seen in both CP men and women, which speak in favour of "leptin resistance", which may promote energy intake and storage and reduce energy expenditure (Lustig *et al*. 2006). However, in comparison to controls, neither the CP patients nor the TGTV patients had very high insulin or lipid levels. GH therapy was probably of importance to counteract cardiovascular risk by an increase in muscle mass, resulting in normal fasting blood glucose and in some improvement in serum lipid levels (Maison *et al.* 2004).

Subclinical hypothyroidism or glucocorticoid overdose could increase CV risk (Owen and Lazarus 2003, Beshyah *et al.* 1999) and glucocorticoids also induce hypothalamic obesity (Tiosano et al 2003). But plasma free T4 was significantly higher in both CP women and men, and in patients with TGTV, and can thus hardly explain the increased cardiovascular risk. Thyroxine and glucocorticoid doses and the percentage on replacement were also similar in CP men and women in this study why differences between genders cannot be explained either by these replacements. GH therapy to GHD subjects normalise 11β HSD1 activity (Giavoli *et al.* 2004).

Thus, this mechanism was probably not behind the much increased fat mass in the TGTV patients compared to the non-TGTV group, as no difference was recorded regarding number of patients with ACTH deficiency, without GH therapy, or in hydrocortisone dosing between groups.

Oral estrogen replacement in GHD women decreases the action of administered GH, why these patients required twice the GH dose, compared to patients with trans-dermal estrogens (Mah et al 2005). Most of the CP women in the present study had oral contraceptives, but they also had higher GH doses, as proved by serum IGF-I levels, at the levels of controls and higher GH doses than men.

The CP women had "androgen deficiency" with significantly lower levels of serum DHEA and testosterone, as compared to controls. A negative correlation has been shown between serum levels of DHEA and markers of inflammation in postmenopausal women (Straub *et al.* 1998), and substitution with DHEA decreases serum lipid levels in some, but not all studies (Lasco *et al.* 2001, Arlt 2006). Thus, in the CP women, "androgen deficiency" may have contributed to the higher cardiovascular morbidity.

Both CP men and women had more visual impairments than controls in this study. Only CP men had lower physical activity than controls. No difference was recorded in visual acuity, or physical activity in patients with or without TGTV. Harz *et al.* (2003) suggested that obesity in children with CP was due to reduced physical activity, and also associated with visual impairment. In the present study, these variables cannot explain the increased cardiovascular risk among the CP women or in the TGTV group.

In paper III we also discovered that about 60% of CP women and 30% of men had increased risk for CVD, as calculated from hs-CRP and LDL-cholesterol levels (Rifai and Ridker 2003). Raised hs-CRP and insulin predicts DM and cardiovascular events, which both can be improved by statins and aspirin (Fernadez-Real *et al.* 2003). The apoB/apoA-I ratio indicated medium or high risk for CVD in 60% of women and 46% of men, thus in line with the previous risk calculation.

GHD and ITT

In paper V we showed that in 16 patients with a high suspicion of GHD, 5 had hypoglycemia with unawareness, and the rest had vague and few symptoms during an ITT. Irrespective of

symptoms they all reached similar nadir blood glucose levels. Unawareness in this patients group has not been discussed in the literature and we have only found one article from 1971 (Merimee *et al* 1971.) reporting on this; and that was in dwarfs. We did not have a control group in this study, but lots of information on symptoms of hypoglycemia in normal subjects is already available and at 3.2 mmol/l of blood glucose, autonomic response to hypoglycemia is commenced in normal subjects (Mitrakou *et al.* 1991). Neuroglycemic symptoms such as hunger, tingling, blurred vision, difficulty in thinking and faintness, begin at blood glucose level of 2.8 mmol/l (Mitrakou *et al.* 1991). All patients in this study reached far below these thresholds, and still had few or no symptoms at all. Another important finding was that despite a reduction in insulin dose (0.07 IU/kg) in the present study very low nadir and long duration of hypoglycemia was present. The currently recommended insulin dose is 0.15 IU/kg. To our knowledge there is no recommendation in reduction of insulin dosing in adults, but it has been recommended in children (Shah *et al.* 1992, Sizonenko *et al.* 2001).

The reason for unawareness in GHD patients is not fully understood. It is possible that GHD per se is responsible, as it has been shown improvement in awareness among diabetics on insulin treatment after one week of GH replacement (Wurzburger *et al*. 1992). These results could however not be reproduced in another study with as long GH treatment period in the same type of patients (Sachon *et al*. 1993). Another reason could be ACTH deficiency, which has been suggested together with GHD (Garg *et al.* 2000), but in this study there was no difference in the duration of hypoglycemia between patients with or without ACTH deficiency.

The importance of this small study is to stress the need for uniform recommendations for the ITT to avoid future complications.

Conclusions

- A doubled fracture incidence risk for non-osteoporotic fractures was recorded in CO GHD women with confirmed GHD on conventional hormone substitution, including GH therapy. This finding was most likely explained by an interaction between incomplete or deficient GH replacement and deficient sex steroid replacement during adolescence. No increased fracture incidence was observed among CO GHD men or AO GHD women, but a significantly decrease in fracture incidence in AO GHD men, which might be explained by an adequate substitution rate of testosterone and GH.

- In patients with confirmed AO GHD on GH therapy since 6 years, a higher prevalence of type 2 DM and lipid-lowering medication was recorded in GHD women and of antihypertensive medication in GHD men. This cardio-protective medication, together with the GH therapy may have resulted in the decline in non-fatal stroke risk, particularly noted in GHD women, and in significantly lower non-fatal cardiac risk that was seen in GHD men.

- At 28 yr of age and 16 yr since diagnosis, patients with a CO CP on complete hormone therapy, including GH, had increased cardiovascular morbidity, and particularly women were at risk. Of patients with hypothalamic damage from the tumor 40% had manifestations of the metabolic syndrome. 60% of CP women had medium or high risk for CVD, but no such risk increase was seen for CP men. Thus, besides the main-stays of diet and physical activity we propose that together with GH therapy, prescription of statins and aspirin should be considered. Whether androgen deficiency in CP women is an additive cause of the gender related cardiovascular risk and if energy balance is altered in CP patients need to be further investigated.

- At 28 yr of age and 16 yr since diagnosis, CO CP women, but not CP men, on complete hormone substitution, including GH, had significantly decreased BMD. The cause is not known but insufficient sex steroid and GH replacements particularly during adolescence are possible explanations. Continuous surveillance of BMD is recommended in particularly CO CP women. Together with physical activity, some of the patients should be recommended Bisfosfonates together with sufficient calcium and D-vitamin intake.

- In 16 consecutively recruited patients with confirmed GHD subjected to the ITT, a very low nadir blood glucose level (1.3 mmol/l) was recorded, the overall symptoms of hypoglycaemia were scarce and a slow recovery of hypoglycaemia was recorded, irrespective of ACTH deficiency. These results emphasize the recommendations from the Growth Hormone Research Society (GRS 1998) that the ITT should only be performed in experienced units. If the ITT is still going to be recommended as the "gold standard" for diagnosing GHD we ask for more uniform recommendations, e.g. dose of insulin; considering BMI, and whether concomitant pituitary hormone deficiencies are present. Also, to avoid future complications during the ITT, recommendations for intervention with i.v. glucose, at unacceptable low blood glucose levels or at prolonged hypoglycaemia, is highly needed.

Populärvetenskaplig sammanfattning på svenska

Hypofysen är en ärtstor körtel placerad strax under hjärnan, som styr hormonproduktionen i kroppen. Bristande hypofysfunktion diagnostiserad i vuxen ålder beror oftast på hypofystumörer eller tumörer intill hypofysen och deras behandling (kirurgi och/eller strålbehandling). Hos barn är det vanligast att orsaken är okänd (idopatisk) tillväxthormonbrist (GHD) eller kraniofaryngiom (särskild form av hypofystumör). Tillväxthormon (GH), luteiniserande hormon, follikelstimulerande hormon (LH och FSH, styr könshormonproduktionen), tyroidea stimulerande hormon (TSH, styr ämnesomsättningen) och adenocorticotropt hormon (ACTH, styr kortisolproduktionen) utsöndras från hypofysens framlob som svar på stimulering från hypotalamus och styr i sin tur hormonproduktionen i kroppen. GHD är ofta den första hormonbrist som uppträder vid hypotalamus eller hypofys skada. Att GH behövs hos barn är självklart, men GHD hos vuxna ger också symtom. Tillväxthormon påverkar nästan alla vävnader och organ i kroppen, antingen direkt eller via IGF-I (insulin-like-growth-factor-I) produktion, som till största delen sker i levern. Symtom på GHD inkluderar, förutom hämmad längdtillväxt hos barn, bukfetma, muskelsvaghet, allmän trötthet och sänkt livskvalitet hos barn och vuxna. Det har också visats i studier att förändringar av kroppssammansättningen sker med minskad muskel- och ökad fettmassa, minskad förmåga att svettas, försämrad hjärtfunktion, blodfettrubbningar, försämrad glukostolerans, och sänkt bentäthet, mest uttalat hos de med GHD sedan barndomen.

Flera studier har visat att livslängden är kortare än förväntat hos patienter med GHD. Man har sett ökad dödlighet, ffa hos kvinnor, i hjärtkärlsjukdom och speciellt slaganfall.

Frakturrisken hos patienter med hypofysinsufficiens är inte riktigt fastställd än. Sänkt bentäthet (bone mineral density, BMD) är kopplat till ökad risk för frakturer och som nämnts ovan har patienter med GHD sänkt bentäthet ffa om de drabbats i barndomen. Hos dem som diagnostiserats avseende GHD först på äldre dagar har man däremot inte sett någon skillnad i bentäthet jämfört med friska jämngamla kontroller. Det finns tre studier som hävdar att det är ökad frakturrisk hos patienter med GHD men dessa resultat är osäkra.

GH-behandling reverserar de flesta negativa effekterna av GHD. Kroppssammansättningen förbättras liksom livskvaliteten, hjärtat förbättras avseende muskelmassa och slagvolym och blodfetterna, ffa total- och LDL – kolesterol, förbättras. Några har visat att glukostoleransen

(känsligheten för socker) förbättras, andra att den blir oförändrad eller försämrad.

GH behandling ökar benresorptionen varför BMD sjunker under de första 6 månadernas behandling för att efter 12 månader återgå till ursprungsnivå och efter 18 månader stiga.

Trots dessa positiva effekter av GH behandling finns det ännu ej några studier som visar positiva effekter på hur många som drabbas av frakturer och slaganfall samt endast en som visar minskning av hjärtinfarkter. Det är också osäkert vad som händer med glukostoleransen med tiden som patienter behandlas med GH och vad händer med diabetesförekomsten. Ingen studie har visat att GH behandling har någon effekt på livslängden hos dessa patienter.

Avhandlingen innehåller fem delarbeten, där de två första är epidemiologiska (läran om sjukdomsförlopp) studier avseende incidenserna (antal fall under en viss tid i en befolkning) av fraktur, icke-dödligt slaganfall (stroke) och kardiovaskulära händelser hos patienter med verifierad tillväxthormonbrist och i de flesta fall mångårig GH substitution, i jämförelse med matchade kontroll grupper, samt i den andra studien även prevalens talen (antal fall vid ett tillfälle i en befolkning) av diabetes mellitus och förskrivning av hjärt-kärl läkemedel.

De två följande studierna är tvärsnittsstudier av vuxna patienter opererade för kraniofaryngiom i barndomen, som hade fullständig hormonsubstitution inklusive GH. Kraniofaryngiom är en ovanlig tumör, som pga. sitt växtsätt och den behandling med kirurgi och strålbehandling som oftast ges, hos de flesta patienter resulterar i total hypofyssvikt och till följd av hypotalamuspåverkan även svår övervikt. I den första studien bedömdes graden av hjärtkärlsjuklighet och riskfaktorer för hjärtkärlsjukdom hos kraniofayngiomopererade patienter jämfört med kontroller matchade för ålder, kön, rökning och bostadsort. I den andra studien jämfördes bentätheten (bone mineral content = BMC och bone mineral density = BMD) mätt vid bentäthetsmätning (DXA) mellan kraniofaryngiom patienterna och kontrollerna. I den femte studien registrerades blodsockernivå och symtom under en insulintoleranstest (ITT) hos 16 patienter. ITT är förstahands metod för att fastställa GHD diagnosen men potentiellt farlig testmetod då patienter har drabbats av krampanfall och t.o.m. dödsfall inträffat hos barn. Metoden är dessutom kontraindicerad vid känd förekomst av krampanfall eller vid misstanke om kärlkramp. Tillstånd som förekommer oftare bland de patienter som man kan överväga att testa, än bland den allmänna befolkningen.

Delarbete I

Detta arbete baseras på uppgifter ur frågeformulär som 832 patienter med verifierad GHD och 2581 matchade kontroller besvarat. Patienterna kom från samtliga universitetssjukhus i Sverige och CSK i Kristianstad. Frågorna i formuläret gällde frakturförekomst och frågor om vid vilken ålder den inträffat, vilken del av skelettet som brutits och om den orsakats av en olyckshändelse (trafik eller skidolycka). Även bakgrundsinformation om intag av mjölkprodukter, alkoholkonsumtion, rökvanor, fysisk aktivitet, födelseland, kroppsvikt och längd besvarades av deltagarna.

Av patienterna hade 100 stycken GHD diagnostiserad före 18 års ålder (childhood onset = CO) och resterande 732 vid eller efter 18 års ålder (adult onset = AO). Huvudsakliga orsakerna till GHD hos CO GHD patienterna var kraniofaryngiom och idiopatisk GHD och hos AO GHD patienterna hypofysadenom. Fyra kontroller per patient, matchade för ålder, kön, bostadslän och födelseland, valdes ut via Statistiska centralbyråns (SCB) register. Svarsfrekvensen var 77% jämfört med patienternas svarsfrekvens på 82%. Fraktur risken (Incidence risk ratio: förekomst av första fraktur i relation till personår under risk hos patienterna i relation till kontrollerna) beräknades för tiden efter att GHD diagnosen fastställts hos patienterna och motsvarande tidsperiod hos kontroller. 98% av männen med CO GHD och 100% av kvinnorna hade haft GH behandling någon gång och median-tiden med GH behandling var 12 år (5:e -95:e percentilen 5-21 år)) för männen och 15 år (5-34) år för kvinnorna. Motsvarande siffror för män med AO GHD var 94% någonsin på GH behandling under i median 6 år (0-12) och för kvinnorna 91% under 5 år (0-12).

Resultatet visade att kvinnor med CO GHD hade en mer än dubblad risk för frakturer medan män med CO GHD och kvinnor med AO GHD inte hade någon riskökning. Hos AO GHD män fann vi en signifikant minskad fraktur risk.

Konklusionen är att den ökade frakturincidensen hos kvinnor med CO GHD skulle kunna bero på otillräcklig GH substitution i ungdomsåren tillsammans med otillräcklig könshormonsubstitution. Den minskade frakturincidensen hos män med AO GHD skulle kunna förklaras med försiktig livsstil i kombination med tillräcklig substitution med testosteron (90%) och GH (94%).

Delarbete II

Liksom i föregående arbete baseras uppgifterna i denna studie på frågeformulär där nyckelfrågorna gällde om respondenterna hade diabetes (kost, tablett eller insulin behandlad), behandling med blodtryckssänkande, blodfettsänkande eller tromocytaggregationshämmande (ASA) mediciner. Dessutom frågades om genomgångna kardiovaskulära händelser (stroke, hjärtinfarkt, ballongvidgning av kranskärlsförträngning eller bypass-kirurgi) och när de inträffat. För incidensberäkning användes två utfallsmått: 1) stroke och 2) kardiovaskulär-händelse.

Här undersöktes incidenserna enligt ovan för tre olika perioder: 1)livslångt, från födelsen till händelse eller slutet av studien 2) från år då första hypofysinsufficiensen diagnostiserades till slutet av studien och 3) från år då GHD diagnosen ställdes till slutet av studien. Incidenserna för stroke och hjärthändelser beräknades var för sig. Förekomsten av diabetes, behandling med blodtryckssänkande, blodfettsänkande och tromocytaggregationshämmande (ASA) mediciner, vid insamlingen av frågeformulären, beräknades också.

I detta delarbete inkluderades endast patienter med AO GHD eftersom för få kardiovaskulära händelser hos patienter med CO GHD omöjliggjorde incidens- och prevalensberäkningar. 750 patienter med AO GHD inkluderades och 2314 kontroller. 94% av männen med AO GHD och 92% av kvinnorna hade någonsin haft GH behandling under i median 6 år (5:e till 95:e percentilen 1-12 år för män och 1- 13 år hos kvinnor). Under större delen av perioderna efter diagnos av första hypofysinsufficiens och diagnos av GHD hade patienterna GH-behandling.

Resultaten visade att den livslånga incidensen av icke-dödlig stroke var trefaldigt ökad hos kvinnliga patienter och dubblad hos männen jämfört med kontroller. Under de två andra uppföljningsperioderna minskade incidensen och var inte längre signifikant ökad för vare sig kvinnor eller män, dock var antal fall hos kvinnor väldigt litet vilket försvårar korrekt värdering av perioderna.

Under livslång uppföljningen var incidensen av icke-dödlig hjärtsjukdom samma hos både manliga och kvinnliga patienter som hos kontroller. Under perioderna efter att första hypofysinsufficiens respektive GHD diagnostiserats, fann vi en signifikant minskning i incidensen av icke dödlig hjärtsjukdom, hos män med AO GHD, men inte hos kvinnor.

Prevalensen av diabetes, vid tiden för insamlandet av frågeformulären, var dubbelt så stor hos kvinnor med AO GHD som hos kontrollerna, medan det för männen inte var någon skillnad

jämfört med kontrollerna. Eftersom de flesta hade diet, tablett eller kombinationsbehandling (tabletter och insulin) för sin diabetes kan man utgå ifrån att det gäller typ 2 diabetes. Kvinnorna hade dubbelt så ofta blodfettsänkande behandling som kontrollerna. Män med AO GHD hade högre förskrivning av blodtryckssänkande jämfört med kontroller Akromegali (överproduktion av GH) och Cushings sjukdom (överproduktion av cortisol) ökar risken för och diabetes. När dessa exkluderades ur beräkningarna och confounders (faktorer som kan störa resultaten) inkluderades i modellen skiljde sig inte diabetesprevalensen hos kvinnliga patienter och kontroller åt. De confounders som föll ut var BMI (Body Mass Index = Vikt i kg/längd i meter i kvadrat) och fysisk aktivitet, vilket innebär att den ökade diabetesrisken hos kvinnor delvis kan förklaras av högre BMI och lägre grad av fysisk aktivitet.

Konklusionen är att efter 6 års GH substitution såg man en minskning i stroke (icke-dödlig) risk, speciellt hos kvinnor med AO GHD och en lägre risk för hjärtsjukdom hos män med AO GHD, vilket kan bero på GH behandlingen och den högre förskrivningen av blodfettsänkande medicin hos kvinnor och blodtryckssänkande medicin hos män. Kvinnorna med GHD hade ökad prevalens av diabetes, delvis pga. högre BMI och lägre fysisk aktivitet än kontrollerna.

Delarbete III

42 patienter, varav 20 kvinnor, opererade i barndomen för kraniofaryngiom, deltog i studien och jämfördes med kontroller matchade för ålder, kön, rökvanor och bostadsort (storstad/landsbygd). Målet med studien var att värdera förekomsten av kardiovaskulär sjuklighet och kardiovaskulära riskfaktorer hos patienter jämfört med kontroller. En neurokirurg graderade inväxt av tumören utifrån operationsberättelserna som intrasellär (intill hypofysen, 3 st), suprasellär (ovanför hypofysen, 14 st) eller suprasellär in mot tredje ventrikeln (in i hypotalamus, 25 st).

De flesta patienter hade flera hypofysinsufficienser och hög grad av substitutionsbehandling. Alla utom 3 kvinnor och 3 män hade GH behandling. Kvinnorna hade inte GH brist vid testning och av männen hade två tidigare haft GH behandling under vardera 6 år, men inte sedan 1 respektive 8 år. Kvinnorna hade haft GH behandling i median 10 år (0-19) och männen i 12 år (0-33). GH doserna var högre hos kvinnorna [0.8 mg (0.4-1.6)] än hos männen [0.5 mg (0.2-1.0)].

Vi jämförde patienterna med kontroller matchade för kön, ålder och bostadsort som slumpmässigt valts ut från den allmänna befolkningen och som sedan kontaktades per telefon och mat-

chades avseende rökning.

Patienter och kontroller undersöktes avseende midja-höftmått (WHR), vikt i förhållande till längd (BMI), kroppskonstitution med hjälp av bioelektrisk impedance (BIA) och blodtryck. Blodprover togs för analys av hormoner, insulin, leptin, blodsocker och blodfetter. Grad av fysisk aktivitet på arbete och fritid värderades mha frågeformulär, intervju och stegräknare. Alla resultat jämfördes mellan manliga respektive kvinnliga patienter med kontroller var för sig. Vi fann att både manliga och kvinnliga patienter vägde mer än kontrollerna, hade högre BMI och mer fettmassa, men kvinnorna hade också mer muskelmassa än kontrollerna. Blodtrycket skiljde sig inte mellan patienter och kontroller. Endast kvinnliga patienter hade sämre blodfetter än kontrollerna. Både manliga och kvinnliga patienter hade högre leptin och insulinnivåer, men samma blodsockernivåer som kontrollerna. Patienterna hade lägre TSH och kortisol nivåer än kontrollerna, medan T4 var högre hos både manliga och kvinnliga kontroller. DHEA (dihydroepiandrostendion) och testosteron var lägre hos kvinnliga patienter än kontroller. Männen rörde sig mindre än kontrollerna, medan för kvinnorna var det inte någon större skillnad. 11 patienter (8 män och 3 kvinnor) hade behandling för kardiovaskulär sjukdom eller uppfyllde kriterierna för det metabola syndromet (ökat midjemått samt två av följande kriterier: påverkade blodfetter – förhöjda triglycerider eller sänkta HDL nivåer, förhöjt blodtryck eller förhjt fasteblodsocker) och jämfört med 3 kontroller. Vi delade upp patienterna i två grupper, en med tumörinväxt i hypotalamus (25 st) och en med dem utan tumörinväxt i hypotalamus (17 st). Dessa grupper hade samma könsfördelning, men de med tumörinväxt hade varit med om fler operationer och var oftare strålbehandlade än de andra. Tio av de 11 patienter med kardiovaskulär sjukdomar eller metabola syndromet hade tumörinväxt i tredje ventrikeln, men det var inga skillnader avseende lipider mellan de med och utan tumörinväxt. Patienterna med tumörinväxt hade signifikant högre nivåer av insulin och insulin per kilo fettmassa, men inga skillnader för blodsocker, leptin eller leptin per kilo fettmassa noterades. Patienter med tumörinväxt vägde mer, hade mer fett och muskelmassa än de utan tumörinväxt men ingen skillnad för grad av fysisk aktivitet noterades mellan grupperna.

Slutsatsen i detta delarbete är att vid 28 års ålder och 16 år efter att diagnosen ställdes, hade patienter med kraniofaryngiom diagnostiserat i barndomen, trots fullständig hormonsubstitution inkluderande GH, fler riskfaktorer för hjärtkärlsjukdom än kontroller och speciellt kvinnor hade ökad risk. Patienter med tumörinväxt i tredje ventrikeln uppfyllde i 40% av fallen kriterierna för det metabola syndromet. 60% av kvinnorna och 40% av männen hade ökad kardiovaskulär risk

enligt riskbedömning utifrån blodfetter och hs-CRP (högkänsligt C-reaktivt protein) respektive ApoB/ApoA-I kvot. Vi föreslår att tillsammans med allmänna råd om kost och motion samt GH behandling, skall lipidsänkande behandling och ASA övervägas till dessa patienter. Huruvida androgen brist hos kvinnor kan ha betydelse för könsskillnaderna eller om energibalansen är störd återstår att studera.

Delarbete IV

Samma patienter som i föregående studie, utom 3 män som vägde för mycket för att DXA skulle kunna genomföras, deltog i denna studie. Målet var att undersöka BMD och BMC med hjälp av DXA (lågdos röntgen av skelettet) och att jämföra resultaten med kontroller på samma sätt som i föregående studie. Resultaten avseende BMI och fett och muskelmassa var samma som i föregående arbete för kvinnorna, men eftersom de som exkluderades pga. övervikt (DXA gräns > 130kg) var män, var det endast högre fettmassa hos manliga patienter än kontroller, men samma muskelmassa och BMI. BMD och BMC skiljde sig inte mellan manliga patienter och kontroller på någon plats i skelettet, men kvinnliga patienter hade lägre BMD för helkropp och L2-L2 (ländrygg) samt lägre BMC för L2-L4 jämfört med kontrollerna. Vår slutsats är att kvinnor med kraniofaryngiom diagnostiserade i barndomen, med fullständig hormonsubstitution inkluderande GH, hade signifikant sänkt BMD trots högre muskel och fettmassa än kontrollerna. Trolig förklaring är otillräcklig könshormon- och tillväxthormon substitution under ungdomsåren. Vi rekommenderar regelbunden kontroll med DXA-mätning av kvinnorna med kraniofaryngiom diagnostiserade i barndomen och att råd ges om tillräcklig fysisk aktivitet samt att det till en del patienter förskrivs bisfofonater, d-vitamin och kalk.

Delarbete V

16 patienter (7 kvinnor), 22-59 år gamla, remitterade för hög misstanke om tillväxthormonbrist, genomgick en insulinbelastning (ITT – insulin tolerance test) som visade att de hade tillväxthormonbrist (högsta GH värde <3µg/l). Dosen insulin (Actrapid®) som gavs intravenöst var 0.1 E/kg, men reducerades ofta med 10-40%, speciellt om patienten hade många hypofysinsufficienser och ffa ACTH insufficiens. Blodsocker analyserades var 5:e-10:e minut men vid blodsockervärden kring 2.2 mmol/l togs blodsocker varannan minut. Under denna period intervjuades patienten också om eventuella symtom på lågt blodsocker. Puls, tendens att svettas och blekhet noterades. När blodsockret var under 2.0 mmol/l gavs druvsocker eller juice, och vid

blodsocker kring 1.3 mmol/l, eller vid långvarig hypoglykemi (≥ 25 min) gavs intravenös bolus med 30%-ig sockerlösning. Insulindosen som gavs var i median 0.07 E/kg (0.06-0.1). Lägsta blodsocker var i median 1.3 mmol/l (1.0-1.9), och kom efter 25 min (20-33). Hypoglykemin (< 2.2) varade i median i 25 min (15-33). Konklusionen blev att bland de 16 patienter som undersöktes med ITT var symtomen få på hypoglykemi och hos fem patienter saknades det helt symtom. Dessutom noterades lågt lägsta blodsocker och lång duration av hypoglykemi oavsett om patienterna hade ACTH brist eller ej. Dessa resultat förstärker ytterliggare rekommendationerna från the Growth Hormone Research Society att ITT endast skall utföras av erfarna centra. Om ITT fortfarande skall vara förstahands test vid misstanke om GHD efterfrågar vi mer enhetliga rekommendationer t.ex. avseende insulindos. För att förhindra framtida komplikationer vid ITT bör det dessutom finnas rekommendationer för när intravenös sockerlösning skall ges vid oacceptabelt lågt blodsocker eller vid långvarig hypoglykemi.

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