Part C

Long-term outcome of recombinant human GH therapy in GH deficient adults

X.

Therapy of Endocrine Disease Long-term effects of recombinant human Growth Hormone (rhGH) replacement in adults with Growth Hormone Deficiency (GHD): a Systematic Review

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ABSTRACT

BACKGROUND: The beneficial effects of recombinant human growth hormone (rhGH) therapy in growth hormone deficient (GHD) adults are well-established in the short term. However, data documenting the effects during prolonged follow-up are relatively scarce.

OBJECTIVE: To evaluate the reported effects of rhGH replacement (≥5 years) in GHD adults on biochemical and anthropometric parameters, quality of life (QoL), bone metabolism, muscle strength, serious adverse events (SAEs) and mortality.

METHODS: We conducted a systematic literature search. Quality assessment of retrieved papers was performed using a quality assessment based on the modified STROBE statement.

RESULTS: We included 23 prospective studies with a rhGH treatment duration ranging from 5 to 15 years. Overall, beneficial effects were reported on QoL, body composition, lipid profile, carotid intima media thickness and bone mineral density. In contrast, the prevalence of the metabolic syndrome, glucose levels, BMI and muscle strength were not, or negatively influenced. Most of the studies were uncontrolled, lacked the presence of a control group (of non-treated GHD patients), and reported no data on lipid-lowering and anti-diabetic medication. Overall mortality was not increased.

CONCLUSION: rhGH treatment in adult GHD patients is well-tolerated and positively affects QoL in the long term. However, the metabolic and cardiovascular effects during long-term treatment are variable. The low numbers of long-term studies and studied patients and lack of control data hamper definite statements on the efficacy of prolonged treatment. Therefore, continuous monitoring of the effects of rhGH replacement to enable an adequate risk-benefit analysis that may justify prolonged, potentially life-long, treatment is advisable.

INTRODUCTION

Growth hormone deficiency (GHD) in adults has been associated with an adverse metabolic profile and increased cardiovascular mortality by inducing abdominal obesity, hypercholesterolaemia and hypertriglyceridaemia (1;2). Consistent beneficial effects of treatment with recombinant human growth hormone (rhGH) were reported on body composition and lipid profile, resulting in reduction of fat mass combined with an increase in lean body mass (LBM) and a reduction in total cholesterol (TC) levels (3). In addition, favorable effects were reported on bone turnover, bone mineral density (BMD), muscle strength, cognitive function and quality of life (QoL) (3-5). Most of these effects have been documented in short-term studies, sometimes placebo-controlled, with a follow-up duration ranging from 6 to 18 months.

The beneficial effects of rhGH therapy have been reported to be sustained for at least 5 years of treatment; however data with a longer follow-up duration are scarce. With respect to cardiovascular disease, a direct improvement of several cardiovascular risk factors was noted within the first treatment year, which was reported to be sustained during prolonged rhGH treatment (6;7). In a small study, sustained improvement of lipid spectrum and diastolic blood pressure (DBP) was reported over 7 and 10 years, suggesting ongoing beneficial effects even beyond 5 years (6;7). However, it is presently unknown whether rhGH therapy has favorable effects on the incidence of cardiovascular events, including cardiovascular death. With respect to bone, rhGH replacement therapy increases bone remodeling, indicated by an increase of biochemical markers of both bone formation and resorption. This results in an initial decline in BMD, followed by a significant increase in BMD, reaching a plateau phase after 5 to 7 years of treatment (8-10).

In general, rhGH replacement therapy in adults is regarded as safe. Recently, Van Bunderen *et al.* reported no differences in overall or malignancy-related mortality rate between GHD-treated patients and the background population (11). However, an increased risk in cardiovascular death was observed in rhGH-treated women. This increased cardiovascular mortality, however, is not easily explained. These recent findings accentuate the necessity to critically evaluate long-term efficacy and safety data.

We performed a systematic review of all available papers on long-term rhGH replacement in adult GHD patients. We aimed to evaluate the effects of long-term rhGH replacement in GHD adults on cardiovascular parameters, bone metabolism, muscle strength, QoL, mortality and adverse events.

MATERIALS AND METHODS

SEARCH STRATEGY AND ELIGIBILITY CRITERIA: To identify studies that investigated the long-term effects of rhGH replacement in GHD adults, we searched the following databases, up to September 2012 (Date of initial search 9th March, 2012): Medline, EMBASE, Web of Science, and the Cochrane Library, in collaboration with a trained clinical librarian (J.S.). We composed a search strategy focusing on long-term rhGH treatment, GHD adults, bone metabolism, QoL, metabolic effects, muscle strength, mortality, and adverse events. Chronic stable replacement was arbitrarily defined as replacement for ≥5 years, since such a period will reflect a stable metabolic situation without confounding by ongoing metabolic changes induced by the start and titration of rhGH replacement (3;12). We used all relevant keyword variations, including free text words. Duplicated articles were excluded. The complete search strategy is provided in *Appendix 1*. References of relevant articles were checked for additional articles. Only original articles were included.

Studies were eligible when all of the following criteria were met: (1) they investigated effects on biochemical, metabolic/anthropometric parameters, bone metabolism, QoL, muscle strength, mortality, or adverse events, (2) in adult patients with either childhood-onset (CO) or adult-onset (AO) GHD and (3) with at least 5 years of rhGH treatment and (4) were written in English. Only human studies were evaluated.

DATA EXTRACTION AND ANALYSIS: Data extraction and eligibility were assessed by two independent investigators (K.M.J.A.C. and N.M.A.-D.). Inconsistencies were resolved by consensus. The following data were extracted: (1) study population (patient characteristics, population size, GHD assessment, control population); (2) exposure to rhGH (duration of rhGH treatment, rhGH dose), (3) outcome (biochemical and anthropometric parameters, QoL, bone metabolism, muscle strength, mortality, serious adverse events (SAEs)) (4) potential confounders (age, sex, CO/AO).

METHODOLOGICAL QUALITY ASSESSMENT: The quality of each included paper was assessed in consensus by two independent reviewers (K.M.J.A.C. and N.M.A.-D.), using a standardized set of criteria based on the STROBE statement (*Appendix 2*), which was modified to cover the topic of our review (12). A maximum score of 10 points could be obtained. According to the total quality score, articles were considered as having poor (0-2), intermediate (3-5), good (6-8), or excellent methodological quality (9-10).

RESULTS

IDENTIFICATION AND SELECTION OF THE LITERATURE: From the initial 841 potentially relevant studies identified, 803 were excluded on the basis of title and abstract. Thirty-eight papers were retrieved for detailed assessment: 9 were excluded because of rhGH treatment duration <5 years, 2 studies described pediatric patients only, 1 study did not report original data and 3 studies did not address the long-term effects of rhGH suppletion. Consequently, 23 studies were eligible for inclusion, several describing part of the same patient cohort (Göteborg) (4;6;9;10;13-16) (KIMS database, (5;17)) (*Figure 1 & Table 1*). Therefore, we also mention the specific study centre.

QUALITY ASSESSMENT: According to the predefined quality criteria, no studies were classified as being poor quality. Two studies scored intermediate (18;19), 15 studies were good quality (4-6;9;11;15;18;21-28) and 6 studies had an excellent methodological quality (7;10;13;15;16;20) (*Table 1*). Mean quality score was 7.3 (range 5.0-9.0), indicating that overall methodological quality was good. However, especially the items on potential bias, handling with missing data, reasons for non-participation at each study stage and the use of/handling with co-medication were not clearly described.

METHODOLOGY: All selected studies (n=23) were prospective follow-up studies, and eleven of these had a controlled design. Healthy controls were included in six studies (5;13;19-22), of which two studies used National Registries. Four other studies included GHD patients with intermittent rhGH treatment or untreated GHD patients as controls (11;22;24;25). Gibney *et al.* compared rhGH-treated patients with both untreated GHD patients and healthy controls (23). Four reports involved multi-center studies (5;11;17;24). No randomized controlled trials (RCT) were available.

ENDOCRINE EVALUATION: Different established stimulation tests were used for the diagnosis of severe GHD. The insulin tolerance test (ITT) was used as standard test in 16 studies and the combined GHRH-arginine test, glucagon stimulation test or stimulation with clonidine alone, in the case of contraindications for ITT. Eight studies did not specify which stimulation tests were used (5;11;14;17;22;24-26). Thirteen studies applied the generally used cut-off value for severe GHD of a peak GH<3µg/L (4;6;7;9;10;13;15;16;19;20;23;27;28), in seven studies no exact cut-off value was reported (5;11;14;17;22;24;26), and three studies used, respectively, <1.7µg/l (21), <5µg/l (18) and <10mU/l (25) as cut-off. In all studies, except for one (25), information on additional pituitary deficiencies was given. Most patients had multiple anterior deficiencies, especially gonadotrope deficiency, but thyreotrope and corticotrope deficiency was also frequently encountered.

Duration of rhGH treatment: Mean treatment duration with rhGH ranged from 5 years to 15 years: 8 studies reported 5-year effects, 2 studies had a 6-year follow-up (11;18), 3 studies investigated 7-year effects (7;21;28), 1 study investigated the 9-year effects (25), 8 studies reported 10-year effects (6;10;13-15;23;24;26), 1 study described 15 years of rhGH suppletion (4). In one study, treatment duration varied among the different countries from 4-8 years (5).

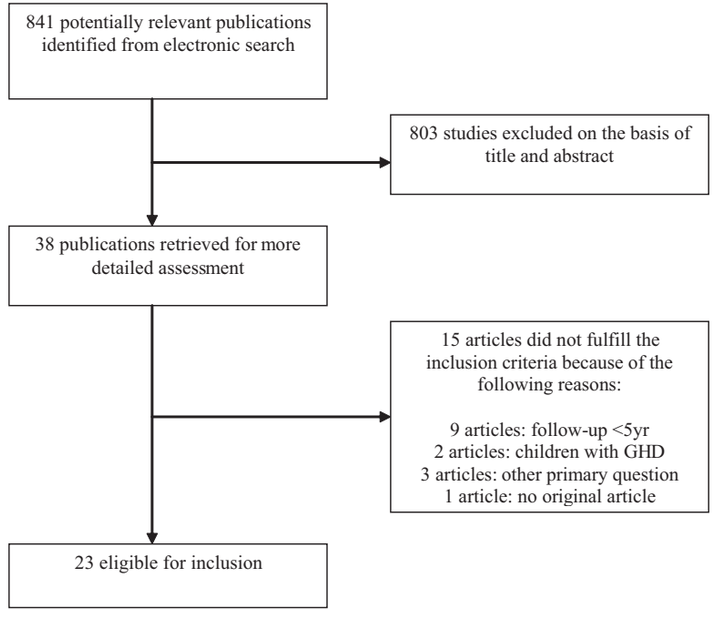

Figure 1: Flow diagram of study selection and exclusion stages.

Table 1. Included studies on the effects of long-term rhGH suppletion

Author (yr (ref))Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Controls	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)
Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	Untreated GHD (N=11) Healthy controls, age, sex & BMI matched (N=12)	0.025IU/kg/d	10
Chrisoulidou (2000(20))	12 (6/6) 10/2	52 ± 10	GHD, 0.5-1.5yr rhGH (N=11) Untreated GHD (N=10)	NR	7
Clanget (2001(18))	12 (8/4) 9/3	42.5 (R 24-61)	-	µ 2.4IU (0.80mg)	6
Götherström (2001(9))** **Göteborg**	118 (70/48) 118/0	49.3 (R 22-74)	-	0.3 (median)	5
Svensson (2002(26))** **Göteborg**	11 (7/4) 9/2	48.0 (R 20-62)	Healthy controls, age & sex & BMI & WHR matched (N=11)	initial 1.10 end 0.61	10
Gilchrist (2002(23))	61 (27/34) 43/1	37.9 ± 11.8	Group A = continuously Group B = no Group C = intermittently	0.25IU/kg/wk	9
Svensson (2003(17))** **Göteborg**	109 (61/48) 109/0	50.0 (R 22-74)	-	initial 0.88 end 0.46	5

Author (yr (ref))Centre	Tested parameters							Quality score (points)
	Glucose	Cardio vascular#	Anthro-pometric/Body composition##	Muscle strength	Bone	QoL	SAE/ Mortality	
Gibney (1999(22)	x	x	x	x		x		7.5
Chrisoulidou (2000(20))	x	x	x					6.5
Clanget (2001(18))					x			5.0
Götherström (2001(9))** **Göteborg**	x	x	x		x			7.0
Svensson (2002(26))** **Göteborg**	x	x	x					8.0
Gilchrist (2002(23))						x		6.0
Svensson (2003(17))** **Göteborg**			x	x				9.0

Table 2. Outcome of long-term of rhGH therapy in GHD adults

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Anthropometric parameters N=13	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↑ weight & BMI, = WHR
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	0.3 (median)	5	↑ BMI
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	10	= weight & WHR & BMI
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	= weight & BMI
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 0.98 end 0.47	5	= BMI
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	= weight & BMI
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	= WC & WHR ↑ BMI
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= waist circ & WHR ↑ BMI
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	7	= weight, ↑ BMI
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	= weight & WC & WHR & BMI
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	= weight & BMI
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	↑ BMI & WC & HC = WHR
	Elbornsson (2012(4))** Göteborg	126 (72/54) 126/0	49.4 (R 22-74)	initial 0.63 end 0.41	15	↑ weight & BMI
Body composition N=11	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	= BF ↑ LBM ↑ thigh muscle area
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓ BF ↑ LBM ↓ skinfold thickness (subscap)
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓ BF ↑ LBM
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	↓ BF ↑ LBM
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↓ BF ↑ LBM
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 3-8 µg/kg/d end 0.3	5	↓ BF ↑ LBM
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↓ BF ↑ LBM
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓ BF ↑ LBM
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ visceral fat
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	= BF ↑ LBM
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BF = LBM

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
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	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓ BF ↑ LBM ↓ skinfold thickness (subscap)
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓ BF ↑ LBM
	Svensson (2002(26))** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	↓ BF ↑ LBM
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↓ BF ↑ LBM
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 3-8 µg/kg/d end 0.3	5	↓ BF ↑ LBM
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↓ BF ↑ LBM
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓ BF ↑ LBM
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ visceral fat
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	= BF ↑ LBM
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BF = LBM

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion TC HDL LDL TG
Lipid metabolism **N=10**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	=
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	7	↓
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	initial 0.98 end 0.48	5	↓
	Svensson (2002(26))**** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 1.10 end 0.61	7	=
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	↓
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	NR
	Götherström (2007(6))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↓
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	=
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	initial 1.10 end 0.61	10 (2-42)	=
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	=

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Glucose **N=11**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025 IU/kg/d	10	= glucose, = insulin
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	initial 0.98 end 0.48	7	= NEFA, = glucose, = insulin
	Götherström (2001(9))** Göteborg	118 (70/48) 118/0	49.3 (R 22-74)	0.3 (median)	5	↑ glucose, = insulin, ↓ HbA1c
	Svensson (2002(26))**** Göteborg	11 (7/4) 9/2	48.0 (R 20-62)	initial 3-8 µg/kg/d end 0.3	7	= glucose & insulin sensitivity
	Giavoli (2004(28))	20 (11/9) 20/0	44 ± 14	initial 0.98 end 0.47	5	↑ glucose & insulin = HOMA-IR & QUICKI
	Van der Klaauw (2006(7))*** Leiden	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.2 end 0.5	7	= glucose
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= glucose
	Götherström (2007(6))** Göteborg	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	10	↑ glucose, ↓ HbA1c
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.6	5	= glucose & insulin & HOMA-IR
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	initial 1.10 end 0.61	10 (2-42)	= all glucose parameters (including glucose, insulin, HbA1c, insulin sensitivity, insulin resistance, β-cell-function, HOMA)
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	= glucose

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Cardiovascular parameters *Including Metabolic syndrome* **N=5**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025 IU/kg/d	10	= SBP & DBP = LVM & ESM & EDM ↓ carotid IMT
	Chrisoulidou (2000(21))	12 (6/6) 10/2	52 ± 10	NR	5	= SBP & DBP = IVRT, = LVM = HR, ↓ resting DBP, = SBP = exercise time
	Van der Klaauw (2006(7))*** Leiden	63 (30/33) 52/11	46.7 ± 14.3	initial 0.2 end 0.5	7	= SBP & DBP
	Van der Klaauw (2007(29))*** Leiden	50 (24/26) 50/0	45.2 ± 9.1	initial 0.2 end 0.5	5	= SBP & DBP ↑ MS prevalence
	Cenci (2008(20))	14 (10/4) 14/0	R 33-62	initial 0.87 end 0.64	5	↓ carotid IMT
Neuromuscular function **N=5**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	= muscle strength
	Svensson (2003(17))** Göteborg	109 (61/48) 109/0	50.0 (R 22-74)	initial 0.88 end 0.46	5	↑ knee flexor & extensor strength ↑ hand grip strength
	Götherström (2005(14))** Göteborg	26 (12/14) 26/0	65.0 (R 61-74)	initial 0.73 end 0.36	5	↑ knee flexor & extensor strength ↑ peak hand grip strength
	Götherström (2009(15))** Göteborg	109 (61/48) 109/0	50 R (22-74)	initial 0.88 end 0.47	10	↑ knee flexor & extensor strength ↑ hand grip strength
	Götherström (2010(16))** Göteborg	24 (11/13) 24/0	65.2 ± 3.4	initial 0.72 end 0.37	10	↑ knee flexor strength = hand grip strength = muscle endurance

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
Bone **N=5**	Clanget (2001(19))	12 (8/4) 9/3	42.5 (R 24-61)	µ 2.4IU (0.80mg)	6	↑ BMC & BMD (lumbar spine, trochanter, = femur neck)
	Götherström (2001(9))** Göteborg	118 (70/48) 118	49.3 (R 22-74)	initial 0.98 end 0.48	5	↑ BMC & BMD = osteocalcin & Ca2+ & PTH
	Götherström (2007(10))** Göteborg	87 (52/35) 87/0	44.1 (R 22-74)	initial 0.98 end 0.47	10	↑ BMC & BMD (total body, lumbar spine, prox femur) ↑ osteocalcin & Ca2+
	Roemmler (2010(24))*	22 (14/8) 16/6	51.5 (R 33-75)	0.3 (median)	10 (2-42)	= BMD
	Elbornsson (2012(4))** Göteborg	126 (72/54) 126/0	49.4 (R 22-74)	initial 0.63 end 0.41	15	↑ BMC & BMD (total body, lumbar spine, plateau) ↑ BMC, = BMD (prox femur, peak at 7yr, thereafter ↓) Fractures: 1 hip, 1 symp VF
Quality of life **N=4**	Gibney (1999(22))	10 (7/3) NR	38 (R 21-48)	0.025IU/kg/d	10	↑ overall psychological well-being, energy, emotional reaction
	Gilchrist (2002(23))	61 (27/34) 43/1	37.9 ± 11.8	0.25IU/kg/wk	9	**NHP**: ↑ energy levels, = sleep & emotional reaction & social isolation & physical mobility & pain **PGWB:** ↑ vitality score

Outcome parameters Nr of studies	Author (yr (ref)) Centre	Total (M/F) AO/CO	Mean age (yr ± SD)	Mean rhGH dose (mg/day)	Duration of rhGH treatment (yr)	Conclusion
	Koltowska-Häggström (2006(5))***** KIMS database	UK 758 (363/395) 704/54 NL 247 (123/124) 222/25 Spain 197 (75/122) 176/21 Sweden 484 (247/237) 453/31	UK 48.5±12.6 NL 48.2±13.3 Spain 45±11.1 Sweden 51.4±13.0	NR	UK 7 NL 6 Spain 4 Sweden 8	**QoL-AGHDA:** ↑ in 1st yr, after that regression to country-specific mean (normalization)
	Spielhagen (2011(25))***** KIMS database	440 (224/216) 301/139	♀ 36.7 ± 8.4 ♂ 35.1 ± 8.1 (R 20-49)	♀ initial 0.27 end 0.46 ♂ initial 0.31 end 0.40	10	**QoL-AGHDA:** ↑ in 1st yr, sustained during longer follow-up
Mortality/SAE N=3	**Svensson (2004(27))**** Göteborg	289 (186/103) 225/64	47.6 ± 14.8	N=80 11.9µg/kg/d N=209 indiv dose titration based on IGF1	5	= overall mortality & malignancy rate ↓ MI rate, ↑ CVA rate
	Van Bunderen (2011(11))	2229 (1160/1069) 1718/511	42.6 ± 16.3	µ 0.48	5.7 (median)	= mortality compared to background pop (after exclusion high-risk) = malignancy mortality ↑ CVD mortality in ♀
	Gaillard (2012(18))***** KIMS database	13983 (7174/6809) 10767/3216	CO 26.9 ± 9.9 AO 48.8 ± 13.0	NR	4.9	↑ overall mortality in ♀ (moderate) = malignancy & CVD mortality ↑ CVA & infection mortality ↑ progression primary tumour & endocrine complications

* *3 patients treated <5yr*

** *Overlapping patient cohorts Götherström 2010, Götherström 2005, Götherström 2001, Götherström 2007, Götherström 2007, Götherström 2009, Elbornsson 2012, Svensson 2003, Svensson 2004*

*** *Overlapping patient cohorts Van der Klaauw 2006 & Van der Klaauw 2007*

**** *Control group at baseline differed from controls at 10 year*

***** *Overlapping patient cohorts KIMS database Koltowska-Häggström 2006, Gaillard 2012, Spielhagen 2011*

rhGH, recombinant human growth hormone; GHD, growth hormone deficiency; AO, adult-onset GHD; CO, childhood-onset GHD; R, range; BF, body fat; LBM, lean body mass; ITT, insulin-tolerance test; GHRH, growth hormone releasing hormone; BMD, bone mineral density; BMC, bone mineral content; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BP, blood pressure; MS, metabolic syndrome; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin check index, Carotid IMT, intima media thickness of carotid arteries; QoL, quality of life; NHP, Nottingham Health Profile; PGWB, psychological general well-being schedule; QoL-AGHDA, quality of life-assessment of GH deficiency in adults; LVM, left ventricular mass; ESM, end systolic diameter; EDM, end diastolic diameter; Ca2+, calcium; NEFA, nonesterified fatty acids; Lp (a), lipoprotein (a); APU, apparent phalangeal ultrasound transmission velocity; CVD, cardiovascular disease; IVRT, isovolumic relaxation time; LVM, left ventricular mass; VF; vertebral fracture; UK, United Kingdom; NL, Netherlands; MI, myocardial infarction; CVA, cerebrovascular attack; SAE, serious adverse event; NR, not reported.

DOSE OF RHGH TREATMENT & IGF-1 LEVELS: Fourteen studies (nine from Göteborg) calculated the GH starting dose based on body weight (4;6;9;10;13-16;19;21-23;25;27). Of the remaining nine studies, three studies used dose titration of rhGH on an individual basis in the beginning of treatment, with the aim to reach IGF-1 levels within the normal age- and sex-related range, and adjusted the rhGH dose accordingly (7;20;26). Six studies did not report their rhGH dosage regimen (5;11;17;18;24;28). GH start dose ranged from 0.2mg/day (7;20) to 1.1mg/day (21). Twelve studies reported mean GH dose after dose titration (at 1 year) (6;9;10;13-17;19;21;24;27), ranging from 0.3mg/day (19) to 0.85mg/day (21) (median dose 0.54 mg/day).

Sixteen studies reported mean IGF-1 levels (4;7;9;10;13-16;18;19;21-24;26;28), and 13 studies described IGF-1 SDS to evaluate treatment response (4;6;9;10;13-17;20-22;24) (*Appendix 3)*. Based on available IGF-1 values, we can conclude that all patients were physiologically substituted at least after 5 to 7 years treatment with rhGH, independently of the applied starting dosage regimen.

Outcome of long-term rhGH replacement (*Table 2*)

ANTHROPOMETRIC PARAMETERS: Thirteen studies (N=1185) assessed the effects on body weight, body mass index (BMI) and/or waist-hip ratio (WHR) (4;6;7;9;10;13;14;20;21;24;26-28). rhGH suppletion did not affect WHR or waist circumference (7;20;21;26-28), except for one study reporting an increased waist circumference (24). Seven studies reporting BMI found a significant increase during rhGH suppletion ≥5years, while the other six studies reported no change (4;6;7;9;13;14;16;19-21;24;27;28). Two of the eight studies reporting body weight found an increase in weight during rhGH suppletion; the other studies reported no change (4;10;13;14;16;21;27;28).

In conclusion, results on long-term effects on BMI appear to be inconclusive with seven studies reporting an increase, and six reporting no change. In addition, there is little evidence for an increase in body weight.

BODY COMPOSITION: Eleven studies (N=538) (6;9;13;14;16;19;21;23;26-28) assessed the effects of rhGH suppletion on body composition using dual-energy X-ray absorptiometry (DEXA) scan (6;9;13;14;16;26;28), bioelectrical impedance analysis (BIA) (19;21) or a helicoidal CT scan (27). Gibney et al. measured body composition with radioisotope potassium (23). The majority of studies (n=9) reported consistent effects with an increase in LBM and a decrease in total body fat (BF) (6;9;13;14;16;19;21;23;28). One study could not detect any effect of rhGH on body composition (26). Cenci et al. reported only a decrease in visceral fat (27).

In conclusion, rhGH suppletion had favourable effects on body composition, with an increase in LBM and a decrease in total BF. Several methods are available to evaluate body composition, which may account for differences between studies. These differences might have influenced the results significantly. In literature, DEXA is reported to be the best currently available technique for measuring body composition (29); however, in this respect, it has to be noted that DEXA cannot distinguish between body cell mass (BCM) and water. Therefore, the LBM data may not be accurate, since rhGH replacement is associated with an increase in the intracellular water component (30-33). However, also when using other modalities to evaluate body composition, positive long-term effects of rhGH replacement were found.

LIPID METABOLISM: Ten studies (N=827) assessed the effects of rhGH suppletion on fasting plasma lipid profiles (6;7;9;20;21;23;24;26-28). Three studies did not find any effect of rhGH therapy on plasma lipids (24;26;27). The seven remaining studies reported generally similar results, *i.e.* reduction of total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol, an increase in high-density lipoprotein (HDL) cholesterol and no effect on triglycerides (6;7;9;20;21;23;28). In 6 patients, new-onset hyperlipidaemia was reported (Göteborg). However, only four studies reported the number of patients on lipid-lowering medication, especially statins, (6;7;20;26) but without excluding these patients from analysis.

In summary, there are ongoing beneficial effects of rhGH suppletion on plasma lipids. However, a strong limitation is the lack of data concerning lipid-lowering treatment, which has a considerable influence on the outcome parameter. Due to the lack of information on lipid-lowering drugs and the overlap in patient groups, meta-analysis was not an option.

GLUCOSE METABOLISM: Eleven studies (N=847) assessed the effects of rhGH suppletion on glucose and insulin levels (6;7;9;19-21;23;24;26-28). Seven studies showed no effect on glucose (7;20;23;24;26-28), three an increase (6;9;19) and one study found a transient increase of glucose levels only during the first year of rhGH therapy (21). Insulin levels increased only in one study (19). HbA1c was evaluated in three studies, two of which observed a decrease (6;9); the other found no change (26). Three studies investigated insulin resistance by the homeostasis model assessment (HOMA) (19;26;27), reporting no significant effects of rhGH suppletion. Insulin sensitivity was not affected in two studies (21;26), while another study reported a transient decrease, using the quantitative insulin check index (QUICKI), showing a trend to return towards basal values afterwards (19). In at least ten patients, new-onset diabetes mellitus type 2 (DM2) was diagnosed (4 Göteborg, 6 KIMS (24)). Five studies reported the number of patients starting anti-diabetic medication during follow-up (6;9;21;26;27). Only one study excluded diabetic patients from analysis (9), while the other studies did not report the handling with anti-diabetic medication in their statistical analysis.

In conclusion, there is moderate evidence for an increase in mean glucose levels after chronic rhGH treatment; however, insulin sensitivity appeared not to be affected in the long term. Due to the lack of information on anti-diabetic medication and the overlap in patient cohorts, we were not able to perform a meta-analysis on this parameter.

CARDIOVASCULAR PARAMETERS: Four studies (N=135) investigated the effects of rhGH suppletion on blood pressure (7;9;20;23;28). No effect was found on systolic blood pressure (SBP); one study reported a decrease in resting DBP (28). Only two studies described the number of patients on antihypertensive medication (7;20), without excluding them from analysis. Prevalence of the metabolic syndrome (MS) was assessed in one study, reporting a strong increase when compared to healthy controls, despite 5 years of rhGH treatment (38.0% *vs* 15.7%) (20).

Two studies (N=22) evaluated the long-term effects on cardiac function (23;28) using echocardiography; Chrisoulidou *et al.* also performed an exercise test (28). Left ventricular mass (LVM) was not affected. In addition, no effect on end diastolic or systolic diameter (23) nor on isovolumetric relaxation time (IVRT) or LVM was demonstrated (23;28). Two studies (N=24) evaluated carotid intima media thickness (IMT), reporting a decrease in IMT up to 18% after long-term rhGH therapy (23;27).

In conclusion, no effect on SBP of LVM was reported. In addition, conclusions on the prevalence of MS cannot be made, since this parameter was only evaluated in one study. Long-term rhGH suppletion had positive effects on IMT.

MUSCLE STRENGTH: Five studies (N=278, all AO-GHD) investigated the effects of rhGH suppletion on muscle strength (13-16;23). In general, 5 years of rhGH improved knee flexor and extensor strength and hand-grip strength. However, these effects were not sustained after 10 years. One study also investigated muscle endurance; no significant effects of rhGH suppletion were demonstrated (15). In addition, rhGH suppletion did not affect muscle strength in the elbow, shoulder or hip (23). Compared with population-based controls, rhGH was found to be protective to the normal age-related decline in muscle performance and neuromuscular function (13;14).

In general, rhGH suppletion improved muscle strength during the first 5 years of treatment; however, these effects were not sustained after prolonged follow-up. Since almost all studies described one patient group, we were not able to perform a meta-analysis on this parameter.

BONE PARAMETERS: Four studies (N=343) evaluated the long-term effects of rhGH on bone (4;9;10;18). After an initial decline, all studies reported an increase in both BMD and BMC within the first 5 years of treatment. After 5 years, all studies reported a plateau phase, which lasted for the entire study period (*i.e.* sustained effects) (4;9;10;18). Only

one study investigated (symptomatic) fracture prevalence, reporting 1 hip fracture and 1 symptomatic vertebral fracture (4). None of the studies described the use of calcium or Vitamin D suppletion, or the use of bisphosphonates, selective estrogen receptor modulator (SERM), teriparatide or strontium ranelate. Again, most patients were from the same study centre, prohibiting the performance of a meta-analysis.

QOL: Two large cohort studies (both KIMS database, N=1686 and N=440, respectively) and two smaller studies (N=71) addressed the effects on QoL and neuropsychological well-being, by QoL-Assessment of GHD in Adults (QoL-AGHDA), Nottingham Health Profile (NHP), and Psychological General Well-Being (PGWB) questionnaires (5;23-25). Within the first year of treatment, a direct beneficial response on well-being was noticed, as assessed by QoL-AGHDA, with marginal changes after the dose-titrating phase (5;24). Improvement was related to the degree of QoL impairment at baseline (5). Scores on NHP and PGWB improved in the long-term, especially with respect to energy level and emotional reaction (23;25).

In conclusion, QoL seemed to improve during long-term rhGH replacement, but positive effects on QoL were especially seen within the first year of treatment. Since studies are scarce and none of them had a placebo-controlled randomized controlled design, which is of paramount importance for the interpretation of QoL, no firm conclusions can be drawn on the effects of long-term rhGH replacement on QoL.

EFFECTS OF RHGH ON MORTALITY: Three cohort studies (N=16501) reported data on mortality after long-term rhGH suppletion (11;17;22), of which one study reports KIMS data (N=13983). In Gaillard *et al.*, overall mortality rate was moderately increased, especially in females, young attained age, patients with aggressive tumours and lower IGF-1 SDS during therapy (17). The other studies did not report differences in overall mortality or malignancy-related death. In two studies (17;22), the rate of cerebrovascular accidents (CVAs) was increased, but the incidence of myocardial infarction was decreased (risk ratio (RR) 0.27, 95%CI 0.03-0.99) when compared with the background population (22). In addition, rates of death due to infections (standardized mortality ratio (SMR) 4.97, 95%CI 3.98-6.14), primary tumour progression (SMR=6.96, 95%CI 4.45-10.3) and endocrine complications (SMR=2.79, 95%CI 1.30-5.23) were increased (17). Van Bunderen *et al.* (N=2229; median rhGH treatment of 5.7 yr) reported an increase in cardiovascular-related deaths in GHD-treated women (SMR 2.36) (11). This was not supported by the other long-term studies (17;22).

In summary, there is evidence for a moderate increased mortality rate despite long-term rhGH replacement, especially in females. Differences in pituitary disease, hormone replacement- and radiation therapies may account for differences between studies. However, since long-term studies on mortality are scarce, at this moment, no firm conclusions on the safety of long-term rhGH replacement can be drawn.

SERIOUS ADVERSE EVENTS (SAES): Twelve studies reported SAEs during long-term rhGH treatment (n=9 from Göteborg) (4;6;7;9;13-16;21;24;28). Since several studies described the same patient cohort, the total number of SAEs could not be extracted.

Malignancies: A total of 6 malignancy cases were reported in the GHD cohort of Göteborg: colonic carcinoma (N=1), prostate carcinoma (N=1), chronic lymphatic leukaemia (N=1), renal carcinoma (N=1), bladder carcinoma (N=1), and pulmonary carcinoma (N=1). In addition, 12 cases of *de novo* malignancies, not further specified, were reported (7;24).

Cardiovascular events: Three cases of myocardial infarction (Göteborg, Van der Klaauw *et al.* (7)) were reported, of which two with fatal outcome. Five patients suffered from a CVA (Göteborg, Van der Klaauw *et al.* (7)), resulting in death in four cases, and one patient died from a subarachnoid hemorrhage (Göteborg). In addition, angina pectoris was reported (N=3) (Göteborg), and 1 patient died from a pulmonary embolism (Göteborg). In at least ten patients, new-onset DM2 was diagnosed (4 Göteborg, 6 KIMS (24)). In, respectively, 6 and 4 patients new-onset hyperlipidaemia and hypertension were reported (Göteborg, (28)).

Other SAEs: Two studies reported primary tumour growth (N=11), either recurrence of the pituitary or central nervous system tumour (7;24).

In conclusion, half of the studies reported SAEs during rhGH therapy. Due to the lack of a control group including non-treated GHD patients, no firm conclusions can be drawn.

DISCUSSION

The short-term effects of rhGH treatment are well-established, showing favourable effects of rhGH therapy within the first 3 years on body composition, lipid profile, bone turnover, BMD, muscle strength, cognitive function, and QoL. This is the first systematic review focusing on the long-term effects of rhGH treatment (beyond ≥5 years of treatment). Overall, long-term rhGH therapy beneficially affected QoL and BMD. In addition, sustained beneficial effects were reported on lipid spectrum and on carotid IMT after 10 years of treatment. Consistent effects on body composition were reported with an increased LBM and a decreased total BF. Muscle strength increased after 5 years of rhGH suppletion, but these effects were not sustained during longer follow-up. Negative effects were reported on mean glucose levels; however, insulin sensitivity appeared not to be affected by long-term rhGH treatment.

The effects of rhGH replacement on bone turnover, and thus on BMD, are dynamic over time. Initially, BMD decreases due to increased bone resorption and formation, followed by an increase within the first years of treatment, finally resulting in a plateau phase. The five studies addressing this topic showed a significant increase within the first 5 years of treatment. Thereafter, BMD was conserved but did not further increase (4;9;10). Only one study described the number of symptomatic fractures (4), however, without radiographic assessment of the vertebral column. As a consequence, the reported fracture rate is most probably underestimated, since 21% of the fractures in patients with osteopenia or osteoporosis are asymptomatic (34). Fracture incidence was also studied by Holmer *et al.* (35), but a considerable proportion of patients were treated <5 years. In CO-GHD women, not in AO-GHD women or CO-GHD men, fracture risk was doubled, and in men with AO-GHD fracture incidence was even decreased (35). Further research has to establish the efficacy of rhGH treatment with respect to fracture risk.

Presently, in clinical practice, QoL is the most frequently addressed outcome parameter, but in the long-term, QoL was studied only in four studies (5;23-25). The two largest studies (KIMS database) showed improvement within the first year of treatment, with thereafter steady progress towards the country-specific mean (*i.e.* normalization) (5;24). The other two studies found ongoing beneficial effects on energy level and emotional reaction (23;25). Unfortunately, none of these studies had a placebo-controlled randomized controlled design. Thus, based on current literature, long-term effects on QoL are inconclusive, and therefore, in daily clinical practice, QoL should be individually evaluated in each patient.

With respect to plasma lipids, seven out of ten studies investigating the long-term effects of rhGH replacement on the lipid profile report ongoing beneficial effects, with a reduction of TC and LDL-C levels, an increase in HDL-C and no effect on triglycerides. However, in this respect, a strong limitation is the lack of data concerning lipid-lowering treatment, prohibiting to draw firm conclusions on this outcome parameter. When comparing these long-term results to short-term studies on plasma lipids, it has be noted that there were also several (randomized controlled) short-term studies reporting no change or minimal effects on lipid levels, and that even these effects were limited to men (36;37). Overall, however, as shown in a meta-analysis of Maison *et al.* investigating 37 placebo-controlled randomized controlled studies (follow-up up to 18 months), rhGH replacement was shown to improve LDL-C and TC levels in the short term (3).

Concerning the safety of rhGH suppletion, in children, long-term safety data have shown conflicting data, raising the question whether long-term use of rhGH is safe. Several large studies did not find an increased mortality in paediatric GHD patients after rhGH replacement, especially not due to new-onset malignancies or recurrence of central nervous system tumors. Cause of death was assessed as unrelated to rhGH replacement in the majority of patients (38-42). In contrast, a recent study of Carel *et al.* showed an increased incidence in bone tumor-related mortality and mortality due to cardiovascular diseases in a large cohort of French children, especially in those with highest GH doses (>50µg/kg/day) (43). Cancer mortality was also increased in a large paediatric UK cohort, especially in high-risk patients (44). Moreover, Bell *et al.* reported an increase in the occurrence of second tumors in children with a prior history of malignancy, especially in case of prior exposure to radiotherapy (38). These discrepant results could be possibly explained by the different dosage regime, with higher GH doses in the French study. A recent meta-analysis underlines this hypothesis, establishing that both low and high IGF-1 levels are associated with increased mortality (hazard ratio (HR) 1.18, 95%CI 1.04-1.34) (45). Adult studies did not show an increased mortality or malignancy rate (11;22), except for one study showing moderate increase in overall mortality in females (17). However, the studies do not represent unselected patients, as initiation of rhGH replacement is not likely in patients with an a priori increased cancer risk, thus creating a clear selection bias. With respect to cardiovascular outcomes, an increase in cardiovascular deaths and number of CVAs was reported in women, whereas the myocardial infarction rate decreased (11;17;22). The increased incidence in cardiovascular deaths in

women might partly be attributed to the more atypical presentation of cardiovascular diseases in women (46). Other factors such as inadequately substituted hypogonadism causing preterm menopause or hypocortisolism may also have influenced this risk. In this respect, it has to be noted that available data suggest that both mortality risk and the causes for mortality are related in both patients receiving and those not receiving rhGH replacement. However, it is of paramount importance to accentuate that all observed increased mortality rates reported to date have been associated only with the presence of hypopituitarism *per se*, but that it is still uncertain if, and to what extent, this can be attributed to GHD.

We assessed the methodological quality of all studies using a modified STROBE list (12), classifying 21 of the 23 included studies as having a good or excellent methodological quality. Several quality criteria were not clearly described; specifically, information on potential bias, handling with missing data and reasons for dropout was lacking in most studies. In addition, the use of co-medication, especially lipid-lowering and anti-diabetic drugs, was not clearly reported. It is of importance to emphasize this limitation, because the effects of rhGH therapy on lipid profile, glucose levels and other cardiovascular parameters could not be interpreted without this information. In addition, no RCTs were identified with a follow-up duration >5 years and only a limited number of centres have reported their data, with half of the long-term studies describing (part of) the same patient cohort (Göteborg). Other studies were relatively small and might have been, therefore, underpowered to allow firm conclusions. Due to these limitations, we were not able to perform a meta-analysis.

Another limitation is the report of outcomes only for patients that had completed 5 or 10-years of follow-up, instead of describing the original cohort. This may introduce a significant selection bias, since patients discontinued rhGH therapy for specific reasons, and, therefore, are likely to differ from patients with complete follow-up. Furthermore, a major limitation of most studies is the lack of a control group and thereby the lack of control for ageing. This limitation is important to notice when interpreting the long-term effects of rhGH replacement on for example body weight, BMI, blood pressure, lipid profile, body composition and BMD (47-49). Consequently, it is very difficult to discriminate between effects as a consequence of natural ageing processes and effects by rhGH replacement *per se*. Firm conclusions on the effects of rhGH *per se* can therefore not be drawn from present results. Another feature that needs to be addressed is the change in patient characteristics and approach to rhGH suppletion over time, which may represent an important confounder for any analysis (50). This emphasizes the need of ongoing monitoring during rhGH treatment.

In summary, available literature on long-term rhGH replacement in adult GHD patients shows inconsistent results with respect to its expected beneficial effects, in the presence of several drawbacks to enable a definite interpretation. First, long-term studies were generally uncontrolled and lacked a control group (of non-treated GHD patients) enabling adjustment for subjective changes or changes due to ageing. Second, only a limited number of centres have reported their data, resulting in a low number of evaluable patients with a follow-up duration of ≥5 years of rhGH replacement with half of the long-term studies describing (part of) the same patient cohort. Especially the course of QoL during ongoing therapy is unestablished. With respect to the metabolic profile, rhGH therapy has shown prolonged, beneficial effects on the long-term for body composition, lipid profile, carotid IMT and BMD, but overall cardiovascular risk, as assessed by the prevalence of the metabolic syndrome, glucose levels, BMI appeared not to be influenced or were even negatively affected. Therefore, the benefit of long-term rhGH treatment should be a matter of ongoing research to enable adequate risk-benefit analyses and, in clinical daily practice, the benefits of rhGH should be considered carefully in each patient.

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APPENDIX 1.

Search strategy used for systematic literature search

The following combined key words were used: *((“recombinant growth hormone” OR “recombinant growth hormones” OR “recombinant human growth hormone” OR “recombinant human growth hormones” OR “r-hGH” OR ((“Growth Hormone”[mesh] OR “growth hormone” OR “growth hormones” OR Somatotropin OR Somatotropins OR Somatropin OR hGH OR Serostim OR Zomacton OR “Cryo-Tropin” OR “Cryo Tropin” OR CryoTropin OR “r-hGH-M” OR Humatrope OR Umatrope OR Maxomat OR Norditropin OR Norditropine OR Nutropin OR Omnitrope OR Saizen OR Genotropin OR Genotonorm) AND (“Recombinant Proteins”[mesh] OR recombinant[tw]))) AND (“long term effects” OR “long term effect” OR “longterm effects” OR “longterm effect” OR longterm OR “long term” OR ((“Twenty years” OR “Twenty year” OR “20 years” OR “20 year” OR “Nineteen years” OR “Nineteen year” OR “19 years” OR “19 year” OR “Eighteen years” OR “Eighteen year” OR “18 years” OR “18 year” OR “Seventeen years” OR “Seventeen year” OR “17 years” OR “17 year” OR “Sixteen years” OR “Sixteen year” OR “16 years” OR “16 year” OR “Fifteen years” OR “Fifteen year” OR “15 years” OR “15 year” OR “Fourteen years” OR “Fourteen year” OR “14 years” OR “14 year” OR “Thirteen years” OR “Thirteen year” OR “13 years” OR “13 year” OR “Twelve years” OR “Twelve year” OR “12 years” OR “12 year” OR “Eleven years” OR “Eleven year” OR “11 years” OR “11 year” OR “Ten years” OR “Ten year” OR “10 years” OR “10 year” OR “Nine years” OR “Nine year” OR “9 years” OR “9 year” OR “Eight years” OR “Eight year” OR “8 years” OR “8 year” OR “Seven years” OR “Seven year” OR “7 years” OR “7 year”) AND (effect[tiab] OR effects[tiab] OR outcome OR outcomes))) AND (adult OR adults)) OR ((“recombinant growth hormone” OR “recombinant growth hormones” OR “recombinant human growth hormone” OR “recombinant human growth hormones” OR “r-hGH” OR ((“Growth Hormone”[mesh] OR “growth hormone” OR “growth hormones” OR Somatotropin OR Somatotropins OR Somatropin OR hGH OR Serostim OR Zomacton OR “Cryo-Tropin” OR “Cryo Tropin” OR CryoTropin OR “r-hGH-M” OR Humatrope OR Umatrope OR Maxomat OR Norditropin OR Norditropine OR Nutropin OR Omnitrope OR Saizen OR Genotropin OR Genotonorm) AND (“Recombinant Proteins”[mesh] OR recombinant[tw]))) AND (“long term effects” OR “long term effect” OR “longterm effects” OR “longterm effect” OR longterm OR “long term” OR ((“Twenty years” OR “Twenty year” OR “20 years” OR “20 year” OR “Nineteen years” OR “Nineteen year” OR “19 years” OR “19 year” OR “Eighteen years” OR “Eighteen year” OR “18 years” OR “18 year” OR “Seventeen years” OR “Seventeen year” OR “17 years” OR “17 year” OR “Sixteen years” OR “Sixteen year” OR “16 years” OR “16 year” OR “Fifteen years” OR “Fifteen year” OR “15 years” OR “15 year” OR “Fourteen years” OR “Fourteen year” OR “14 years” OR “14 year” OR “Thirteen years” OR “Thirteen year” OR “13 years” OR “13 year” OR “Twelve years” OR “Twelve year” OR “12 years” OR “12 year” OR “Eleven years” OR “Eleven year” OR “11 years” OR “11 year” OR “Ten years” OR “Ten year” OR “10 years” OR “10 year” OR “Nine years” OR “Nine year” OR “9 years” OR “9 year” OR “Eight years” OR “Eight year” OR “8 years” OR “8 year” OR “Seven years” OR “Seven year” OR “7 years” OR “7 year”) AND (effect[tiab] OR effects[tiab] OR outcome OR outcomes))) NOT (“child”[mesh] NOT “Adult”[mesh])) NOT (“Animals”[mesh] NOT “Humans”[mesh]).*

APPENDIX 2.

List of criteria used for methodological quality assessment, modified STROBE statement

Item	Criterion
1.	Clear presentation of key elements of study design.
2.	Description of setting, locations, relevant dates including periods of recruitment, exposure, follow-up, and data collection.
3.	Description of eligibility criteria, and the sources and methods of selection of participants. i) Description of methods of follow-up (cohort study) ii) Rationale for the choice of cases and controls (case-control study)
4.	Clear definition of all outcomes, exposures, predictors, potential confounders.
5.	Description of any effects to address potential sources of bias.
6.	Clear description of statistical methods and handling with missing data.
7.	Report of reasons of non-participation at each stage of the study (for example, flow diagram).
8.	Clear description of characteristics of study participants.
9.	Clear description of follow-up time.
10.	Presentation of unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (*i.e.* 95% confidence interval).

APPENDIX 3.

Mean serum IGF-1 levels and IGF-1 SDS at start of rhGH suppletion and during follow-up, specified per study

Study(ref)	Baseline	1yr	5yr	7yr	10yr	15yr
Götherström 2010(16)						
IGF-1 ug/l						
IGF-1 SDS	87.0 (54.0)	245.0 (123.0)	200.0 (98.0)	208.0 (98.0)	170.0 (83.0)	NR
	-1.10 (1.08)	2.05 (2.40)	1.17 (2.06)	1.33 (1.76)	1.17 (1.52)	NR
Svensson 2003(17)						
IGF-1 ug/l	109.0 (6.0)	300.0 (13.0)	256.0 (11.0)	NR	NR	NR
IGF-1 SDS	-1.54 (0.12)	2.35 (0.27)	1.53 (0.21)	NR	NR	NR
Götherström 2005(14)						
IGF-1 ug/l	88.0 (10.0)	246.0 (13.0)	202.0 (18.0)	NR	NR	NR
IGF-1 SDS	-1.10 (0.20)	2.06 (0.46)	1.21 (0.39)	NR	NR	NR
Götherström 2009(15)						
IGF-1 ug/l	109.6 (62.4)	300.0 (135.2)	256.0 (114.0)	256.0 (114.0)	202.0 (94.0)	NR
IGF-1 SDS	-1.54 (1.25)	2.35 (2.81)	1.53 (2.18)	1.34 (1.70)	1.12 (2.18)	NR
Roemmler(25)						
IGF-1 ug/l	NR	NR	NR	NR	167.0 (R74.0-319.0)	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Van der Klaauw 2007(29)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	-2.00 (0.8)	NR	0.80 (1.9)	NR	NR	NR
Van der Klaauw 2006(7)						
IGF-1 nmol/l	9.1 (4.6)	NR	NR	25.5 (9.9)	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Giavoli(20)						
IGF-1 nmol/l	8.4 (4.4)	24.8 (9.6)	22.2 (8.0)	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Götherström 2007(6)						
IGF-1 ug/l	NR	NR	NR	NR	NR	NR
IGF-1 SDS	-1.80 (0.12)	3.10 (0.28)	1.88 (0.24)	1.51 (0.19)	1.29 (0.25)	NR
Gibney(23)						
IGF-1 nmol/l	12.3 (3.0)	NR	NR	NR	26.9 (4.0)	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR

Study(ref)	Baseline	1yr	5yr	7yr	10yr	15yr
Götherström 2001(9)						
IGF-1 ug/l	102.0 (6.0)	313.0 (13.0)	268.0 (11.0)	NR	NR	NR
IGF-1 SDS	-1.73 (0.11)	2.55 (0.25)	1.66 (0.20)	NR	NR	NR
Chrisoulidou(22)						
IGF-1 nmol/l	12.4 (R9.2-18.1)	NR	NR	29.1 (R16.5-41.9)	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Cenci(21)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Svensson 2002(27)						
IGF-1 ug/l	74.0 (14.0)	361.0 (35.0)	NR	260.0 (47.0)	NR	NR
IGF-1 SDS	-2.94 (0.36)	2.90 (0.78)	NR	1.89 (0.89)	NR	NR
Götherström 2007(10)						
IGF-1 ug/l	99.5 (6.6)	341.6 (14.1)	281.0 (13.0)	279.6 (14.5)	223.3 (9.8)	NR
IGF-1 SDS	-1.81 (0.12)	3.10 (0.28)	1.88 (0.24)	1.51 (0.19)	1.29 (0.25)	NR
Spielhagen 2010(26)						
IGF-1 ng/ml (M)	144.3 (11.8)	248.0 (12.3)	224.9 (11.6)	203.1 (12.4)	177.3 (11.7)	NR
IGF-1 ng/ml (F)	114.6 (9.0)	187.6 (10.1)	160.2 (9.2)	155.2 (9.5)	153.4 (NR)	NR
IGF-1 SDS (M)	-1.9 (0.3)	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.1 (0.2)	NR
IGF-1 SDS (F)	-2.4 (0.2)	-0.6 (0.2)	-0.6 (0.2)	-0.3 (0.2)	0.0 (0.2)	NR
Elbornsson 2012(4)						
IGF-1 ug/l	103.0 (6.0)	NR	273.0 (10.0)	246.0 (8.0)	206.0 (8.0)	183.0 (7.0)
IGF-1 SDS	-1.69 (0.11)	NR	1.89 (0.21)	1.47 (0.18)	0.84 (0.17)	0.62 (0.16)
Clanget(19)						
IGF-1 ug/l	95.4 (16.2)	304.3 (49.6)	235.0 (24.0)	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Koltowska(5)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Gilchrist(24)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Svensson 2004(28)						
IGF-1 ug/l (M)	125.0 (6.0)	322.0 (11.0)	283.0 (11.0)	275.0 (16.0)	NR	NR
IGF-1 ug/l (F)	83.0 (6.0)	224.0 (11.0)	229.0 (15.0)	231.0 (21.0)	NR	NR
IGF-1 SDS (M)	-1.39 (0.12)	2.74 (0.21)	2.06 (0.22)	1.88 (0.34)	NR	NR
IGF-1 SDS (F)	-2.12 (0.13)	0.51 (0.22)	0.83 (0.29)	0.87 (0.43)	NR	NR
Van Bunderen(11)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS	NR	NR	NR	NR	NR	NR
Gaillard(18)						
IGF-1	NR	NR	NR	NR	NR	NR
IGF-1 SDS (M)	NR	0.54 (1.77)	NR	NR	NR	NR
IGF-1 SDS (F)	NR	-0.16 (1.67)	NR	NR	NR	NR

Data are presented as mean (SD), unless specified otherwise.
IGF-1, insulin-like growth; NR, not reported; R, range; M, males; F, females