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Chapter 6

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

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ABSTRACT

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

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

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

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

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

INTRODUCTION

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

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

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

PATIENTS AND METHODS

Study design

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

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

Patient selection

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

Treatment protocol

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

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

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

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

General population

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

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

Study parameters

The following efficacy parameters were studied:

1. Biochemical parameters: fasting glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels. LDL-C concentrations were calculated by the Friedewald formula. Blood samples were taken after an overnight fast.
2. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressures (SBP and DBP, respectively) were measured. BMI (in kg/m2) and waist-hip ratio were calculated.
3. Additional information on medication use and co-morbidity was gathered from patient records.

Metabolic Syndrome (MS)

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

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

Assays

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

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

Statistical analysis

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

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

RESULTS

Characteristics of patients and controls

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

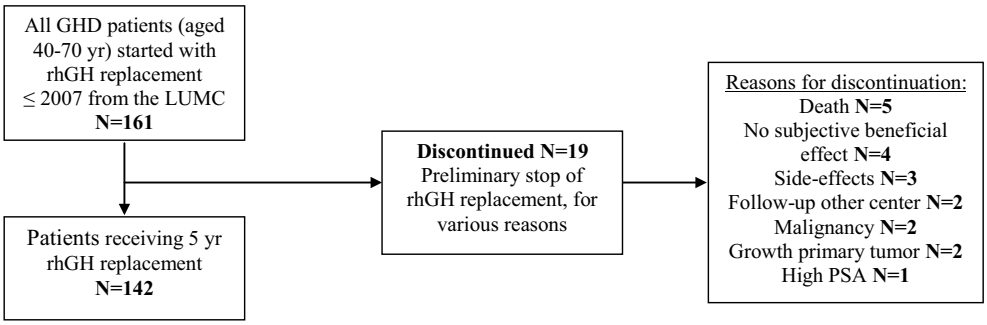


FIGURE 1.

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

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

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

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

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

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

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

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

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

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

Mean GH doses after dose titration (1 year) and 5 years of rGH replacement were, respectively, 0.35±0.16mg/day and 0.37±0.19mg/day (p=0.399). After 5 years of rGH replacement, females received higher mean GH doses than males (0.40±0.23mg/day and 0.34±0.17mg/day, respectively, p=0.05).

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

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

MS proportion in untreated GHD patients

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

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

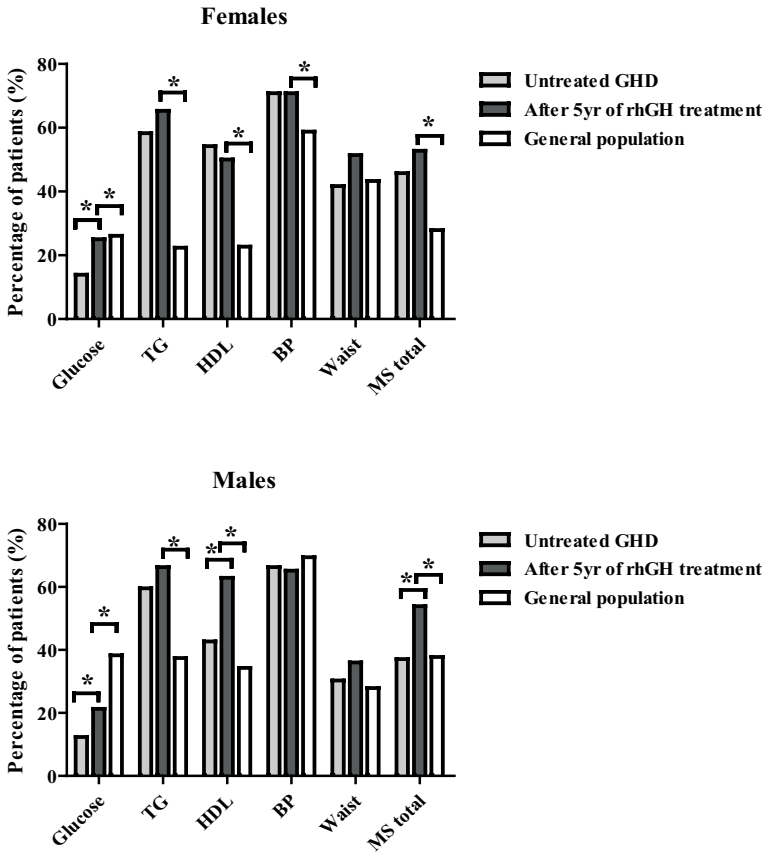
The MS proportion increased significantly from 41.0% before to 53.4% after 5 years of rGH replacement ($p=0.007$), especially due to an increased proportion of hyperglycaemia ($p=0.02$). In males, HDL-C levels decreased significantly ($p<0.001$). As indicated in *Figure 2*, the increase in MS proportion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

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

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

FIGURE 2.

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



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

*, $p<0.05$

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

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

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

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

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

Values are expressed as mean ± SD.

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

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

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

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

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

Values are expressed as number (percentage). Chronic rhGH replacement was defined as 5 years. MS was defined according to the NCEP-ATP III criteria (23;24). Log-binominal regression models were applied with robust standard errors, allowing direct estimations of PRs adjusted for age and sex, and, subsequently, with additional adjustments for BMI. N, number of patients; rhGH, recombinant human Growth Hormone replacement; GHD, Growth Hormone Deficiency; HDL-C, high-density lipoprotein cholesterol; waist, waist circumference; BMI, body mass index; PR, prevalence ratio; 95%CI, 95% confidence interval. RR1, adjusted for age and sex. RR2, adjusted for age, sex and BMI. *, $p<0.01$.

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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