The growth hormone system and cardiac function in patients with growth hormone disturbances and in the normal population

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THE FOUR ORIGINAL PAPERS ARE:

1
Mikkel Andreassen, Henrik Vestergaard, Jens Faber, Caroline Kistorp, Lars Østergaard Kristensen. NT-ProBNP in patients with growth hormone disturbances. Clinical Endocrinology (2007) Vol 66, page 619-627 (1).

2
Mikkel Andreassen, Jens Faber, Andreas Kjær, Claus Leth Petersen, Lars Østergaard Kristensen. Cardiac function in growth hormone deficient patients before and after one year with replacement therapy: A magnetic resonance imaging study. Pituitary 2010 epub ahead of print August 21 (2).

3
Mikkel Andreassen, Jens Faber, Andreas Kjær, Claus Leth Petersen, Lars Østergaard Kristensen. Cardiac effects of three months treatment of acromegaly evaluated by magnetic resonance imaging and B-type natriuretic peptides Pituitary 2010 epub ahead of print August 10 (3).

4
Mikkel Andreassen, Ilan Raymond, Caroline Kistorp, Per Hildebrandt, Jens Faber, Lars Østergaard Kristensen. Insulin-like growth factor-I as predictor of all cause mortality and cardiovascular disease in an elderly population. European journal of Endocrinology (2009) 160, page 25-31 (4).

INTRODUCTION

The most prominent physiological feature of the Growth Hormone/Insulin-like Growth Factor I system (GH/IGF-I system) is its proliferative actions on bone tissue creating longitudinal growth in children. Within the last decades numerous other effects on different tissues and organs as well as on substrate metabolism have emerged. The influence of the GH/IGF system has been explored in different areas of medical research including glucose, lipid and protein metabolism (5), cancer (6,7), neurodegenerative diseases (8) and inflammatory diseases (9).

Another area that has attracted attention and has been subject to some controversy is a possible role of GH and in particular IGF-I in the complicated mechanisms involved in development of cardiovascular diseases (CVDs). Studies in patients with GH disturbances as well as in populations with normal GH-secretion have shown that the interaction between the GH/IGF-I system and CVD is complicated (10-12). The acromegalic cardiomyopathy has been intensively investigated and is now widely accepted as a unique entity (13), whereas the influence on cardiovascular structures of subnormal levels of IGF-I and variation within normal range are more questionable (14).

The aim of this PhD thesis was to investigate cardiac function in patients with GH disturbances and the prognostic role of IGF-I in relation to development of CVD in a normal population. Detection and monitoring of CVD was based primarily on cardiac magnetic resonance imaging and measurements of B-type natriuretic peptides which are very sensitive and precise methods to evaluate cardiac function.

THE GH/IGF-I SYSTEM

As illustrated in Figure 1 the secretion of the different hormones which constitute the GH/IGF-I system is regulated through feedback mechanisms. Pituitary GH secretion is stimulated by hypothalamic Growth Hormone Releasing Hormone (GHRH) and Ghrelin from the stomach and inhibited by hypothalamic somatostatin as well as by IGF-I (15,16). GH stimulates hepatic and local IGF-I production in other GH target tissues (17). Growth hormone has IGF-I independent effects on e.g. substrate metabolism (5), but the primary mediator of the actions of the GH/IGF-I system is IGF-I.

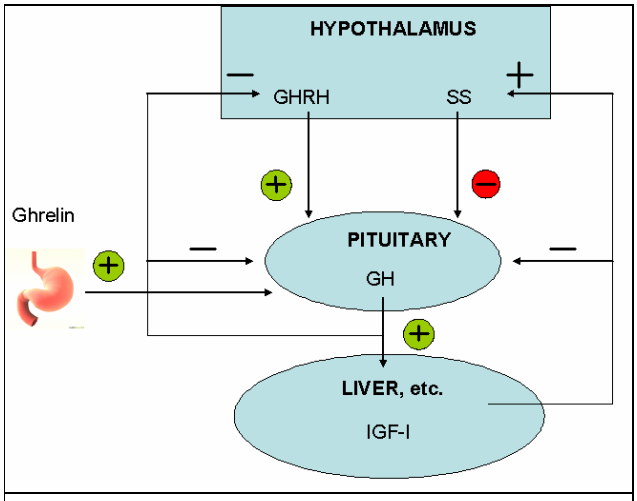

Figure 1
Regulation of the GH/IGF-I system through negative feedback-mechanisms.
SS=Somatostatin.

Circulating levels of GH and IGF-I are the two principal biochemical markers for evaluation of the activity of the GH/IGF-I system. The clinical utility of a single measurement of GH is limited by a short half life (about 18 minutes) and pulsative secretion (18). Therefore dynamic testing of GH levels is necessary to diagnose excess or deficient GH production. Circulating IGF-I has a longer half life, (about 7-20 hours) and exhibits only minor diurnal variations (17), implying that single measurements of IGF-I can be used for scientific and screening purposes and in monitoring of treatment in patients with GH-disturbances.

Less than one percent of IGF-I molecules are circulating in a free non-protein bound form whereas the remaining molecules form complexes with a family of six different IGF-I binding proteins (IGFBP1-6) and acid labile subunit (ALS) (17).

For clinical and scientific use measurement of total extractable IGF-I with immunoassays is by far the most widely used method. As a rough estimate of the free, bioavailable fraction, the molar ratio between IGF-I and its major binding protein IGFBP3 can be employed (19). Within the last decade different analyses of direct measurements of free IGF-I have become available. Frystyk has recently reviewed the clinical utility of free IGF-I compared to total IGF-I, and concluded that in most cases free IGF-I do not represent an analytical advantage (20). Measurements of total IGF-I levels are highly assay dependent, implying that cut off values and reference ranges obtained by one assay can not be extrapolated to other assays (21,22)

EVALUATION OF CARDIAC FUNCTION

Cardiac Imaging

The most widely used technique to assess cardiac volume, mass and function is two-dimensional echocardiography. The advantages of this method are that it is quick to perform, has low costs and can be performed at bedside without cooperation from the patients. As disadvantages echocardiography is highly operator dependent and blinding of the observer is difficult (23). Furthermore it is dependent on obtaining a sufficient acoustic window, and quantifications of volume and mass are limited by the use of geometric assumptions. Therefore use of echocardiography for scientific intervention studies requires large sample sizes to achieve a sufficient statistical power (24).

Left ventricle (LV) contractility can also be evaluated by radionuclide angiography. This technique is operator independent and does not make use of geometric assumptions, and it is considered to be superior to echocardiography for evaluation of ejection fraction (EF) (25). Measurements of cardiac muscle mass such as LV mass (LVM) can not be performed by radionuclide angiography.

An alternative to echocardiography is cardiac magnetic resonance imaging (CMRI). It is a method with a high accuracy, high intra- and interobserver reproducibility, and blinding during the reading process is standard (26). Therefore CMRI is considered the gold standard for assessing mass and volume of left and right ventricle (27,28). Furthermore it is a non-invasive method without exposure to ionizing radiation and without use of contrast agents. Compared to echocardiography a large reduction in sample sizes can be attained (24), implying that the method is very useful for intervention studies in rare diseases where only minor changes of cardiac function is expected as in e.g. growth hormone deficiency (GHD) (29).

As disadvantages CMRI is time consuming, it can not be performed at bedside, it requires a regular heart rhythm and patients need to cooperate by holding breath for 10-15 seconds approximately 10 times during one examination of 15 minutes duration. There are so far no published data where CMRI has been used for cardiac evaluation in patients with GHD. In acromegaly CMRI has been applied in two previous studies (30,31).

Natriuretic peptides

Measurements of natriuretic peptides have emerged as a useful diagnostic tool to evaluate the cardiac function. It has been widely used in scientific studies and it has now been implemented in daily clinical practice for evaluation of cardiac disease, especially left heart ventricle function (32).

The natriuretic peptides consist of a family of structurally similar peptide hormones: Atrial natriuretic peptide (ANP) and B-type

natriuretic peptide (BNP) which are mainly of cardiac origin, and C-type natriuretic peptide (CNP) (Figure 2) (33).

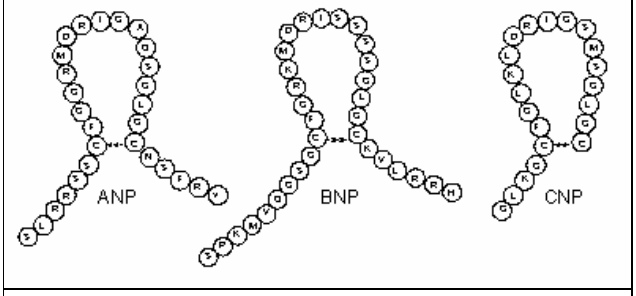

Figure 2
The structure and amino acid sequences of ANP (28-amino acids), BNP (32 amino acids) and CNP (22 amino acids). The Figure shows the biological active C-terminal part of the three pro-molecules

Elevated plasma concentrations of ANP and BNP are found in response to increased wall stretch as primarily observed in systolic heart failure, but also hypertrophy, hypertension and diastolic dysfunction with preserved systolic function increase secretion of natriuretic peptides from cardiac tissue (33). ANP and BNP seem to counteract some of the negative consequences of heart failure by promoting natriuresis, vasodilatation, inhibition of the renin-angiotensin-aldosterone system, and modulation of cardiac hypertrophy (Figure 3) (34). The role of CNP in cardiovascular physiology is questionable.

Measurements of BNP seem to be superior to ANP in detection of cardiac dysfunction (35). In case of BNP, measurement can also be performed on the co-secreted biological inactive N-terminal fragment (NT-proBNP, 76 amino acids) which has a longer half-life (32).

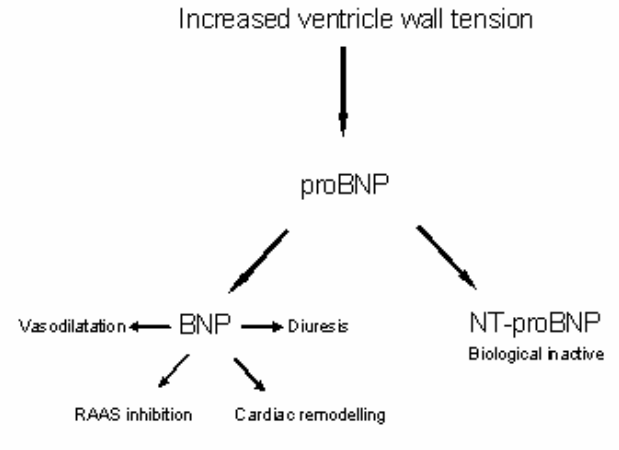

Figure 3
Secretion and biological actions of BNP. RAAS= Renin-Angiotensin-Aldosterone System

Natriuretic peptides are very sensitive markers of cardiac dysfunction, and BNP and NT-proBNP concentrations are correlated to the severity of heart failure and reflect improvement or aggravation during treatment (32,36).Thus, BNP or NT-proBNP measurements are now recommended as daily routine (32).

THE GH/IGF-I SYSTEM AND CARDIOVASCULAR DISEASE

Epidemiological studies of acromegalic patients (37-39) and of hypopituitary patients with GHD (40-44) have suggested that pathological increased as well as reduced GH secretion and IGF-I production seems to be related to increased risk of CVDs. The studies suggest that the interaction between GH/IGF-I effects and development of CVD probably involve many different mechanisms. Direct actions on cardiomyocytes and endothelium have been proposed as well as indirect effects through changes in cardiovascular risk factors such as body composition, blood pressure, glucose and lipid metabolism and inflammation (10,12,45-47). The cardiovascular risk factors may be influenced in different directions by the GH/IGF-I system. The multiple interrelated effects give rise to a very complex scenario, as will be summarized in the following sections considering cardiac and vascular aspects.

DIRECT EFFECTS ON CARDIAC TISSUE

Although cardiomyocytes are considered to be terminally differentiated cells and thus unable to proliferate it is well documented that the GH/IGF-I system has the potential to influence cardiac size, as well as contractility. In animal models it has been shown that both GH and IGF-I receptors are expressed on myocardial tissue (48-50). The data obtained in animals and also experimental in vivo data obtained in humans suggest that cardiac effects of the GH/IGF-I system are primarily mediated by circulating or locally secreted IGF-I (50-55).

Cardiac mass

Numerous observations in acromegaly have clearly indicated that prolonged GH/IGF-I excess increases cardiac mass. The underlying mechanisms seem to involve increased protein synthesis in cardiomyocytes, increased connective tissue synthesis, reduced apoptosis and probably also oedema (31,56-59).

In humans there are very limited data concerning the relationship between cardiac size and physiological levels of IGF-I. Two studies of hypertensive patients have shown a positive association between LVM and serum IGF-I (60,61), a third study showed no association in normotensive type 2 diabetes patients (62) and finally a fourth study detected an inverse association between LVM and IGF-I levels in hypertensive patients (63). Thus whether or not variations of IGF-I within the normal range influence cardiac mass is not clarified.

In untreated GH-deficient patients reduced (64-70) or unchanged (71-74) LVM have been observed. During treatment some studies have reported an increase in LVM (64-66,68,70,72,75-78), whereas others have been unable to detect any influence of increased levels of IGF-I (71,73,79-84).

In acromegaly increased LVM is a very consistent observation (30,56,85-88). This specific cardiac hypertrophy seems to be related to the severity and duration of IGF-I excess (89). It may be aggravated in case of hypertension, but it is also present in normotensive acromegalic patients (90). Long-term treatment (5 years) seems to reduce or even normalize cardiac mass (91).

Cardiac contractility
Experimental in vitro models (54,92), animal models (93) and GH/IGF-I treatment in healthy volunteers (55,94,95) have all suggested that IGF-I excess increases cardiac contractility. The underlying mechanisms seem to involve increase in intracellular calcium content and maybe also an increased sensitivity to calcium (54,96).

By contrast there are very limited data concerning the physiological influence of endogenous IGF-I on cardiac contractility in normal healthy individuals. To my knowledge only one previous study has addressed this question. Colao et al. showed that in young top level rowers there was a positive correlation between IGF-I and the ejection fraction (EF), supporting that the inotropic effects observed in experimental models are also operating under normal physiological conditions in humans (97).

Based on the positive inotropic effect of IGF-I several small short term studies investigating GH treatment in chronic heart failure (CHF) have been conducted with very conflicting results. In a recently published meta-analysis of 11 studies including a total of 230 patients a positive effect of GH treatment on cardiac contractility and symptoms assessed by the NYHA classification was found (98). However, a large scale placebo controlled long term study including mortality data is lacking.

Concerning patients with long-term exposure to either excess or reduced levels of endogenous IGF-I it has not been possible in a consistent way to show an association between IGF-I levels and cardiac contractility as evaluated by the EF. A hyperkinetic initial phase of acromegaly with increased cardiac contractility and cardiac output (CO) has been suggested (87,99). However in general acromegalic patients are diagnosed after many years of disease with concomitant remodelling of cardiac tissue leading to severe hypertrophy, and they often present with normal or reduced EF at time of diagnosis (30,86,89,91,100). If left untreated the acromegalic cardiomyopathy progresses to an end-stage with reduced EF and overt cardiac failure (101,102). Several studies have reported a beneficial effect of long-term treatment (up to 5 years) on systolic function (88,100-103).

In untreated GHD very divergent results have been reported with normal (68,71-74,81), reduced (64,66,69,70,104) and in a single study increased EF (67). In line with these equivocal results there is no consensus as regard the effect of treatment (14,29).

Vascular structures and development of atherosclerosis
Until 2002 most studies investigating atherosclerosis and IGF-I action focused on the expression of IGF-I receptors on vascular smooth muscle cells (VSMC) (105). IGF-I and the IGF-I receptor has been detected in atherosclerotic lesions in humans, and in vitro it has been shown that IGF-I exerts proliferative actions on VSMC (106-108). Therefore it was suggested that IGF-I might be a promoter of arterial obstructive lesions (109).
Intervention studies employing somatostatin analogues have been carried out in humans for secondary prevention after percutaneous transluminal coronary angioplasty (PTCA). One study showed beneficial effects on restenosis but no improvement in clinical variables (110). Another study found a reduction in cardiovascular events without improvement of arterial lumen diameter (111). A third study reported no effect on restenosis or cardiovascular events (112). Taken together reduction of GH-action to prevent further development of atherosclerosis has not shown very promising results and to my knowledge no trials of GH/IGF-I lowering therapies have been published since 1997 (113).

In 2002 Juul et al. reported that low levels of IGF-I predicted the overall development of ischemic heart disease (IHD) in a general population (114). The result was supported by a study on cardiovascular mortality from 2004 (115) and another from 2005 showing increased incidence of ischemic stroke with low IGF-I levels (116). These studies led to an increased attention on beneficial vascular effects of a high GH/IGF-I action. As possible protective mechanisms direct actions of IGF-I on endothelium and cardiomyocytes as well as indirect actions through a favourable influence on lipid and glucose metabolism have been suggested (117-121). Moreover a study from 2003 showed that low IGF-I levels specifically predicted development of CHF (122). However, more recent studies have not been able to identify low IGF-I as a risk factor for development of IHD, stroke or CHF (123-125). These discrepancies are also reflected in cross sectional studies of prevalent CVD where low (117), high (126,127) and unchanged IGF-I levels (128) have been reported.

Studies in patients with GHD have supported that lack of GH/IGF-I activity might have unfavourable influence of cardiovascular risk factors such as body composition, lipids and inflammation (46,47,70,129-132). In one study premature atherosclerosis as judged by increased intima-media thickness (IMT) in the coronary arteries was reported (133) whereas another study found unchanged IMT compared to healthy control subjects (134).

Concerning vascular involvement and development of atherosclerosis in acromegaly the literature is more controversial. One study using cardiac computed tomography (CT) for detection of calcified plaques in the coronary arteries and one using ultrasound for measurement of IMT observed evidence of increased atherosclerosis (135,136). By contrast two other investigations employing the same diagnostic modalities did not observe increased numbers of calcified plaques or increased IMT (137,138). In fact the ultrasonic study reported reduced formation of atherosclerotic changes as judged by IMT in acromegalic patients. The authors suggested that increased local nitric oxide (NO) production might exert a beneficial effect on the endothelium (138). As another possible mechanism anti-inflammatory effects of high IGF-I levels could have a favourable influence on the endothelium (47,139). Moreover a large autopsy study has shown reduced atherosclerosis in acromegaly compared to what would be expected based on the CV risk profile (56). Thus, acromegalic patients might actually be protected from atherosclerosis.

The GH/IGF-I system has also direct effects on the endothelium. It has been shown that IGF-I stimulates the release of NO with subsequent vasodilatation (140,141). This physiological effect cannot be brought into context with observation of blood pressures in patients with GH-disturbances. In untreated GHD both increased (142), unchanged (69,73,74) and reduced blood pressure has been reported (66,71) as well as equivocal effects of replacement therapy (10). Furthermore it is well known that acromegaly is associated with hypertension (90).

Taken together the GH/IGF-I system interacts with the cardiovascular system in many different ways. In acromegaly the pathologi-

cal involvement seems primary to be related to a direct effect of IGF-I on cardiac structures. In GHD and in populations with intact GH-secretion both direct cardiac effects as well as effects of vascular structures might be of importance.

AIMS AND HYPOTHESES

The present PhD thesis investigated the influence of different levels of IGF-I in relation to development of CVD. We aimed to test the hypotheses that

- pathological reduced and increased levels of IGF-I exert harmful influences on cardiac function
- treatment of the GH-disturbances improve cardiac function
- low levels of IGF-I in the normal population increase the risk of developing CVDs

Specifically the following objectives were studied:

- Cardiac function in untreated GHD and acromegaly and effects of two years of treatment evaluated by serial measurements of B-type natriuretic peptides
- Cardiac function in untreated GHD and effects of one year of GH replacement therapy evaluated by measurements of B-type natriuretic peptides and CMRI
- Cardiac function in untreated acromegaly and effects of three months of treatment evaluated by measurements of natriuretic peptides and CMRI
- The influence of endogenous IGF-I in regard to development of CVDs in a normal population

To investigate the above objectives we conducted four studies:

Study I NT-proBNP in patients with growth hormone disturbances (1)

Study II Cardiac function in growth hormone deficient patients before and after one year with replacement therapy: A magnetic resonance imaging study (2)

Study III Cardiac effects of three months treatment of acromegaly evaluated by magnetic resonance imaging and B-type natriuretic peptides (3)

Study IV Insulin-like growth factor-I as predictor of all cause mortality and cardiovascular disease in an elderly population (4)

STUDY POPULATIONS

Study I

This was a retrospective study of 10 acromegalic (age 48 ± 12 years) and 10 GH-deficient patients (age 41 ± 14 years) from the outpatient clinic at Department of Endocrinology, Herlev Hospital. At baseline all patients were without prevalent CVD except hypertension. Serum NT-proBNP was measured in stored serum samples obtained before and 3, 6, 12 and 24 months of treatment. In GHD the treatment consisted of daily injections of GH with a dose titrated according to IGF-I levels. In acromegaly transphenoidal surgery as well as medical therapies were employed.

Study II

Sixteen consecutive patients (8 males and 8 females, mean age 49 years (range 18-75)) with severe GHD and 16 matched control subjects were included and prospectively examined. All patients were recruited from the outpatient clinic at Department of Endocrinology, Herlev Hospital. CMRI was performed at baseline and after one year of GH-replacement therapy. IGF-I, BNP and NT-proBNP were measured before and after 1,2,3,6 and 12 months of treatment.

Study III

Eight patients (5 males and 3 females, mean age 53 ± 12 years (range 30-70) from the outpatient clinic at Department of Endocrinology, Herlev Hospital and 8 matched control subjects were included. CMRI was performed at baseline and after three months of treatment employing different routine treatment modalities. IGF-I, BNP and NT-proBNP were measured at baseline and after 1,2 and 3 months of treatment.

Study IV

It was a population based prospective study comprising 642 individuals from a central part of Copenhagen. The participants were included in the study in the period from September 1998 until January 2000 (143). A physical examination was performed and an extensive medical history including data on hospital administrations and current medication was obtained. All participants underwent a transthoracic echocardiographic examination. Systolic function was evaluated in a blinded fashion by two experienced cardiologists.

Development of CHF was evaluated in individuals with normal left ventricle ejection fraction (LVEF ≥ 50%) and no history of CHF or previous hospital administrations for the diagnosis of acute myocardial infarction (MI). These criteria were met by 576 participants. The analysis of the incidence of first major cardiovascular event was evaluated in 504 participants without prevalent CVD defined by LVEF ≥ 50% and no previous hospital administrations for the diagnoses of acute MI, unstable angina pectoris, stroke, transient ischemic attack (TIA) or a history of CHF or angina pectoris.

METHODS

Immunoassays

IGF-I measurements were performed at Endocrine Research Laboratory at Herlev Hospital. In study I by an immunoradiometric assay from Nichols Institute Diagnostics (San Clemente, CA, USA). The normal gender and age matched reference intervals were adopted from a Scandinavian study using the same method (144).

In study II, III and IV IGF-I was measured by an immunosorbent assay (ELISA) from R&D Systems (Minneapolis, MN, USA). The intra- and interassay CVs were 3 and 7%. The normal ranges and Z-scores for IGF-I were based on our own age- and gender appropriate reference ranges obtained in 724 individuals (145).

IGFBP3 was measured by an ELISA from R&D Systems (Minneapolis, MN, USA). The intra- and interassay CVs were 3 and 4%.

GH was measured by a fluoroimmuno assay (Delphia, PerkinElmer, Turku, Finland) with a lower limit of detection of 0.04 ng/ml. The intra- and interassay CVs were 1% and 6%

In study I and IV serum NT-proBNP was measured by an immunoassay from Roche Diagnostics (Mannheim, Germany). Lower limit of detection was 5 pg/mL. The intra- and interassay CVs were 2% and 5%.

In study II and III NT-proBNP was measured by a solid double antibody sandwich technique with Chemiluminescense as signal (Immuno 2500, Siemens Healthcare diagnostics, Deerfield, IL,

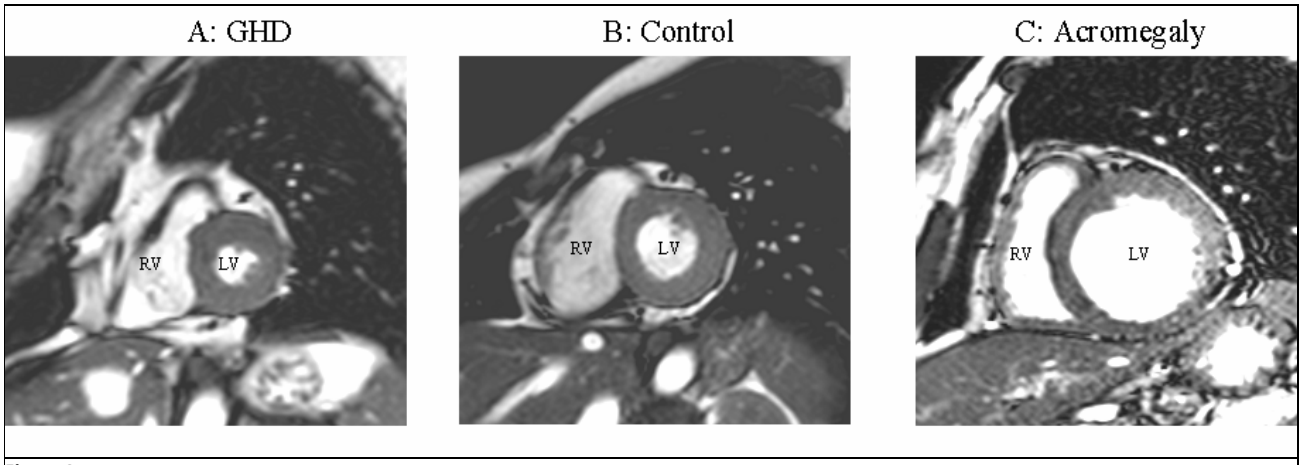

Figure 4
Basal short axis slice through right (RV) and left ventricle (LV) in end-systole in untreated GHD (A, EF=80%, ESV=10 ml, LVM=58 g), healthy control subject (B, EF=73%, ESV=34 ml, LVM=93 g), and untreated acromegaly (C, EF=17%, ESV=440 ml, LVM=359 g).

USA). Measuring range was < 20-35000 pg/mL with intra- and interassay CVs both < 5%.

Plasma BNP was measured by an automated two-site sandwich immunoassay technique using chemiluminescence (Siemens, ADVIA Centaur, Germany). The sensitivity of the assay was <2.0 pg/mL and the intra- and interassay CVs were 1.2% and 2.3% (146).
All variables were measured in duplicate with exception of IGF-I in study IV where only single measurements were obtained.

Cardiac magnetic resonance imaging
The recordings were done at Frederiksberg Hospital, University of Copenhagen in accordance with a standard protocol for CMRI as essentially described previously (147). CMRI was performed on a 1.5 Tesla whole body scanner (Intera; Philips Medical Systems, MN, USA). Following localization of the long axis of the heart continuous true short-axis slices were acquired using breath-hold ECG-triggered cine MRI gated prospectively (Figure 4). The heart was covered by 10-15 slices of 10 mm.

The endocardial contours were drawn at the end-diastole and end-systole frame in all slices. End-diastolic volume (EDV) and end-systolic volume (ESV) were calculated by adding volume measurement in end diastole and end systole. To obtain measurements of LV myocardial volume epicardial contours were drawn at the end-diastolic frame in all slices. Myocardial volume was subsequently calculated by adding the differences between epi- and endocardial volumes. All investigations were read by two independent observers in a blind manner. In case of discrepancies of more than 15% the investigation was re-evaluated by a third blinded observer and the final result calculated as the mean of the two readings with the highest level of agreement.

In Table 1 and Figure 5 the level of agreement between the two primary investigators is presented. Figure 5 (A, B and C) gives the interobserver variability for EDV, ESV and LV myocardial volume by Bland-Altman analyses. The two dashed lines in each figure represent 95% CI. As reflected in the median values (Table 1) as well as in the Bland-Altman plots there were no signs of any systematic differences between the two observers.

In Table 1 the Intraclass Coefficients of Variation (ICV) (Two way random – absolute agreement model) for the three variables are also given. For LV myocardial volume ICV was 0.98 (0.97-0.99) indicating that 98% of the total variation can be explained by variation within the variable and that observer variation only account for approximately 2% of total variation. For EDV and ESV the ICV were even higher.

All CMRI measurements presented in study II and III with the exception of EF are indexed (I) to body surface area.

Criteria for cardiovascular disease and chronic heart failure
In study IV development of CVD were ascertained after a median of 5-years (range 2-63 months) follow-up. Cardiovascular events requiring hospitalization were used as outcome. All events were recorded by the discharge registry of the Danish National Board of Health (148). The codes of diagnosis were assigned according to the International Classification of diseases 10th revision (ICD-10). Development of CHF was defined by a discharge diagnosis code I50. First major cardiovasculare event was a combined outcome including non-fatal MI, fatal coronary heart disease, unstable angina pectoris, CHF, stroke and TIA (ICD-10 codes I20.0-I22, I24, I42, I46, I50, I63, I65 and I66).

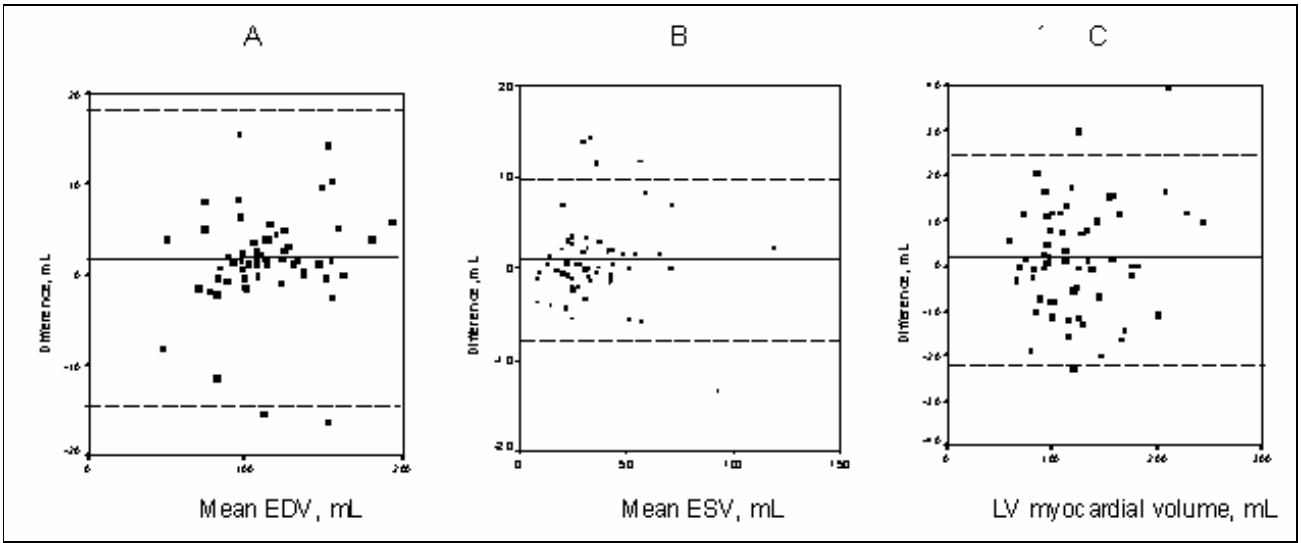

Figure 5
Bland-Altman analyses for interobserver variability of LV EDV (A), ESV (B) and myocardial volume (C). Solid lines indicate the mean difference and dashed lines 95%CI. One acromegalic patient with severe hypertrophy and dilatation of LV is not represented in the figure.

Echocardiography
The images obtained by echocardiography in study IV were evaluated off-line in a blinded fashion by two expert cardiologists. The procedure has been described in detail previously (143).

Diagnostic tests
GHD:
The diagnosis of GHD (study I and II) was based on the 2-hours pyridogstigmin-GHRH test, with a zenith GH level of 6.5 ng/mL as cut-off.

Acromegaly:
The diagnosis of active acromegaly (study I and III) was based on clinical features, elevated gender- and age-adjusted IGF-I levels and an insufficient GH suppression (nadir GH>0.3 ng/mL) during oral glucose tolerance test.

SUMMARY OF RESULTS

Study I
N-Terminal Pro-B-Type Natriuretic Peptide in patients with growth hormone disturbances.

In acromegalic patients baseline NT-proBNP were lower as compared to healthy matched control subjects (P<0.001). NT-proBNP levels changed during treatment (P=0.002). After 3 months of treatment serum NT-proBNP peaked with a 4-fold increase. In GHD NT-proBNP levels did not differ from control subjects (P=0.19), and did not change during treatment (P=0.39) (Figure 6) (1).

Study II
Cardiac function in growth hormone deficient patients before and after one year with replacement therapy: A magnetic resonance imaging study.

Left ventricle mass index (LVMI), EF, cardiac output index (CI) and stroke volume index (SVI) as well as levels of BNP (P=0.09) and NT-proBNP (P=0.91) were similar at baseline compared to control subjects. The patients had significantly smaller LV EDVI and ESVI (Table 2). In untreated patients EF was inversely associated to EDVI (P<0.001) (Figure 7). LVMI was positively associated with baseline IGF-I levels (P=0.03). No significant changes in any CMRI variable occurred during one year of GH-treatment. BNP levels were unchanged (P=0.88), whereas NT-proBNP tended to decrease (P=0.052).

n=63	Investigator 1	Investigator 2	P-value	ICV (95% CI)
EDV, mL	112 (91-142)	114 (98-137)	0.98	0.99 (0.99-1.00)
ESV, mL	31 (24-47)	33 (24-45)	0.90	0.99 (0.99-1.00)
LV myocardial volume, mL	121 (92-154)	117 (95-158)	0.95	0.98 (0.97-0.99)

Table 1
Comparisons between investigator 1 and 2 as concern LV EDV, ESV and myocardial volume. The P-values are obtained by T-Test. ICV=Intraclass Coefficient of Variation

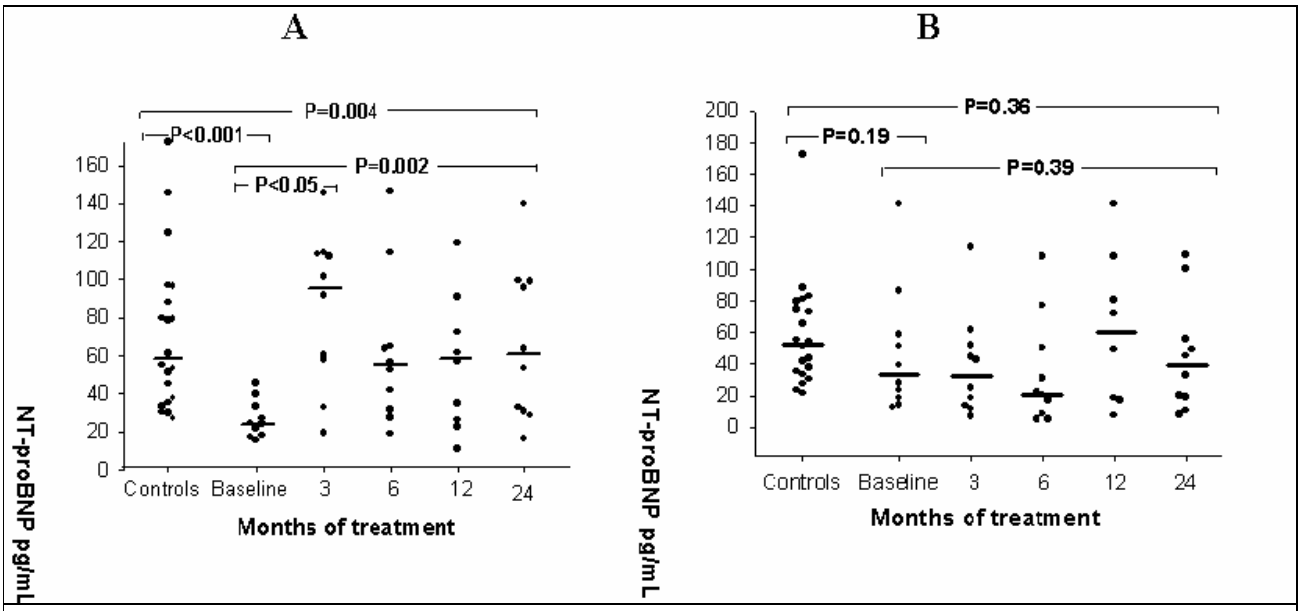

Figure 6
NT-proBNP in acromegalic patients (A), GH-deficient patients (B) and in control subjects. The horizontal line in each column represents the median value.

Additional results:
The association between EDVI and EF vs. IGF-I is shown in Figure 8 for the GH-deficient patients and control subjects separately and combined (N=32). The combined analysis showed a significant positive relationship between EDVI and IGF-I (P=0.004) and a significant inverse relationship between EF and IGF-I (P=0.05). The association between EDVI and IGF-I remained statistical significant after adjustment for LVMI (P=0.010). All analyses were adjusted for age.

Study III
Cardiac effects of three months treatment of acromegaly evaluated by magnetic resonance imaging and B-type natriuretic peptides.

At baseline the patients had increased LVMI compared to control subjects. LVMI was positively associated with the IGF-I Z-score (r = 0.72, P=0.041). LVMI was a strong predictor of EF. Adjusted for age an increase in LVMI of 10 g/m2 led to a decrease in EF of 6% (95%CI, 4-8%,P<0.001). Baseline levels of BNP and NT-proBNP were inversely associated with EF and positively to LVMI.

After three months of treatment there was an increase in EDVI, a decrease in heart rate and an increase in levels of BNP and NT-proBNP (Table 3).

Study IV
Insulin-like Growth Factor-I as predictor of all cause mortality and cardiovascular disease in an elderly population

During follow-up 58 individuals (11.5%) without prevalent CVD developed a major cardiovascular event. Plasma IGF-I was not associated with the development of a cardiovascular event (Table 4 and Figure 9) and age-adjusted IGF-I levels did not differ be-

	Controls (N=16)	GHD Baseline (N=16)	GHD 12 months (N=14)	P value controls vs. GHD baseline	P value GHD baseline vs. treatment
LV EDVI, mL/m^2	64.3 ± 14.9	53.6 ± 11.8	55.6 ± 10.5	0.032	0.42
LV ESVI, mL/m^2	20.9 ± 7.4	15.8 ± 5.6	15.4 ± 6.0	0.038	0.97
LV SVI, mL/m^2	43.4 ± 9.5	37.7± 6.7	40.2 ± 7.0	0.063	0.29
LV EF, %	68 ± 7	71 ± 5	72± 8	0.13	0.58
LVMI, g/m^2	58.7 ± 8.7	55.2 ± 9.2	58.3 ± 11.9	0.28	0.059
Heart rate	68 ± 14	73 ± 13	72 ± 10	0.38	0.75
CI, L/min/m^2	2.85 ± 0.49	2.68 ± 0.56	2.81 ± 0.55	0.37	0.25

Table 2
Comparison of cardiac characteristics obtained by CMRI in control subjects vs. untreated GH-deficient patients and effects of 12 months GH-treatment. Values are presented as mean ± 1 SD or median (IQR)

tween the individuals who developed a CV event and those who did not (71 (55-92) vs. 72 (59-87) ng/mL; P= 0.78).

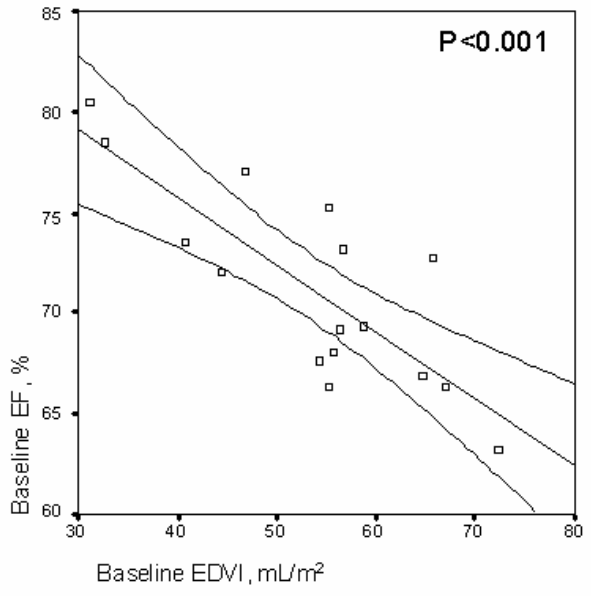

Figure 7
Baseline association between EF vs. EDVI. The p-value is obtained by linear regression analyses adjusted for age.

Individuals who developed CHF as their primary cardiovascular event (n=19) had significantly higher age-adjusted IGF-I levels as compared to those who did not develop CHF (89 (66-117) vs. 71 (58-86) ng/mL; P= 0.009). Adjusted for confounding variables high IGF-I levels were associated with increased risk of developing CHF (Figure 9 and Table 4) (4).

In the total cohort there was no significant relationship between EF and IGF-I levels. However in this study EF above 60 % was not further quantified. In the sub-group of individuals with EF below 60% there was a significant inverse association between EF and IGF-I (age-adjusted) (P=0.04).

In the publication data on the influence of plasma IGF-I in relation to all cause mortality were also presented. However, the relationship between IGF-I and all cause mortality was not the aim of the PhD thesis and these result are therefore not presented or discussed in the thesis.

DISCUSSION

Hypotheses and major results

The aim of this PhD thesis was to test the hypotheses that pathological low and high levels of IGF-I exert harmful influences on cardiovascular structures and functions and that correction of the GH-disturbances improve cardiac function. Concerning the normal population the hypothesis was that low levels predict CVDs.

In the GH-deficient patients LVMI and systolic function (EF, SVI, CI) were unchanged compared to matched control subjects and no effect of GH replacement therapy could be detected. However we detected reduced EDVI and ESVI in GH-deficient patients before and after treatment. In agreement with the morphological data, GHD and subsequent GH treatment did not influence the levels of natriuretic peptides measured in two different cohorts. In acromegaly there was increased LVMI, but cardiac systolic function (EF, SVI, CI) was similar to healthy control subjects and short-term treatment was associated with evidence of cardiac impairment as judged from increased LV EDVI. The increase in levels of natriuretic peptides observed in the retrospective as well as in the prospective CMRI study was in agreement with the morphological changes. In the normal population high IGF-I levels actually predicted development of CHF but did not influence the overall incidence of CVD. Thus, considering some of the major results we had to reject our hypotheses illustrating the complex interacting between GH/IGF-I activity and cardiovascular structure and function.

One of the advantages of the studies included in this PhD thesis is a detailed assessment of cardiac function. Concerning the prospective studies in patients with GH-disturbances the use of CMRI was considered essential. In GHD only subtle cardiac changes are

	Controls (N=8/16)	Baseline	1 months	2 months	3 months	P-value Baseline vs. Controls	P-value Baseline vs. Treatment
IGF-I, ng/mL	125 ± 37	377 ± 135	225 ± 76	175 ± 65	189 ± 52	0.001	<0.001
IGF-I Z-score	0.6 (-2.5-1.6)	4.5 (2.5-6.4)	2.8 (-1.6-4.0)	1.2 (-2.2-.3.6)	2.3 (-0.1-3.3)	<0.001	0.002
NT-proBNP, pg/mL	51 (20-275)	63 (20-1004)	60 (20-1859)	65 (20-1547)	80 (20-3391)	0.48	0.027
BNP, pg/mL	13 (0.58-35)	7 (0.58-286)	7 (2-544)	7 (4-255)	20 (1-489)	0.93	0.033
LV EDVI, mL/m^2	66 (40-94)	66 (27-238)			72 (43-248)	0.45	0.007
LV ESVI, mL/m^2	19 (9-44)	21 (5-197)			27 (5-177)	0.38	0.69
LV SVI, mL/m^2	44 (30-53)	42 (23-53)			46 (37-71)	0.65	0.15
LV EF, %	71 (54-77)	67 (17-83)			63 (28-89)	0.51	0.99
LVMI, g/m^2	62 (49-76)	92 (53-161)			87 (58-175)	0.016	0.54
Heart rate	65 (58-96)	72 (52-89)			65 (53-74)	0.50	0.04
CI, L/min/m^2	3.0 (2.2-3.5)	2.8 (1.7-3.7)			2.8 (2.4-4.5)	0.82	0.60

Table 3
Comparisons of cardiac characteristics obtained by CMRI and levels of IGF-I and natriuretic peptides in control subjects vs. untreated acromegalic patients and effects of 3 months of GH/IGF-I lowering therapy. Data are presented as mean ± 1SD or as median (ranges).

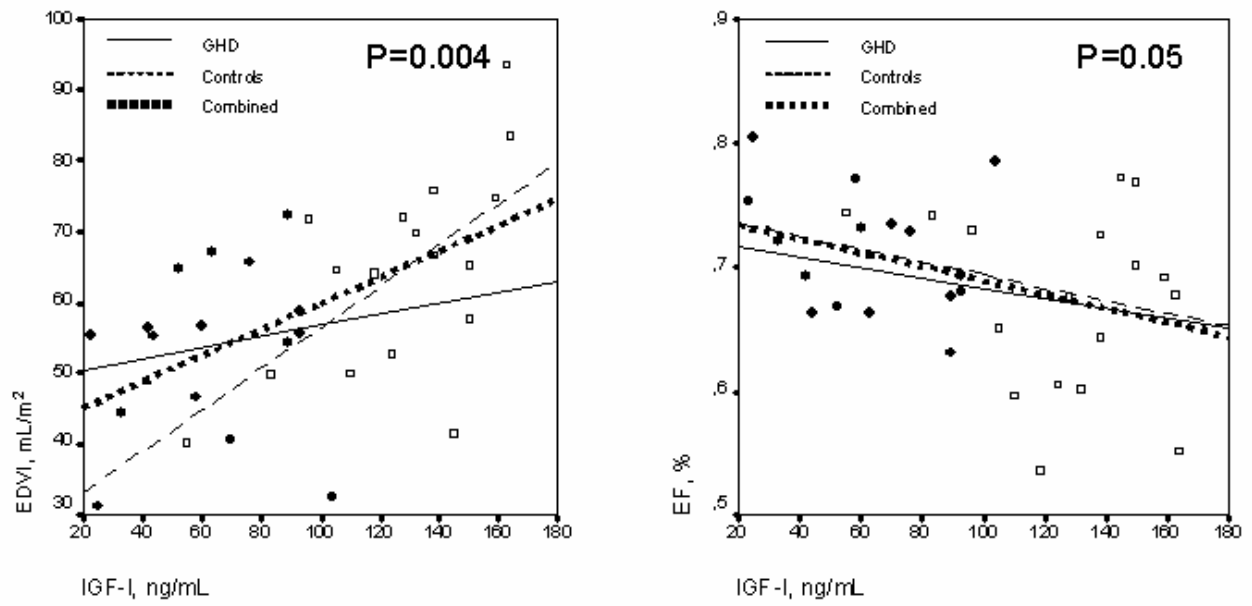

Figure 8
Associations between EDV and EF vs. IGF-I. In each scatter-plot regression lines are provided for untreated GHD, healthy control subjects and a combined analysis. The P-values are obtained in the combined cohort by linear regression analysis adjusted for age.

expected (29) and unless very large study populations can be provided highly sensitive methods are necessary. Based on the accuracy of CMRI we consider the prospective study to have sufficient statistical power to detect clinical relevant changes in cardiac size and function (24). Inaccurate cardiac imaging by echocardiography, in concert with numerous other factors, might contribute to explain the very divergent results reported on cardiac involvement in GHD and effects of replacement therapy.

To my knowledge the population based study (study IV) is the only one investigating IGF-I as predictor of CVD where cardiac imaging systematically has been obtained at baseline. This gave us the opportunity to exclude participants with silent impairment of systolic function thereby increasing the likelihood that observed associations might actually be causally related.

Cardiac size.
In the acromeglic patients our data confirmed that the untreated stage is associated with increased LVMI. In GHD there were no statistical significant differences between patients and control subjects, but there was a positive association between LVMI and IGF-I and a trend to an increase during one year of GH treatment. These data suggest that both pathological high and low levels of IGF-I may influence cardiac mass. Concerning physiological levels of IGF-I and LVMI we could not detect any association in the healthy control subjects (study II).

In GHD, EDVI and ESVI were significantly reduced, suggesting that reduced GH/IGF-I activity influence LV volume. This reduction in LV volume was not accompanied by reduced LVMI. As one possibility reduced GH/IGF-I activity may change the architecture of the cardiac tissue as evaluated by the relationship between LV mass and volume. This hypothesis was to some degree supported by the combined regression analysis of GH-deficient patients and control subjects, which showed that LV EDVI was positively associated to serum IGF-I also when LVM was taken in consideration. Possible hemodynamtic consequences of reduced LV volume will be discussed in the following section.

Cardiac contractility
An important issue concerning GH/IGF-I activity and cardiac function is the influence on cardiac contractility. Consistently, different experimental models have indicated that IGF-I excess increases cardiac contractility (54,55,92-95).

In the acromegalic patients (study III) there was an increase in EDVI and in levels of natriuretic peptides after short-term treatment. We interpret that these changes most likely represent a decrease in cardiac function when levels of IGF-I are suddenly reduced, suggesting that endogenous long-term (median 16 years) excess of IGF-I has a direct positive inotropic effect on the cardiomyocytes as observed in the initial hyperkinetic phase. However this was not reflected in higher EF and CI compared to control subjects. Probably the concomitant increased LVM with increased stiffness had a detrimental effect on the contractility as illustrated by the strong inverse association between EF and LVMI. Therefore our data support that acromegalic heart disease is a complex condition where cardiomyocyte contractility and the degree of hypertrophy are two different GH/IGF-I dependent players operating in opposite directions (13).

By contrast the data obtained in GHD and in the healthy control subjects lend no support of a positive inotropic effect of IGF-I. There was no difference in EF between untreated patients and control subjects (EF GHD=71% ± 5, EF controls=68% ± 7) and there was no evidence of positive inotropic effects of GH replacement therapy. Furthermore in GH-deficient patients as well

Variable	HR (95%CI) for IGF-I values in the 4th quartile	P-value	HR (95%CI) per 1 SD increase in Log IGF-I.	P-value
[1]CHF (n=19): IGF-I				
Age-adjusted	4.25 (1.71-10.57)	0.002	1.66 (1.17-2.36)	0.005
[3]Multivariable model	5.02 (2.00-12.64)	0.001	1.76 (1.14-2.70)	0.010
[2]First major CV event (n=58): IGF-I				
Age-adjusted	1.28 (0.73-2.25)	0.40	0.97 (0.72-1.38)	0.80
[3]Multivariable model	1.05 (0.59-1.90)	0.86	0.95 (0.72-1.23)	0.68

Table 4 Hazard ratios (HR) for risk of CHF and of a major CV event according to plasma IGF-I.

[1]The analyses were restricted to 576 individuals with LVEF ≥ 50% and no history of CHF or MI.
[2]The analyses were restricted to 504 individuals with LVEF ≥ 50% and no history of a major CVD.
[3]Adjusted were made for age, sex, hypertension, DM, atrial fibrillation, smoking status, NT-proBNP and cholesterol.

as in control subjects there were insignificant inverse associations between EF vs. IGF-I, but in the combined analyses it was statistical significant. In agreement with these results IGF-I levels adjusted for age (study IV) were inversely associated with the EF in the sub-group of individuals from the general population with EF<55%. In another study we have examined CHF patients and observed no association between EF vs. endogenous IGF-I (128). Thus, concerning contractility expressed as EF our results ob tained in different populations with normal or low IGF-I activity suggest a discrepancy between reported acute direct actions of IGF-I compared to what can be observed during long-term exposure to endogenous IGF-I.

As discussed previously low levels of IGF-I in GHD seem to reduce LV volume (Figure 8) and this was associated with an increased contractility (Figure 7). The reduced EDV together with increased EF and low IGF-I levels, suggest that GH-deficient patients are able to compensate small LV volumes by increasing cardiac contractility. Increased EF despite low IGF-I levels might be due to an activation of various compensatory processes e.g. neuronal, hormonal, para- or autocrine mechanisms to maintain CO. As an example an IGF-I deficient mouse model showed an increased EF due to increased sensitivity to catecholamines (149). In analogy studies in humans have shown that sympathetic activity measured by muscle sympathetic nerve activity is increased in GHD

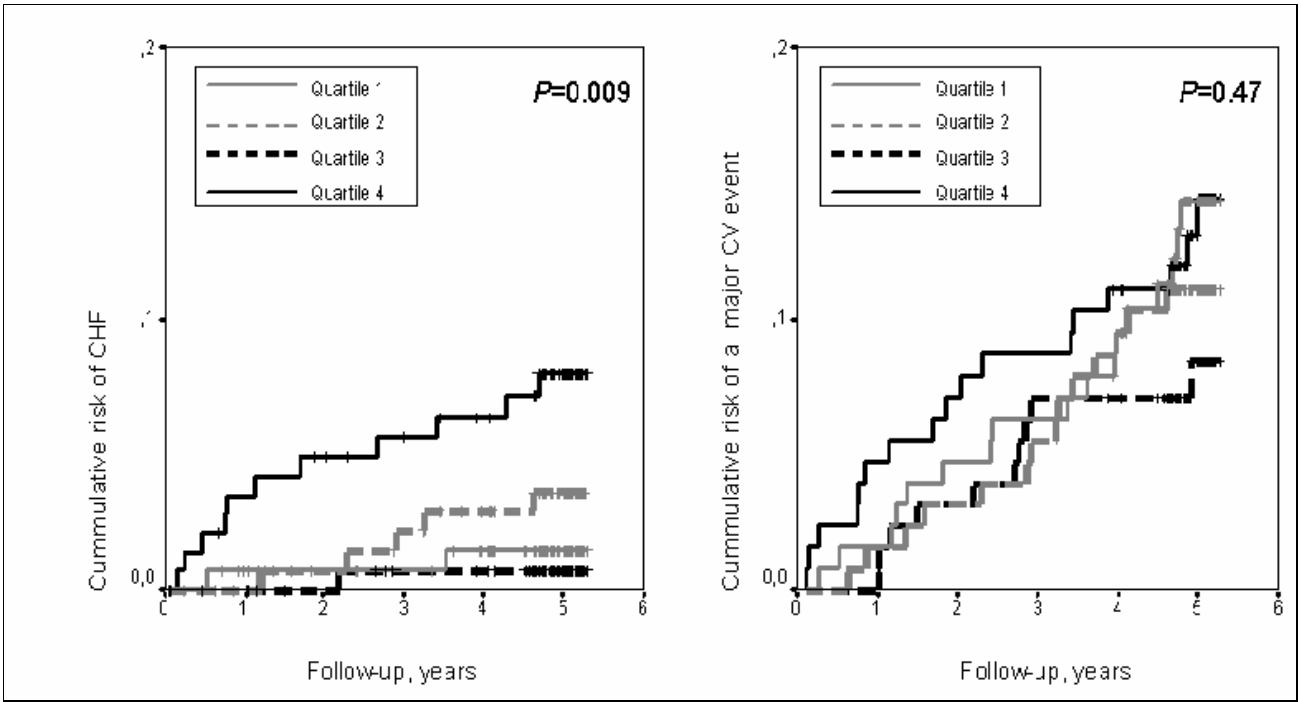

Figure 9
Kaplan-Meier curves of cumulative risk of CHF and cumulative risk of a major cardiovascular (CV) event according to quartiles of age-adjusted IGF-I. P for differences across the quartiles was assessed by the Log rank test

with subsequent improvement during long, but not after short term treatment (150,151). I should be noted that other investigations in rodents have shown that reduced IGF-I activity is associated with reduced contractility (152,153).

Taken together LV mass, volume and contractility are highly inte grated GH/IGF-I dependent factors. The multiple and divergent actions in relation to cardiovascular function seem to complicate prediction of long-term cardiovascular effects of endogenous GH/IGF-I activity. Important from a clinical point of view, cardiac output which is the integrated outcome of ventricle size, contraction, and heart rate did not differ between patients with GH-disturbances and control subjects (study II and III), and no single patient had a cardiac index (cardiac output/m^2) below what can be observed in normal healthy subjects (154). This emphasizes that the overall regulation of cardiac function is very robust. The potential harmful consequences of pathological increased and especially reduced GH and IGF-I seem to a large degree to be counterbalanced by compensatory mechanisms.

The combined analyses comprising untreated GH-deficient patients and control subjects suggested that GH/IGF-I activity influence LV EDV and EF (Figure 8). Obviously caution should be taken when such combined results are interpreted. However GHD seems to be associated only with minor cardiac changes, the regression lines in the two groups for these two variables were quite similar and there was a substantial overlap in IGF-values between patients and control subjects. In my opinion this justifies the use of the combined analyses.

Development of CVD in the normal population
In study IV we observed no association between IGF-I levels and the overall development of CVDs. Different results within this topic have been reported (114-116,123,125), as described previously in the thesis, page 17.

We observed that high levels of IGF-I were an independent risk factor for the development of chronic heart failure. This is in opposition to a study by Vasan et al. who reported that low levels of IGF-I predicted development of CHF (122) and another by Kaplan et al. reporting no influence of IGF-I (124). In contrast to the two previous studies our patients were only classified as having developed CHF, if it was the primary discharge diagnosis on the first admission for a cardiovascular event. Consequently CHF after e.g. a myocardial infarction would in our study not be classified as a CHF event. This difference in regard to definition of development of CHF might have favoured a higher proportion of CHF on a non-atherosclerotic basis in our study. Thus, in patients with a primary diagnosis of CHF IGF-I might be a pathogenetic factor. As a possible mechanism high levels of endogenous IGF-I through many years might in a subgroup of susceptible individuals leads to hypertrophy and subsequent impairment of systolic function. This suggestion is somewhat supported by the literature where it has been shown that high IGF-I levels within normal range increases LVM (60,61). In general increased LVM seems to be harmful and associated with decreased contractility and increased risk of developing CHF (155-158).

Increased risk of primary CHF associated with high levels of IGF-I might have masked a protective role of IGF-I on development of other CVDs of purely atherosclerotic origin. Among the individuals in study IV who developed a CVD (n=58), baseline levels of age-adjusted IGF-I were higher in those who developed CHF compared to those who developed other kinds of CVDs (89 (66-117) vs. 69 (51-85) ng/mL, P=0.020). To state it more simple, the relationship between IGF-I and CVD could be U-shaped (127) with low values increasing the risk of atherosclerotic disease and high values increasing the risk of hypertrophy and primary heart failure.

Natriuretic peptides
Study I showed reduced levels of NT-proBNP in untreated acromegalic patients and a 4-fold increase after short term treatment. In the manuscript (1) we proposed different explanations for this somewhat unexpected observation: GH/IGF-I excess might directly or indirectly change levels of NT-proBNP in such way that the normal relationship between NT-proBNP levels and heart function could be disturbed. Alternatively the low baseline levels might reflect a hyperkinetic phase with subsequent reduced systolic function after treatment.

The CMRI data showed that generally the levels of natriuretic peptides reflect cardiac function independent of the GH-disturbances. Based on this knowledge, the low levels of NT-proBNP in untreated acromegalic patients observed in Study I probably reflected a stage of hypercontractility. The increase with a peak value after 3 months was most likely caused by a temporary decrease in cardiac function when the positive inotropic effect of excess of IGF-I was suddenly reduced and cardiac size was still increased. Finally the subsequent decrease in NT-proBNP levels from 3-24 months of treatment could represent cardiac remodelling and normalization of cardiac function and size. The reason for the discrepancy between baseline levels of NT-proBNP in untreated acromegaly in study I vs. study III could probably be due to a more severe cardiac involvement among some patients in the latter study.

As one difference from the general population increased LVM in acromegalic patients seems not to be accompanied by increased levels of B-type natriuretic peptides. Whether this has a pathophysiological importance is unknown. As discussed (1) it is a possibility that untreated acromegalic patients have inappropriately low levels of BNP. Given the cardioprotective actions of natriuretic peptides low levels might contribute to further increase in LVM and it is a possibility that low levels of BNP contribute to the well known water retention observed in acromegaly.

In contrast to BNP, NT-proBNP levels were inversely associated with IGF-I levels at baseline and during treatment in GH-deficient individuals (study II). After multiple adjustments a negative association between NT-proBNP and IGF-I could also be detected in the normal population (study IV). This suggest that there might be a difference between the two bio-markers dependent on IGF-I activity. Changes in body composition associated with GHD might influence levels of NT-proBNP and BNP differently (159,160).

Taken together in patients with GH-disturbances levels of B-type natriuretic peptides seem at least qualitatively to be markers of cardiac dysfunction as in other patients with various cardiac diseases.

LIMITATIONS
As a major limitation to study I cardiac imaging was not available. In study II the GH treatment was not performed blinded and placebo controlled since it was not considered ethical. To con-

clude the investigation the 2nd CMRI-evaluation was performed after only one year of treatment. GH-replacement therapy is considered life-long and cardiac remodelling is a slow-going process. Therefore long-term data would have been desirable and our aim is a further follow-up of these patients. Furthermore the sample size does not allow any conclusion concerning more rare cardiac manifestations of GHD, but based on age and aetiology of pituitary insufficiency the study population was considered representative.

The prospective study (study III) of acromegalic patients involved few patients with very different degrees of heart disease and they may not represent cardiac involvement in acromegaly in general.

In the normal population (study IV) data on height and weight were lacking. It can not be excluded that adjustment for body mass index might have influenced the results.

CONCLUSIONS

- Assessed by high-sensitive methods untreated GHD was not associated with reduced systolic function or reduced left ventricle mass
- Untreated GH-deficient patients had reduced LV EDVI and ESVI. Reduced EDVI was associated with increased contractility. The importance of these latter observations is unclear
- No favourable effect of one year of GH-replacement therapy was observed in GHD
- Short term treatment of acromegaly was associated with increased levels of BNP and NT-proBNP and increased EDVI, suggesting an initial decrease in cardiac function when treatment was initiated
- High levels of endogenous IGF-I predicted development of chronic heart failure but IGF-I did not influence the overall development of CVDs

CLOSING REMARKS:

- After about two decades with GH-replacement therapy it is still unsolved if the treatment is of clinical significance from a cardiological point of view
- There seems to be no doubt that treatment of GHD has favourable effects on several metabolic processes and positive effects on body composition and quality of life
- In acromegaly the present knowledge indicate that control of disease activity and treatment of co-morbidity (diabetes and hypertension) can normalize the relative risk of cardiovascular mortality
- The specific role of IGF-I as biomarker for different diseases is unclear

SUMMARY

Pathological high and low levels of Insulin-like Growth factor I (IGF-I) might exert harmful influences on cardiovascular structures. In the normal population low IGF-I levels might be harmful.

In a retrospective investigation in patients with growth hormone deficiency (GHD), normal levels of NT-proBNP at baseline and no changes during two years of GH treatment could be detected. A subsequent prospective study confirmed normal levels of NT-proBNP and also of BNP. Furthermore cardiac systolic function and left ventricle (LV) mass assessed by cardiac magnetic resonance imaging (CMRI) were unchanged compared to control subjects. One year of GH replacement therapy did not change levels of NT-proBNP, BNP or any of the variables obtained by CMRI.

In a retrospective study of acromegalic patients we found reduced serum NT-proBNP in the untreated stage and a 4-fold increase after 3 months of treatment. A subsequent prospective CMRI investigation confirmed an initial increase in natriuretic peptides after 3 months treatment, and showed that the increase in natriuretic peptides was accompanied by an increase in end diastolic volume.

In a normal population followed prospectively for 5 years, high plasma IGF-I was accompanied by increased incidence of chronic heart failure, whereas IGF-I levels did not seem to influence the overall development of cardiovascular diseases.

In conclusion,: assessed by sensitive methods patients with GHD had normal systolic function, and one year of GH replacement therapy did not change LV function or size. In acromegalic patients short-term treatment was associated with a minor decrease in cardiac function. In the normal population high levels of IGF-I was a risk factor for development of heart failure. The results illustrates that the interaction between the GH/IGF-I system and cardiovascular disease is very complex.

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