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XI.

Metabolic profile in Growth Hormone Deficient (GHD) adults after long-term recombinant human Growth Hormone (rhGH) therapy

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ABSTRACT

BACKGROUND: The metabolic effects of recombinant human GH (rhGH) therapy in adults are well-documented in the short term. The effects of long-term rhGH therapy beyond 5 years on metabolic parameters are presently unknown.

OBJECTIVE: The aim of the study was to evaluate the long-term effects of rhGH treatment on biochemical and anthropometric parameters in a large cohort of GH-deficient adults.

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

RESULTS: Total cholesterol and low-density lipoprotein cholesterol concentrations were lower, and high-density lipoprotein cholesterol levels were significantly higher during long-term rhGH replacement when compared to baseline (all p<0.001). Both waist circumference (p<0.001) and BMI (p=0.018) were significantly higher after 10 years, as were fasting plasma glucose levels (p<0.001). No significant changes were observed in triglycerides, waist-to-hip ratio and blood pressure during follow-up. In the subset of patients with 15-yr rhGH treatment (N=43), generally similar metabolic effects were found. MS prevalence was increased after 10 years of rhGH treatment (57.1% vs 32.7%, p<0.001), especially in males (69.4% vs 32.7%, p<0.001).

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

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INTRODUCTION

Recombinant human GH (rhGH) replacement therapy has been a regular treatment option for adult GH-deficient (GHD) patients since the nineties. Adult GHD is hypothesized to be a cardiovascular risk factor associated with increased mortality, by inducing abdominal obesity, hypercholesterolemia, and hypertriglyceridemia (1;2). In short-term studies, GH replacement reduces some, but not all of these cardiovascular risk factors (3). Consistent 'short-term' effects on body composition and lipid metabolism were documented, resulting in reduction of body fat combined with an increase of fat-free mass, and a reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels (3). In addition, favorable effects on bone mineral density (BMD) and quality of life (QoL) have been reported (3-6).

Sustained improvement of lipid spectrum and diastolic blood pressure (DBP) has been reported over 7-10 years (7-9). However, despite a significant improvement of some, but not all, individual cardiovascular risk factors, overall cardiovascular risk profile, as reflected by the prevalence of the metabolic syndrome (MS), was still increased when compared with the general population and was not affected by rhGH after 5 years of treatment (10). Moreover, a recent study reported an increased risk of cardiovascular death in GHD women treated with rhGH when compared with background population (11).

A recent review addressing the effects of rhGH replacement in elderly patients (>60 years old) with GHD, revealed that rhGH replacement decreases LDL-C levels and improves QoL, but the effects on other parameters were not unequivocal (12). However, sufficient data concerning the use of long-term rhGH therapy in elderly are currently unavailable, as is the case in younger patients. In view of the overall scarce documentation of cardiovascular effects over 10 years (8;13;14), the efficacy of ongoing rhGH therapy in reducing cardiovascular risk in adult GHD still has to be established.

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

PATIENTS AND METHODS

PATIENTS: Since 1994, consecutive patients diagnosed with GHD at the Endocrinology Department of the Leiden University Medical Center were collected in a database, including both adult-onset (AO) and childhood-onset (CO) GHD. Severe GHD had been defined prior to start of treatment by a GH peak response to the Insulin Tolerance Test (ITT) <3μg/l (glucose nadir<2.2mmol/l) or Growth Hormone Releasing Hormone/Arginine-test (GHRH/Arg) (with body mass index (BMI)-adjusted GH cut-offs) in case of contraindications for ITT, according to guidelines (10;15). All patients with rhGH treatment during childhood were retested at time of transition to the adult outpatient clinic, after treatment cessation for more than 3 months. After dose titration, aiming at an IGF-1 level in the normal range, patients were evaluated at least yearly at the outpatient clinic according to a standard protocol.

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

TREATMENT PROTOCOL: All patients were treated with s.c. injections of rhGH (Genotropin Pharmacia/Pfizer, Zomacton Ferring, or Norditropin NovoNordisk) injected in the evening. The initial dose of rhGH was 0.2mg/day, which was individually adjusted each month in the first half year to achieve serum IGF-1 concentrations within the age-dependent laboratory reference range, aimed at an SD score (SDS) between 0 and +2. When stable plasma concentrations were reached, this individualized dose was continued and adjusted as necessary. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value 0.55mmol/l) after a CRH stimulation test or ITT. When secondary amenorrhea was present for more than 1year, premenopausal women were classified as gonadotropin deficient. In men, gonadotropin deficiency was defined as a testosterone level below the reference range (8.0nmol/l). TSH deficiency was defined as total T4 or free T4 level below the reference range (<10pmol/l). Hypopituitarism was supplemented by hydrocortisone, l-T4, testosterone in men, and estrogen in combination with prostagens in premenopausal women only. Dosages were monitored and adjusted as required. Thyroid hormone replacement, lipid-lowering medication and antihypertensive medication were started according to the discretion of the attending physicians.

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

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

II. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressures (SBP and DBP, respectively) were measured. BMI and waist-to-hip (WH) ratio were calculated. Body weight was measured to the nearest 0.1kg, and body height was measured barefoot to the nearest 0.001m. The BMI was calculated as weight in kilograms divided by the square of height in meters.

III. Additional information with respect to medication use, co-morbidity and possible side effects and adverse events was gathered from patient records.

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

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

1. Fasting plasma glucose concentration of at least 100mg/dl or on anti-diabetic drug treatment;
2. TG concentration of at least 150mg/dl or on drug treatment;
3. HDL-C concentration below 40mg/dl in men and below 50mg/dl in women, or on drug treatment;
4. Blood pressure of at least 130/85mmHg or on antihypertensive treatment
5. Waist circumference greater than 102cm in men and greater than 88cm in women.

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

From 1986 to 2005, serum IGF-1 concentrations were determined by RIA (Incstar, Stillwater, MN) with a detection limit of 1.5nmol/l and an interassay CV less than 11%. IGF-1 is expressed as SD score for age- and sex-related normal levels determined in the same laboratory (20). Since 2005, serum IGF-1 concentrations (nmol/l) were measured using an immunometric technique on an Immulite 2500 system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The intra-assay variations at mean plasma levels of 8 and 75nmol/l were 5.0 and 7.5%. IGF-1 levels were expressed as SDS, using lambda-mu-sigma smoothed reference curves based on 906 controls (21;22).

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

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

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RESULTS

A total of 184 adult patients with GHD started rhGH suppletion in 2002 or before (*Figure 1*). Forty-two patients are on current rhGH treatment, but did not complete 10 years of treatment yet. Fourty-four patients discontinued rhGH treatment for various reasons (*Figure 1*). Reasons for preliminary discontinuation of rhGH treatment were: no subjective beneficial effect (N=10), death (N=9), tumor growth (N=7), malignancy (N=12), high age (N=1), new-onset diabetes mellitus type 2 (N=1), other (N=4). Consequently, we included 98 patients (50% female, mean age 59.4yr) for the present analysis, of which 43 patients completed 15 years of rhGH therapy (44% female, mean age 61.3yr). Baseline characteristics are shown in *Table 1*.

No differences were found between the patients with and without complete 10-yr ore more rhGH treatment, with respect to sex, BMI, age of start rhGH therapy, etiological diagnosis, surgery, radiotherapy, pituitary deficiencies, and use of lipid-lowering or antihypertensive medication. Only the number of patients with CO GHD was higher among non-completers (p=0.009) (*data not shown*).
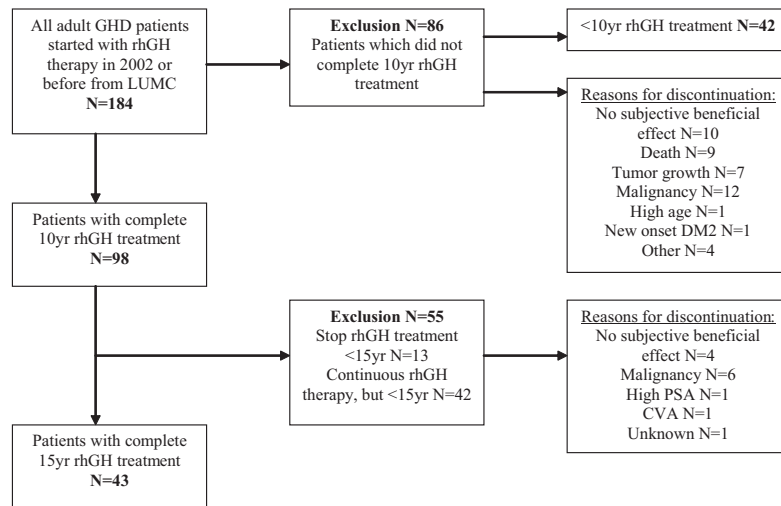
GH DOSE AND IGF-1 CONCENTRATION: Mean GH dose after dose titration (after 1yr) was 0.46±0.20mg/day (range 0.20-1.50mg), 0.48±0.25mg/day (range 0.15-1.50mg) at 5yr. At 10 and 15 years mean GH doses were 0.44±0.26mg/day (range 0.10-1.50mg) and 0.39±0.21mg/day (range 0.10-1.50mg), respectively (5-, 10- and 15-yr GH doses were not significantly different from the GH dose after dose titration). Serum IGF-1 levels remained significantly higher during rhGH replacement for the duration of the study up to 15 years compared with baseline (*Table 2*). During the entire study period, mean IGF-1 SDS was within the normal range, increased from -0.68±2.27 at baseline to 0.20±2.25 at 15 years of rhGH suppletion (*Figure 2*). In males, mean IGF-1 SDS was -0.24±2.26 at baseline and increased to 0.21±2.31 at 15 years (p=0.007); in females, mean IGF-1 SDS increased from -1.13±2.17 at baseline to 0.19±2.25 after 15 years of rhGH treatment (p<0.001).

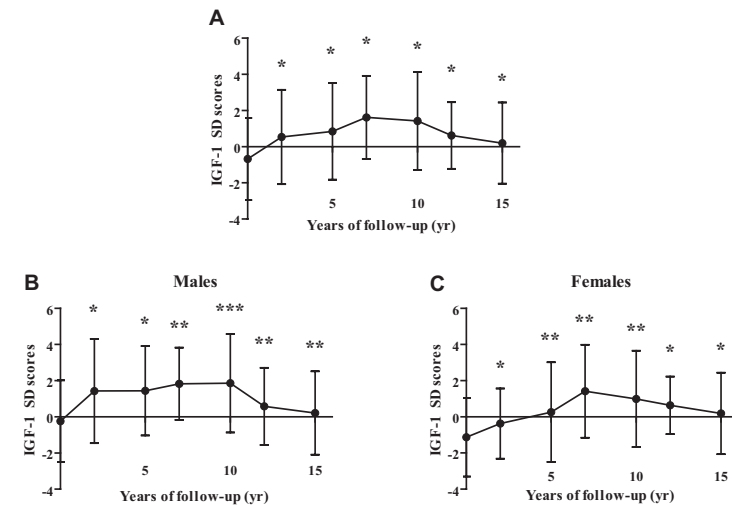Figure 1. Flow chart of patient selection and follow-up

Figure 2. Mean IGF-1 SD scores during 15 years of rhGH therapy, for the total cohort of GHD patients (A, N=98) and separated for males (B) and females (C), respectively

Mean IGF-1 SD scores ± SD during rhGH treatment are presented for the total GHD cohort (N=98), and separately for male (N=49) and female patients (N=49). IGF-1 SD scores were evaluated at start of rhGH therapy, and after 2, 5, 7, 10, 12, and 15 years of rhGH suppletion.

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

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Table 1. Baseline characteristics of 98 patients with GHD, which completed minimal 10 years of rhGH replacement therapy

Patient characteristics	GHD patients with complete 10yr follow-up (N=98)
Sex, female / male (n)	49 / 49
Age at start rhGH therapy, yr (range)	44.9 ± 13.6 (17 – 84)
AO / CO (n)	87 / 11
BMI, kg/m² (range)	28.2 ± 5.9 (16.9 – 45.4)
Etiological diagnosis of GHD (n)	
NFA	30
Functioning adenoma	25
Craniopharyngeoma	14
Cerebral malignancy	5
Congenital	9
Other causes	15
Surgery, TS / TC (n)	53 / 24
Radiotherapy (n)	37
Pituitary deficiencies (n)	
TSH	89
ACTH	88
FSH/LH	85
ADH	28
Isolated GHD	1
Lipid-lowering drugs (n)	10
Antihypertensive medication (n)	10

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

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

Table 2. Effects of 10 years of rhGH replacement in 98 adults with GHD on biochemical and anthropometric parameters

	Baseline[†]	5 years of rhGH replacement[††]	10 years of rhGH replacement[†††]
IGF-1, nmol/l	14.8 ± 10.3	25.4 ± 13.3 *	22.2 ± 10.7 *
Fasting glucose, mmol/l	4.7 ± 0.8	5.0 ± 1.1 **	5.1 ± 1.0 *
TC, mmol/l	6.2 ± 1.4	5.5 ± 1.0 *	5.2 ± 1.0 *, b
LDL-C, mmol/l	4.5 ± 1.4	3.7 ± 0.9 *	3.3 ± 0.8 *, a
HDL-C, mmol/l	1.3 ± 0.5	1.5 ± 0.5 *	1.6 ± 0.6 *
TG, mmol/l	1.8 ± 1.1	1.8 ± 1.0	1.8 ± 1.1
Waist circumference, cm	94.6 ± 13.7	97.0 ± 14.3	98.9 ± 14.2 *, a
WH ratio	0.95 ± 0.07	0.97 ± 0.12	0.96 ± 0.06
BMI, kg/m²	28.2 ± 5.9	28.8 ± 6.3	29.9 ± 6.9 ***, c
SBP, mmHg	129.9 ± 14.7	130.4 ± 17.8	130.9 ± 16.6
DBP, mmHg	83.5 ± 9.7	80.4 ± 9.8 **	80.4 ± 9.1 ***

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

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

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

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

Among the subgroup of patients who completed 15 years of rhGH treatment (N=43), comparable significant effects of rhGH suppletion were found on fasting glucose levels, lipids and waist circumference (*Table 3*). The effects on TC, HDL-C, LDL-C and TG were not affected when patients using lipid-lowering medication at any time point during follow-up were excluded (N=41, 42%). TG, WH ratio, BMI and DBP did not change when compared to baseline; SBP was significantly higher (p=0.001). Within the 15-yr treated group, there were, in general, no differences between 10 and 15 years of rhGH therapy; except for SBP, being significantly higher after 15 years. Excluding patients on antihypertensive medication at any time point (N=41, 42%) revealed no changes in DBP; however, the observed increase in SBP between 10 and 15 years of treatment was not significant any longer.

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

OTHER POTENTIAL INFLUENCING FACTORS: Forty-nine patients (50%) were less than 60 years old, 49 patients (50%) were at least 60 years of age at baseline. The individualized rhGH dose used in older patients did not differ from the dose in younger patients. In addition, there were no significant differences in the rhGH response between younger and older

patients with respect to IGF-1, glucose, lipid profile, WH-ratio, BMI or blood pressure. Furthermore, the number of pituitary insufficiencies did not affect the response to rhGH treatment. Mean hydrocortisone doses were 25.6±7.3mg/day, 23.1±5.8mg/day, 21.4±4.1mg/day, and 21.1±4.3mg/day at baseline and after 5, 10 and 15 years of rhGH suppletion, respectively. However, patients with hydrocortisone substitution did not differ from hydrocortisone-independent patients, except for a greater decrease in LDL-C levels after 10 years (-1.35 *vs* -0.70mmol/l, p=0.021). Patients with CO-GHD did not differ from AO-GHD patients in their response to any of the metabolic parameters studied. In addition, there was no difference between patients with or without cranial irradiation, except for a higher waist circumference (p=0.024) among irradiated patients.

When incorporating all factors in a linear regression model, neither age, sex, CO- *vs* AO-GHD, nor hydrocortisone use significantly influenced any of the metabolic parameters. Radiotherapy, however, influenced waist circumference and DBP negatively. In addition, higher GH dose was associated with higher BMI and waist circumference after 10 years of rhGH suppletion.

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

At baseline, the MS was equally prevalent in men and women (32.7% *vs* 32.7%). After 5 years of rhGH suppletion, 49.0% of the males and 44.9% of the female GHD patients had MS (p=0.687). After 10 years of rhGH suppletion, 69.4% of men fulfilled the criteria of MS *vs* 44.9% of the women, p=0.015. This indicates that males especially drove the increase in MS prevalence over time, and that MS prevalence stabilized in females after 5 years. In a logistic regression model incorporating age, sex, hydrocortisone use, radiotherapy and GH dose, only higher GH dose negatively influenced the MS prevalence after 10 years of rhGH suppletion.

CARDIOVASCULAR EVENTS: We assessed the number of major cardiovascular events in the patients who completed at least 10year of rhGH treatment (N=98). In total, 25 events were reported: myocardial infarction (N=2), progressive angina pectoris and/or coronary bypass surgery (N=7), cerebrovascular attack (N=2), intermittent claudication (N=1), pulmonary embolism (N=1). No cardiovascular death was reported. Consequently, the incidence rate of major cardiovascular

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events in our GHD cohort was 25/16552 (mean duration of rhGH therapy 168.9 months x 98 patients) = 1.5/1000py.

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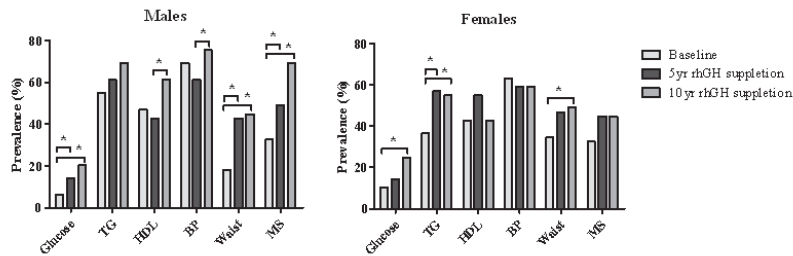

Figure 3. Prevalence of (individual components of) the MS in 98 GHD adults at baseline, and after 5 and 10 years of rhGH suppletion, respectively, separated for males and females

The bars denote the prevalence of the (individual components of) the MS, at baseline and after 5 and 10 years of rhGH treatment, respectively.

TG, triglycerides; HDL, high-density lipoprotein cholesterol; BP, blood pressure; waist, waist circumference; MS, metabolic syndrome. *, p<0.05

Table 3. Effects of 15 years of rhGH treatment in 43 adults with GHD on biochemical and anthropometric parameters

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

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

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

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

DISCUSSION

The present study demonstrates ongoing beneficial effects of rhGH treatment on lipids in patients with GHD after 10 and 15 years in the presence of an increase in anthropometric parameters such as BMI, waist circumference and SBP. As a consequence, overall cardiovascular risk, as assessed by the prevalence of the MS increased significantly after 10 years of rhGH replacement. This increase in MS prevalence was higher than expected as a consequence of ageing alone in non-GHD adults (23).

The short-term effects of rhGH therapy on cardiovascular risk factors are well documented. A meta-analysis of placebo-controlled studies in GHD adults showed favorable effects of short-term (up to 1.5yr) rhGH replacement therapy on TC and HDL-C levels, DBP, as well as on lean body and fat mass, but unfavorable effects on glucose and insulin concentrations (3). Data on the long-term effects of adult rhGH replacement therapy are limited and are based on observational studies. The metabolic changes after at least 10 years of rhGH substitution have been reported in only three studies (8;13;14), including a total of 119 patients, and their conclusions were inconclusive. The first study, Gibney *et al.* (13), reported favorable effects on HDL-C and LDL-C, but no changes in TC, TG, insulin, or blood pressure, whereas the second study, Götherström *et al.* (8), reported a decrease in TC and LDL-C, an increase in HDL-C levels, and an increase in BMI and glucose levels. The third study, Roemmler *et al.* (14), did not demonstrate any effect of treatment on lipids, glucose or anthropometric parameters.

In this study, we documented a decrease in TC and LDL-C levels, and an increase in HDL-C, in accordance with the findings reported by Gibney *et al.* and Götherström *et al.*(8;13). TG levels, however, did not change during rhGH therapy. The pattern of observed changes in lipid concentrations did not change after exclusion of patients using lipid-lowering drugs. The observed mean decrease in TC and LDL-C levels after 15 years of rhGH therapy was 1.3 (20%) and 1.6mmol/l (32%), respectively. In the general population, every 10% decrease in cholesterol levels by statins reduces cardiovascular mortality risk in patients with hypercholesterolemia by 15% (24). Whether lowering of TC levels by rhGH replacement is associated with the same magnitude of reduction in cardiovascular mortality remains to be established. Furthermore, it is crucial not only to ascertain whether the beneficial effects of rhGH suppletion on lipid profile are comparable to the effects obtained with conventional lipid-lowering drugs, but also whether these effects can be superimposed. From a cost-effectiveness point of view, the latter is

of paramount importance, but to date, only one study, including 61 GHD patients on statin treatment, has demonstrated such an additional beneficial effect of rhGH treatment on lipid profile (25). In addition, Schneider *et al.* reported a cardiovascular risk reduction of approximately 50% after 2 years of rhGH treatment, using Framingham and Procam risk scores (26). Because hypopituitarism *per se* is also associated with increased cardiovascular mortality (12,13), any additional effect of rhGH substitution next to conventional lipid-lowering drugs may be beneficial.

Despite improvement of lipid spectrum, the MS prevalence increased after 10 years of rhGH replacement when compared with baseline, especially in males. Previously, we demonstrated an increased MS prevalence in untreated GHD adults, defined by the NCEP-ATP III criteria (27), in comparison to a Dutch historical reference population (data collected between 1993 and 1997) (38.0% *vs* 15.7%) (10). Subsequently, age-adjusted prevalence rates of 29% for the MS were reported by Attanasio *et al.* before start of rhGH treatment in 1420 European adults with AO and CO-GHD (Hypopituitary Control and Complications Study, HypoCCS) (28), and of 41% before start of rhGH suppletion by Verhelst and colleagues among 2479 patients with severe AO-GHD, using the same updated NCEP-ATP III criteria (27). Two studies have shown a persistently high MS prevalence after 3 and 5 years of rhGH treatment, respectively (10;28). In our study, we found a further increase in MS prevalence to 57% after 10 years of rhGH substitution, mainly due to an increase in abdominal obesity, hypertriglyceridaemia and hyperglycaemia.

A limitation of the present study, and a general drawback of long-term follow-up studies, is the lack of a non-treated control group. Since the beneficial effects of rhGH therapy are well-established in the short term, it is unethical to withhold patients with GHD receive rhGH in case of no contra-indications. This makes it difficult, if not impossible, to perform long-term randomized, controlled follow-up studies including GHD patients without rhGH treatment. However, several Dutch population-based studies reported the MS prevalence in the general population. In a Dutch survey among 4000 subjects, conducted in 2009-2010, an overall MS prevalence of 34% and 24% was reported, in males and females, respectively. When stratified by age (per decade), MS prevalence for males and females, respectively, were 20% and 10% (30-39yr), 29% and 17% (40-49yr), 41% and 29% (50-59yr), and 48% and 44% (60-69yr) (23). Another study involving a cohort of Dutch adults aged 65 years or older (Longitudinal Aging Study Amsterdam, LASA) reported an MS prevalence of 37.1% (29). Based on these literature data, the MS prevalence of 57.1%

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in our cohort of GHD patients (mean age 59yr) is strongly increased, despite 10 years of rhGH replacement.

As demonstrated previously (8;30;31), we observed a significant increase in fasting glucose levels during rhGH replacement. This finding is in accordance with the well-known negative effects of GH on peripheral insulin sensitivity, thereby impairing peripheral glucose uptake. These increased fasting glucose levels may have significantly affected cardiovascular morbidity or mortality, because previous reports have indicated that even when glucose levels were below the diabetic threshold, there was a positive correlation with the occurrence of cardiovascular events (32;33).

Previous radiotherapy was shown to negatively influence abdominal obesity and blood pressure in GHD adults. This finding is consistent with earlier studies among long-term survivors of childhood cancer, reporting a higher MS prevalence after cranial irradiation (34;35). A possible explanation might be that, in addition to pituitary damage, cranial irradiation induces damage to the hypothalamic area. Therefore, cranial radiotherapy might be an independent risk factor for cardiovascular disease.

Due to the presence of multiple pituitary hormone deficiencies in almost all patients, it is difficult to examine to what extent the reported effects can be attributed to rhGH treatment, or whether they are the consequence of suboptimal or excessive replacement therapy of other hormones. Isolated GHD provides the ideal model to characterize GHD without interference from other pituitary deficiencies or their treatment. Abs *et al.* showed generally similar clinical presentation and rhGH treatment response in IGHD patients and patients with multiple deficiencies, especially in AO-GHD (36), supporting the concept that GH *per se*, at least in part, affects the metabolic phenotype of substituted patients with multiple pituitary hormone deficiencies. In accordance with these findings, in a recent meta-analysis, both low and high IGF-1 levels increased mortality in the general population (hazard ratio (HR) 1.18, 95%CI 1.04-1.34) (37).

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

Another feature that needs to be addressed is the presence of interactions between glucocorticoids and rhGH during substitution. In the past, hydrocortisone doses used in hypopituitarism resulted in supraphysiological cortisol levels, accounting for at least part of the adverse metabolic profile. Decreasing the glucocorticoid dose from 20-30mg/day to 15mg/day had beneficial effects (42). In our study, mean hydrocortisone dose was lowered during 15 years of follow-up from 25.6±7.3 to 21.1±4.3mg/day. When hydrocortisone use was incorporated in a regression model, no relation to MS presence/worsening was found. Possible explanations for the absence of an effect may be the lower hydrocortisone substitution scheme when compared to Danilowicz *et al.*, in addition to less variation in the hydrocortisone doses used. Furthermore, it has been reported that GH accelerates cortisol metabolism by inhibiting 11β-hydroxysteroid dehydrogenase 1 resulting in decreased tissue exposure to cortisol (43;44), thereby reducing hydrocortisone bioavailability.

IGF-1 SD levels were closely monitored and were titrated on a physiological level, adjusted for age during the entire study period. This is one of the most important differences between our GHD cohort and other GHD cohorts, in which an initial high dose resulted high IGF-1 SDS (over +2SDS) and excessive changes in body composition, especially in men. Our data showed no obvious differences in IGF-1 levels or metabolic effects of rhGH suppletion between men and women, taken into account the need of a higher rhGH substitution dose in women to maintain stable plasma IGF-1 concentrations. In addition, we did not find differences between younger and older GHD patients with respect to response to long-term rhGH treatment. However, we cannot exclude lack of power to detect any differences due to the relatively small number of patients within each age group.

In conclusion, the present study showed ongoing beneficial effects of rhGH therapy in GHD adults on lipid profile, whereas other cardiovascular risk factors continued to deteriorate after long-term rhGH treatment. The increases in glucose levels and BMI were striking and negatively affected the prevalence of the MS in the long-term. Therefore, the net beneficial effects of long-term rhGH treatment on overall cardiovascular risk still need to be established in further studies that adequately address and balance all these factors involved, including cost-effectiveness and QoL.

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