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Clinical Endocrinology (2017) **86**, 297–304 doi: 10.1111/cen.13247

ORIGINAL ARTICLE

Metabolic health profile in young adults with Prader–Willi syndrome: results of a 2-year randomized, placebo-controlled, crossover GH trial

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Summary

Context Patients with Prader–Willi syndrome (PWS) have an increased fat mass and decreased lean body mass. GH-treated young adults with PWS who have attained adult height benefit from continuation of growth hormone (GH) treatment, as GH maintained their improved body composition, whereas fat mass increased during the placebo period. Adults with PWS are predisposed to T2DM and cardiovascular disease. Whether GH affects metabolic health profile of this patient group is unknown.

Objective To investigate the effects of GH *vs* placebo on metabolic health, in young adults with PWS who were GH-treated for many years during childhood and had attained adult height (AH).

Method A 2-year, randomized, double-blind, placebo-controlled crossover study with stratification for gender and BMI in 27 young adults with PWS. Intervention with GH (0·67 mg/m^2/day) and placebo, both for 1-year duration.

Results Compared to placebo, GH treatment resulted in similar glucose and insulin levels during oral glucose tolerance test. Only fasting glucose and insulin were slightly higher during GH *vs* placebo (+0·2 mmol/l and +18·4 pmol/l), although both remained within normal ranges in both phases. Blood pressure and lipid profile were similar after GH *vs* placebo. At baseline (AH) and during GH, no patients had metabolic syndrome, while 1 developed it during placebo treatment.

Conclusions Growth hormone treatment has no adverse effects on metabolic health profile. Thus, GH-treated young adults with PWS who have attained AH benefit from continuation of GH treatment without safety concerns regarding metabolic health.

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Study: Nederlands Trialregister NTR1038.

(Received 7 September 2016; returned for revision 16 September 2016; accepted 25 September 2016)

Introduction

Prader–Willi syndrome (PWS) is a neurogenetic disorder caused by the lack of expression of paternally expressed genes located on the PWS region of chromosome 15,[1,2] with clinical findings that change over age. Infancy is characterized by muscular hypotonia and failure to thrive, while obesity, hyperphagia, psychomotor delay and behavioural problems are prominent during childhood and adulthood.[3,4] The body composition of patients with PWS is abnormal, with increased fat mass (FM) and decreased lean body mass (LBM), even if there is no obesity.[2,4–8]

In children with PWS, the benefits of growth hormone (GH) treatment are well-established without adverse effects on glucose parameters, lipid profile and blood pressure.[5] GH improves body composition, bone mineral density, psychomotor development, cognition, adaptive functioning, linear growth and adult height (AH)[5,9–12] and has substantially changed the phenotype of children with PWS. Currently, when young adults with PWS without GHD have attained AH, they have to discontinue GH treatment. We recently demonstrated an impressive increase in FM during the placebo period in GH-treated young adults with PWS who have attained AH, while continuation of GH maintains the improved body composition, indicating that they benefit from continuation of GH treatment.[13]

Adults with PWS are predisposed to develop type 2 diabetes mellitus (T2DM) due to their abnormal body composition[14] and cardiovascular disease (CVD), because risk factors for CVD such as hypertension and hyperlipidaemia occur more often in PWS.[15] GH has diabetogenic effects, but there are no studies on the metabolic effects of GH *vs* placebo in young adults with PWS who were treated with GH during childhood and had attained AH. Studies in older PWS patients, who were GH-untreated at inclusion, found no major side effects of GH on metabolic health profile.[16,17]

As there were no negative effects of GH on the metabolic health profile in GH-treated children and GH-untreated adults with PWS, we hypothesized that GH *vs* placebo would result in a similar or even more favourable metabolic profile after attainment of AH. We therefore investigated the effects of GH *vs* placebo on the metabolic health profile of young adults with PWS after attainment of AH, in a 2-year, randomized, double-blind crossover study.

Subjects and methods

Subjects

Inclusion criteria were (i) genetically confirmed diagnosis of PWS by a positive methylation test; (ii) GH treatment during childhood for at least 2 years and being on GH at time of inclusion; and (iii) AH attainment, defined as a height velocity <0·5 cm per 6 months and a complete epiphyseal fusion. Exclusion criteria were (i) medication to reduce weight (fat) or (ii) noncooperative behaviour.

From June 2008 to January 2014, 33 young adults with PWS aged 14·1–20·2 years fulfilled the inclusion criteria, but two did not want to continue daily injections and three refused to participate due to too large a burden of hospital visits. Twenty-eight subjects were included, but one 16·7-year-old participant (BMI 25·0 kg/m^2) died due to gastric rupture 3 months after the study start while receiving placebo. Her baseline characteristics were not significantly different from the other patients and were excluded from analysis.

Growth hormone treatment was prescribed during childhood at an initial dose of 1 mg/m^2/day, and the dose was lowered in eight children due to high serum IGF-I levels. In this study, the GH dose was 0·67 mg/m^2/day (≈0·023 mg/kg/day), which was considerably lower than during childhood. The most important other medications were sex steroid replacement therapy in 12 (42·9%) young adults, thyroid hormone supplementation in 8 (28·6%), modafinil in 2 (7·1%) and risperidone and citalopram in 1 (3·6%) adolescent; doses were not changed during the study. All patients were on strict diet and exercise programmes.

Design

A 2-year, randomized, double-blind, placebo-controlled, crossover study investigated the effects of 1-year placebo *vs* 1-year GH on metabolic health profile. Young adults were stratified according to gender and BMI (below/above 25 kg/m^2) and then randomly and blindly assigned to receive 1 year of subcutaneous injections once daily at bedtime of either 0·67 mg/m^2/day GH (Genotropin®, 5 mg/ml; Pfizer Inc, New York, NY, USA) or 1 year of identical appearing placebo (placebo, Pfizer), after which they crossed over to the alternative treatment for another year. According to the FDA, a washout period should be at least three times the half-life of a drug.[18] As the half-life of GH is only 2–3 h and the study duration per phase was 1 year, no washout period was implemented. An independent statistician generated the random allocation sequence. Investigators were blinded for the allocation. An independent physician monitored safety during the study. During the entire study period, unblinding was not necessary.

Measurements

Patients were assessed every 3 months by the PWS team of the Dutch Growth Research Foundation in collaboration with paediatric endocrinologists and paediatricians. At each visit, the injection dose was adjusted to the calculated body surface area. In addition, patients visited the Sophia's Children Hospital at baseline, 6, 12, 18 and 24 months, to obtain: height, weight, waist circumference (WC), blood pressure, fasting blood levels of glucose and insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDLc) and high-density lipoprotein cholesterol (HDLc), triglyceride (TG), IGF-I and IGFBP-3. In addition, a standard 75-g oral glucose tolerance test (OGTT) according to the World Health Organization was performed. To evaluate the overall responses to the oral glucose load, apart from the plasma levels at various time points, the 120-min area under the curve (AUC) for time–concentration for glucose and insulin was calculated using the trapezoidal rule. The insulin/glucose ratio at 0, 30 and 120 min was calculated as an index of relative insulin resistance. Impaired glucose tolerance (IGT) was defined as a fasting glucose level <6·1 mmol/l, and glucose between 7·8 and 11·1 mmol/l (140–200 mg/dl) 120 min after glucose load.[19]

Standing height was measured with a calibrated Harpenden stadiometer, weight was determined on a calibrated scale (Servo-Balance KA-20-150S), and BMI was calculated. Height, weight and BMI were expressed as SDS, adjusted for age and sex.[20,21] SDS values were calculated with GrowthAnalyser 4.0 (www.growthanalyser.org). Systolic blood pressure (BP) and diastolic blood pressure (BP) were measured using an appropriately sized cuff, while patients were in the sitting position. As height is an important determinant of BP, BP was expressed as standard deviation scores (SDS), adjusted for height and sex.[22] Fat mass percentage (FM) was measured by DXA.[13]

Assays

Blood samples were collected after an overnight fast and measured in a single laboratory: glucose, insulin, IGF-I, IGFBP-3, TC, HDLc and TG. LDLc was calculated using the Friedewald formula: LDLc (mmol/l) = TC–HDLc–0·45*TG.[23] Blood samples during the OGTT for glucose and insulin determination were obtained after 30, 60, 90 and 120 min after ingestion and immediately assayed.[24] Levels of IGF-I and IGFBP-3 were measured and expressed as SDS, adjusting for age and gender.[13,25] Lipids were determined as described.[26]

Metabolic syndrome

Revised criteria of the National Cholesterol Education Program (NCEP; Adult Treatment Panel III) were used to determine components of the metabolic syndrome.[27] Metabolic syndrome was defined as having three or more of the following risk

factors: (i) Abdominal obesity: waist circumference in men >102 cm, and in women >88 cm; (ii) TG above 1·7 mmol/l; (iii) HDLc in men below 1·03, in women below 1·3 mmol/l; (iv) BP ≥130/≥85 mm Hg; (v) Fasting glucose above 5·6 mmol/l.

Statistics

Statistical analysis was performed with SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Calculation of sample size based on body composition indicated that 20 subjects would be sufficient for a power of >90% with a significance level of 0·05. To account for attrition, eight more patients were added. As data were normally distributed, parametric tests were used and data expressed as mean [standard deviation (SD)]. Effects of GH *vs* placebo were calculated using linear mixed model analysis with the outcomes measured at the end of the two treatment periods as dependent variable and with an unstructured covariance matrix. Possible carry-over effects were analysed by adding the interaction between period and treatment, but not found. In addition to the 2-year results, we also presented systolic and diastolic BP SDS and lipid levels during the 4 years prior to attainment of adult height, as significant changes in the years prior to the study might influence the interpretation. Linear mixed model analysis was used.

Study approval

Written informed consent was obtained from the patients and their caregivers. The study protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam, and registered at Dutch Trial Register (www.trialregister.nl NTR1038).

Results

Baseline characteristics

Table 1 shows the clinical characteristics of 27 young adults (eight boys, 19 girls) with PWS at adult height (AH). Their mean age was 17·2 (1·8) years and BMI +0·9 (1·3) SDS. Nine (33·3%) patients had a deletion, 15 (55·6%) an mUPD, 2 (7·4%) an ICD and 1 (3·7%) a translocation. During childhood, GH treatment was started at a mean age of 8·5 (3·5) years and continued for 8·7 (3·2) years until AH. Boys had a mean AH of 174·1 (3·0) cm, being −1·4 (0·4) SDS and girls reached 159·9 (7·3) cm, being −1·7 (1·1) SDS. Both treatment regimens had similar baseline characteristics.

Metabolic health profile

Carbohydrate metabolism. Fig. 1 and Table 2 show glucose and insulin levels during OGTT after long-term GH treatment during childhood, at baseline of this study at adult height (AH), after 1 year of GH and after 1 year of placebo. At baseline, none of the patients had a fasting glucose above 5·6 mmol/l, while IGT, defined as a glucose between 7·8 and 11·1 mmol/l at 120 min after glucose load, was present in four patients and none had T2DM.

Compared to placebo, GH treatment resulted in similar glucose and insulin levels at 30, 60, 90 and 120 min after glucose load (Fig. 1). Only fasting glucose and insulin levels were higher after GH treatment *vs* placebo, although both remained within the normal ranges in both phases (glucose 4·7 *vs* 4·5 mmol/l, $P = 0{\cdot}012$, and insulin 65·8 *vs* 47·4 pmol/l, $P = 0{\cdot}037$, respectively) (Table 2). All other carbohydrate parameters were similar after GH *vs* placebo. Mean glucose at 120 min after glucose intake was similar (GH vs placebo 6·0 *vs* 6·1 mmol/l, $P = 0{\cdot}998$). The 120-min AUCs for glucose and insulin during OGTT were not significantly different after both treatment phases ($P = 0{\cdot}343$ and $P = 0{\cdot}457$, respectively), and the insulin/glucose ratios at 30 and 120 min were similar after GH and placebo. IGT was present in two patients after 1 year of GH and in two other patients after 1 year of placebo. None of the patients developed T2DM.

Blood pressure. Fig. 2a,b shows systolic BP and diastolic BP during the 2-year crossover study and the 4 years before. At baseline, mean systolic BP and diastolic BP were significantly higher than height- and sex-matched controls (0·5 and 0·7 SDS, $P = 0{\cdot}016$ and $P < 0{\cdot}001$, respectively) (Table 2), but there were only two patients with a systolic BP above +2 SDS (both +2·2 SDS).

Compared to placebo, GH treatment resulted in a similar systolic BP and diastolic BP ($P = 0{\cdot}547$ and $P = 0{\cdot}779$). Four young adults had a systolic BP above +2 SDS after 1 year of GH, while two of them and one other had an elevated systolic BP after 1 year of placebo. Two young adults had a diastolic BP above +2 SDS after both GH and placebo, and 1 only after 1 year of placebo.

Serum lipid levels. Fig. 2c shows fasting serum lipid levels during the 2-year crossover study and the 4 years before. At baseline, all mean lipid levels were within the normal range (Table 2). Levels above the normal range of TC and LDLc were found in, respectively, two and one patients, while one patient had a HDLc below the normal range.

Compared to placebo, GH treatment resulted in similar levels of TC, LDLc, HDLc and TG ($P > 0{\cdot}415$). One young adult had a TC above the upper limit of 5·5 mmol/l after 1 year of GH, while another had an elevated TC after 1 year of placebo. LDLc and TG were not elevated after 1 year of GH treatment, while after 1 year of placebo, one patient had an LDLc higher than the upper limit of 3·8 mmol/l, and this and another patient had a TG level higher than 1·6 mmol/l. HDLc was not below the lower limit of 0·9 mmol/l in any patient.

Metabolic syndrome. Table 3 shows the different components of the metabolic syndrome (MS). At baseline (AH), none of the patients had MS according to the revised NCEP criteria.[27]

After 1 year of GH treatment, 12 components of MS were present; four patients had central obesity, three low HDLc

Table 1. Baseline characteristics of total group and per treatment schedule

	PWS (n = 27)	GH/Placebo (n = 13)	Placebo/GH (n = 14)	P*
Boys/girls (n)	8/19	4/9	4/10	
Genetic subtype				
Deletion	9	7	2	
mUPD	15	5	10	
ICD/translocation	3	2	1	
Age (yrs)	17·2 (1·8)	17·3 (1·2)	17·2 (2·2)	0·877
Height for age (SDS)	−1·3 (0·9)	−1·2 (0·9)	−1·3 (0·9)	0·695
Adult height (SDS)	−1·6 (1·0)	−1·5 (0·9)	−1·7 (1·1)	0·660
Weight for height (SDS)	0·6 (1·3)	0·5 (1·4)	0·8 (1·2)	0·563
BMI for age (SDS)	0·9 (1·3)	0·7 (1·3)	1·0 (1·2)	0·527
BMI for age PWS (SDS)	−1·4 (1·2)	−1·5 (1·2)	−1·2 (1·2)	0·506
Fat mass percentage (%)	38·0 (10·9)	36·4 (11·0)	39·4 (10·9)	0·487
Age at start GH treatment (yrs)	8·5 (3·5)	8·9 (3·2)	8·2 (3·8)	0·646
Duration of GH treatment (yrs)	8·7 (3·2)	8·4 (2·5)	8·9 (3·8)	0·679

Data expressed as mean with (SD).
*P-value at baseline between the two treatment schedules.

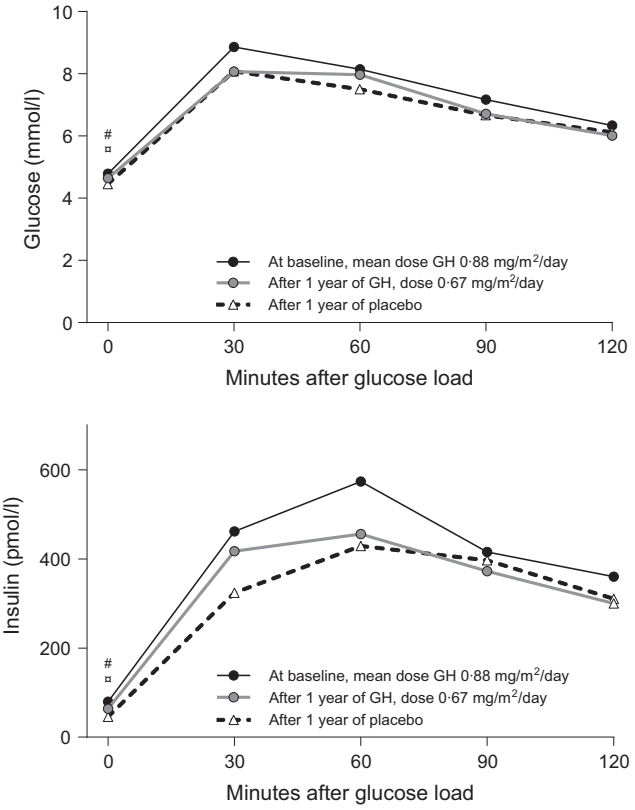

Fig. 1 Glucose and Insulin during oral glucose tolerance test (OGTT). Mean glucose and insulin levels during OGTT after long-term GH treatment during childhood at baseline (AH) (*black circles*), after 1 year of GH treatment (*grey circles*) and after 1 year of placebo (*white triangles*) of 27 young adults with PWS. #GH *vs* placebo P-value <0·05, ¤Baseline *vs* placebo P-value <0·05, other not significantly different.

levels and five a high BP, while none had high TG levels or high fasting glucose. After 1 year of placebo, there were 17 components of MS; five had central obesity, one high TG levels, six low HDLc levels, four high BP and one a high fasting glucose.

Compared to placebo, GH treatment did not result in MS. During the 2 years of study, one girl (3·7%) developed MS. She had no MS symptoms after 1 year of GH, but after the subsequent year with placebo three symptoms were present.

Discussion

To our knowledge, this is the first 2-year, randomized, double-blind, placebo-controlled study in young adults with PWS who were treated with GH during childhood until AH, which investigates the effects of GH *vs* placebo on metabolic health. Our findings demonstrate that glucose-stimulated glucose and insulin levels during OGTT were similar during GH treatment *vs* placebo. Compared to placebo, GH treatment did not affect other carbohydrate parameters such as AUC of glucose and insulin, and insulin/glucose ratios. Fasting glucose and insulin levels were slightly higher during GH *vs* placebo therapy, but remained within the normal ranges during both phases and none developed T2DM. The prevalence of IGT and MS was similar after GH treatment and placebo, and BP and lipid profiles were not significantly different after GH therapy *vs* placebo. This indicates that GH does not affect carbohydrate parameters compared with placebo, with the exception of a slightly higher fasting glucose and insulin, and that GH therapy *vs* placebo does not disturb components of metabolic health.

Compared to placebo, GH treatment did not affect glucose-stimulated glucose and insulin levels, AUC for glucose and insulin, and insulin/glucose ratios. These results of the OGTT are reassuring and demonstrate that glucose and insulin homoeostasis reacts appropriately without developing glucose intolerance during GH therapy *vs* placebo. The minor increase in fasting insulin with minimal influences on glucose metabolism is a well-known and physiological phenomenon during GH treatment.[5,28] Our finding that GH treatment has only minimal effects without being pathologic concur with the conclusion of Höybye *et al.*[16] that GH treatment did not elicit pronounced adverse effect on glucose and insulin homoeostasis in older previously GH-untreated adults with PWS.

Lipid levels remained similar during GH *vs* placebo therapy, while studies in other patient groups such as GHD or obesity found beneficial effects of GH treatment on lipid profiles.[29,30] Our findings might be the result of the already quite favourable lipid profile of patients with PWS and the fact that hypercholesterolaemia does not seem to be frequent, probably due to the smaller amount of visceral fat.[31] Findings in older adults with PWS who were GH-untreated at inclusion were in line with our

Table 2. Metabolic health parameters of 27 PWS adolescents at different stages in the study

		Stage of the study			Mean difference between GH and placebo	*P*-value*
		Baseline	After 1 year GH	After 1 year placebo		
Carbohydrate data						
Fasting glucose (mmol/l)		4·8 (4·6 to 4·9)	4·7 (4·5 to 4·9)	4·5 (4·3 to 4·7)	0·2	**0·012**
AUC glucose (mmol/l*120 min)		897 (824 to 971)	855 (782 to 929)	808 (729 to 887)	47	0·343
Fasting insulin (pmol/l)		79·4 (58·4 to 100·3)	65·8 (49·1 to 82·5)	47·4 (30·7 to 64·1)	18·4	**0·037**
AUC insulin (pmol/l*120 min*10^3)		47·4 (36·6 to 58·2)	43·5 (32·1 to 54·8)	39·4 (27·7 to 51·0)	4·1	0·457
Ratio ins/gluc at 30 min		52·2 (38·5 to 65·9)	50·7 (38·1 to 63·3)	41·2 (28·2 to 54·3)	9·5	0·205
Ratio ins/gluc at 120 min		55·0 (39·4 to 70·7)	50·7 (36·5 to 65·0)	53·5 (38·1 to 68·9)	−2·8	0·752
Blood pressure						
Systolic BP (SDS)		0·5 (0·1 to 0·8)	0·8 (0·4 to 1·1)	0·6 (0·3 to 1·0)	0·1	0·547
Diastolic BP (SDS)		0·7 (0·4 to 0·9)	0·8 (0·5 to 1·1)	0·9 (0·6 to 1·2)	0·0	0·779
Serum lipids						
TC (mmol/l)	3·0 to 5·5*	4·4 (4·1 to 4·7)	4·5 (4·3 to 4·8)	4·6 (4·4 to 4·8)	0·0	0·851
LDLc (mmol/l)	1·7 to 3·8*	2·7 (2·4 to 3·0)	2·8 (2·6 to 3·0)	2·8 (2·6 to 3·0)	0·0	0·711
HDLc (mmol/l)	0·9 to 1·9*	1·5 (1·3 to 1·6)	1·5 (1·3 to 1·6)	1·5 (1·3 to 1·6)	0·0	0·974
TG (mmol/l)	0·4 to 1·6*	0·9 (0·8 to 1·0)	0·9 (0·7 to 1·0)	0·8 (0·7 to 0·9)	0·1	0·415
Growth factors						
IGF-I SDS		2·2 (1·7 to 2·6)	1·8 (1·4 to 2·2)	−0·7 (−1·1 to −0·3)	2·5	**<0·001**
IGFBP-3 SDS		0·5 (0·2 to 0·7)	0·4 (0·2 to 0·6)	−0·6 (−0·8 to −0·3)	1·0	**<0·001**

GH, growth hormone; AUC, area under the curve; ins/gluc, ratio of insulin/glucose; BP, blood pressure; TC, total cholesterol; TG, triglyceride; PWS, Prader–Willi syndrome.
Data expressed as mean with 95% CI.
*Normal range for 13–18 years. *P*-value of mean difference between GH and placebo, bold values are statistically significant ($p<0.05$).

results,[28] although Sode-Carlsen *et al.*[17] reported a small reduction in LDLc only, during GH therapy compared to placebo. Their patients were, however, GH-untreated and heavier at baseline, while our patients had already received 8 years of GH treatment before they participated in the present study, and this might have influenced the findings.

In our cohort of patients who were GH-treated during childhood for many years, none had MS at attainment of AH and none developed MS during GH therapy after AH, while one developed MS during placebo treatment. In obese adults with PWS, a prevalence of MS of 41·4% was reported, while it was only 4·7% in the nonobese PWS subjects.[32] The authors concluded that obesity plays a significant role in metabolic health. Elaborating on this, it might be that the role of body composition, and subsequently GH treatment, is even larger. Continuation of GH prevents deterioration of body composition[13] and thus favourably affects metabolic health.

Patients with PWS have a relatively high insulin sensitivity with high adiponectin levels, which are thought to be protective with regard to T2DM and CVD.[31,33–35] Besides the favourable effects of GH on adiponectin,[34] continuation of GH treatment at AH prevents the deterioration of body composition in young adults with PWS. During placebo, FM rose dramatically with a relative increase of 21·5% in only 1 year, while GH treatment maintained the improved body composition with less FM and more LBM.[13] Thus, when GH treatment stops, the natural course of increasing obesity in PWS is no longer counteracted. As LBM is the primary tissue of insulin-stimulated glucose uptake, disposal and storage,[36] and obesity and FM are associated with MS, T2DM and CVD,[37] it is likely that the deterioration of body composition due to discontinuation of GH will impair glucose and insulin homoeostasis over time. This suggests that discontinuation of GH at AH might result in a worse metabolic health profile in the long term in young adults with PWS, and that the benefits of GH far outweigh the minimal effects on glucose and insulin homoeostasis.

The percentage of patients with an mUPD was higher than previously described,[38] but an mUPD is more commonly found nowadays, probably related to the increasing maternal age at conception in Western countries. It is not only a diagnosis of PWS which influences risk of metabolic disease but also other risk factors such as obesity, advanced age, positive family history and low LBM. By choosing a crossover design, interindividual risk factors were eliminated and this strong design allowed a smaller sample size, especially important as PWS is a rare disorder. Ongoing monitoring of young adults with PWS receiving GH treatment is highly recommended as they are at risk of developing T2DM and CVD, although our study demonstrated reassuring findings. Additional studies are needed to confirm this in the longer term, as our patients are still young and received only 1 year of GH treatment.

In conclusion, this crossover study demonstrates that 1 year of GH treatment has no adverse effects on glucose homoeostasis, with similar glucose-stimulated glucose and insulin levels during OGTT, AUC of glucose and insulin, and insulin/glucose ratios during GH treatment and placebo. Fasting glucose and insulin

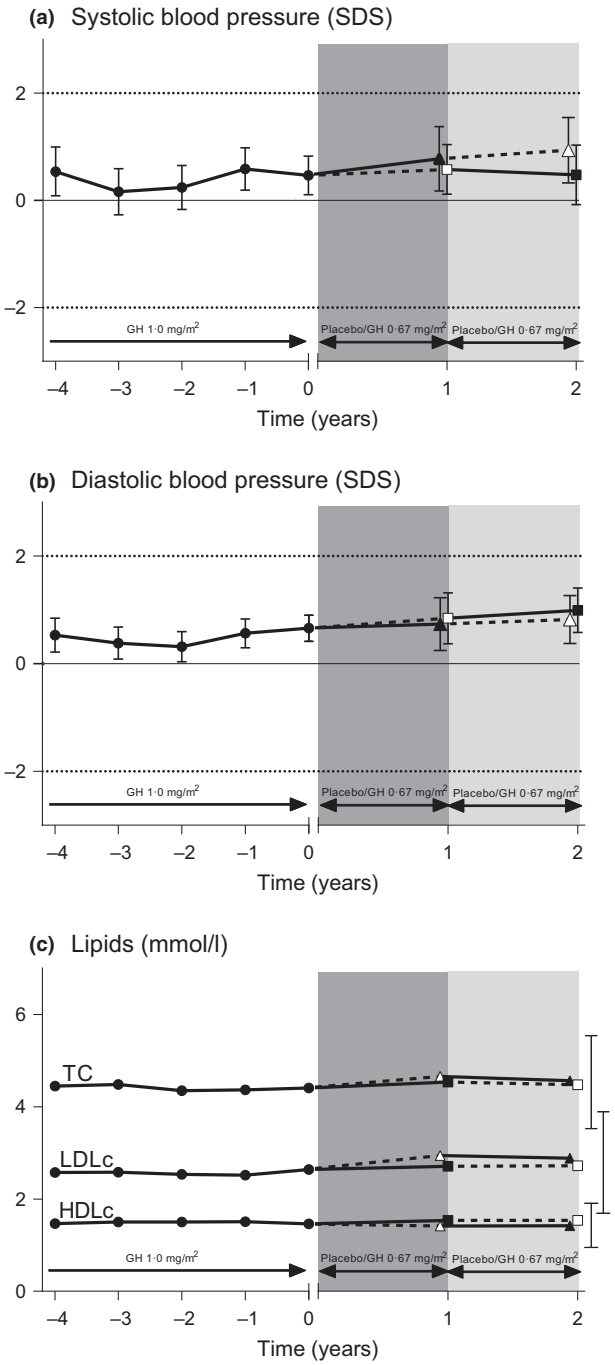

Fig. 2 (a–c) Changes in systolic (a) and diastolic blood pressure (b), and total cholesterol, LDLc and HDLc (c) presented as Means with 95% CI of 27 adolescents with PWS during the 2 years of this crossover study (in dark and light grey). Longitudinal changes in Estimated Marginal Means with 95% CI during the 4 years prior to attainment of AH (in white). BP SDS was calculated according to height- and sex-matched references.[22] Normal ranges for lipids are shown on the right side of Fig. 2c. Black lines with black symbols represent the course during GH treatment, and dotted lines with white symbols represent the course during placebo.

levels remained within the normal ranges and were only slightly higher during GH treatment *vs* placebo. Blood pressure and lipid profile remained similar in both phases. None of the patients developed MS during GH treatment, while one developed MS during placebo. Thus, GH-treated young adults with PWS who have attained AH benefit from continuation of GH treatment without safety concerns regarding their metabolic health profile.

Table 3. Metabolic syndrome components during the study according to ATP III criteria[26]

	Stage of the study		
Symptoms	Baseline	During 1 year GH	During 1 year placebo
Central Obesity	2/27 (7·4%)	4/27 (14·8%)	5/27 (18·5%)
High TG levels	None	None	1/27 (3·7%)
Low HDLc levels	6/27 (22·2%)	3/27 (11·1%)	6/27 (22·2%)
High BP	3/27 (11·1%)	5/27 (18·5%)	4/27 (14·8%)
High fasting glucose	None	None	1/27 (3·7%)
More than 3 symptoms	None	None	1/27 (3·7%)

GH, growth hormone; TG, triglyceride; BP, blood pressure.
Number of patients (%) with MS symptoms. No significant difference between GH and placebo year.

Acknowledgements

We express our gratitude to all young adults and parents for their enthusiastic participation in this study. We thank Mariëlle van Eekelen, Berber Kerkhof and Elise Snikkers for their help. We thank all the collaborating physicians and the paediatricians who referred patients to participate in our study.

Funding

This study was an investigator-initiated study, supported by an independent research grant from Pfizer. Pfizer provided Genotropin and identical appearing placebo without charge.

Declaration of interest

The study was investigator-initiated, ACH received an independent research grant from Pfizer. The other authors have declared that no conflict of interest exists.

References

1 Goldstone, A.P., Holland, A.J., Hauffa, B.P. *et al.* (2008) Recommendations for the diagnosis and management of Prader–Willi syndrome. *Journal of Clinical Endocrinology and Metabolism*, **93**, 4183–4197.
2 Cassidy, S.B. (1997) Prader–Willi syndrome. *Journal of Medical Genetics*, **34**, 917–923.

3 Eiholzer, U., Bachmann, S. & l'Allemand, D. (2000) Is there growth hormone deficiency in Prader–Willi Syndrome? Six arguments to support the presence of hypothalamic growth hormone deficiency in Prader–Willi syndrome. *Hormone Research*, **53** (Suppl 3), 44–52.

4 Holm, V.A., Cassidy, S.B., Butler, M.G. *et al.* (1993) Prader–Willi syndrome: consensus diagnostic criteria. *Pediatrics*, **91**, 398–402.

5 Bakker, N.E., Kuppens, R.J., Siemensma, E.P. *et al.* (2013) Eight years of growth hormone treatment in children with Prader–Willi syndrome: maintaining the positive effects. *Journal of Clinical Endocrinology and Metabolism*, **98**, 4013–4022.

6 Emerick, J.E. & Vogt, K.S. (2013) Endocrine manifestations and management of Prader–Willi syndrome. *International Journal of Pediatric Endocrinology*, **2013**, 14.

7 Brambilla, P., Bosio, L., Manzoni, P. *et al.* (1997) Peculiar body composition in patients with Prader-Labhart-Willi syndrome. *American Journal of Clinical Nutrition*, **65**, 1369–1374.

8 Bekx, M.T., Carrel, A.L., Shriver, T.C. *et al.* (2003) Decreased energy expenditure is caused by abnormal body composition in infants with Prader–Willi Syndrome. *Journal of Pediatrics*, **143**, 372–376.

9 Bakker, N.E., Kuppens, R.J., Siemensma, E.P. *et al.* (2015) Bone mineral density in children and adolescents with Prader–Willi syndrome: a longitudinal study during puberty and 9 years of growth hormone treatment. *Journal of Clinical Endocrinology and Metabolism*, **100**, 1609–1618.

10 Festen, D.A., Wevers, M., Lindgren, A.C. *et al.* (2008) Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader–Willi syndrome. *Clinical Endocrinology*, **68**, 919–925.

11 Siemensma, E.P., Tummers-de Lind van Wijngaarden, R.F., Festen, D.A. *et al.* (2012) Beneficial effects of growth hormone treatment on cognition in children with Prader–Willi syndrome: a randomized controlled trial and longitudinal study. *Journal of Clinical Endocrinology and Metabolism*, **97**, 2307–2314.

12 Lo, S.T., Festen, D.A., Tummers-de Lind van Wijngaarden, R.F. *et al.* (2015) Beneficial effects of long-term growth hormone treatment on adaptive functioning in infants with Prader–Willi syndrome. *American Journal on Intellectual Developmental Disabilities*, **120**, 315–327.

13 Kuppens, R.J., Bakker, N.E., Siemensma, E.P.C. *et al.* (2016) Beneficial effects of growth hormone in young adults with Prader–Willi syndrome: results of a 2-year randomized, double-blind, placebo-controlled cross-over trial. *Journal of Clinical Endocrinology and Metabolism*, **101**, 4110–4116.

14 Nagai, T. & Mori, M. (1999) Prader–Willi syndrome, diabetes mellitus and hypogonadism. *Biomedicine & Pharmacotherapy*, **53**, 452–454.

15 Laurier, V., Lapeyrade, A., Copet, P. *et al.* (2015) Medical, psychological and social features in a large cohort of adults with Prader–Willi syndrome: experience from a dedicated centre in France. *Journal of Intellectual Disability Research*, **59**, 411–421.

16 Hoybye, C., Hilding, A., Jacobsson, H. *et al.* (2003) Growth hormone treatment improves body composition in adults with Prader–Willi syndrome. *Clinical Endocrinology*, **58**, 653–661.

17 Sode-Carlsen, R., Farholt, S., Rabben, K.F. *et al.* (2010) One year of growth hormone treatment in adults with Prader–Willi syndrome improves body composition: results from a randomized, placebo-controlled study. *Journal of Clinical Endocrinology and Metabolism*, **95**, 4943–4950.

18 Senn, S. (1994) The AB/BA crossover: past, present and future? *Statistical Methods in Medical Research*, **3**, 303–324.

19 World Health Organization (2006) Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation. World Health Organization.

20 Fredriks, A.M., van Buuren, S., Wit, J.M. *et al.* (2000) Body index measurements in 1996–7 compared with 1980. *Archives of Disease in Childhood*, **82**, 107–112.

21 Fredriks, A.M., van Buuren, S., Burgmeijer, R.J. *et al.* (2000) Continuing positive secular growth change in The Netherlands 1955–1997. *Pediatric Research*, **47**, 316–323.

22 Rosner, B., Prineas, R.J., Loggie, J.M. *et al.* (1993) Blood pressure nomograms for children and adolescents, by height, sex, and age, in the United States. *Journal of Pediatrics*, **123**, 871–886.

23 Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**, 499–502.

24 Kuppens, R.J., Diene, G., Bakker, N.E. *et al.* (2015) Elevated ratio of acylated to unacylated ghrelin in children and young adults with Prader–Willi syndrome. *Endocrine*, **50**, 633–642.

25 Rikken, B., van Doorn, J., Ringeling, A. *et al.* (1998) Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Hormone Research*, **50**, 166–176.

26 van der Steen, M., Lem, A.J., van der Kaay, D.C. *et al.* (2015) Metabolic health in short children born small for gestational age treated with growth hormone and gonadotropin-releasing hormone analog: results of a randomized, dose-response trial. *Journal of Clinical Endocrinology and Metabolism*, **100**, 3725–3734.

27 Grundy, S.M., Cleeman, J.I., Daniels, S.R. *et al.* (2005) Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*, **112**, 2735–2752.

28 Sanchez-Ortiga, R., Klibanski, A. & Tritos, N.A. (2012) Effects of recombinant human growth hormone therapy in adults with Prader–Willi syndrome: a meta-analysis. *Clinical Endocrinology*, **77**, 86–93.

29 Maison, P., Griffin, S., Nicoue-Beglah, M. *et al.* (2004) Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. *Journal of Clinical Endocrinology and Metabolism*, **89**, 2192–2199.

30 Mekala, K.C. & Tritos, N.A. (2009) Effects of recombinant human growth hormone therapy in obesity in adults: a meta analysis. *Journal of Clinical Endocrinology and Metabolism*, **94**, 130–137.

31 Talebizadeh, Z. & Butler, M.G. (2005) Insulin resistance and obesity-related factors in Prader–Willi syndrome: comparison with obese subjects. *Clinical Genetics*, **67**, 230–239.

32 Grugni, G., Crino, A., Bedogni, G. *et al.* (2013) Metabolic syndrome in adult patients with Prader–Willi syndrome. *Nutrition, Metabolism and Cardiovascular Diseases*, **23**, 1134–1140.

33 Zipf, W.B. (1999) Glucose homeostasis in Prader–Willi syndrome and potential implications of growth hormone therapy. *Acta Paediatrica. Supplement*, **88**, 115–117.

34 Festen, D.A., van Toorenenbergen, A., Duivenvoorden, H.J. *et al.* (2007) Adiponectin levels in prepubertal children with Prader–Willi syndrome before and during growth hormone therapy. *Journal of Clinical Endocrinology and Metabolism*, **92**, 1549–1554.

35 Pagano, C., Marin, O., Calcagno, A. *et al.* (2005) Increased serum resistin in adults with Prader–Willi syndrome is related to obesity and not to insulin resistance. *Journal of Clinical Endocrinology and Metabolism*, **90**, 4335–4340.

36 Smith, A.G. & Muscat, G.E. (2005) Skeletal muscle and nuclear hormone receptors: implications for cardiovascular and metabolic disease. *International Journal of Biochemistry & Cell Biology*, **37**, 2047–2063.

37 Mokdad, A.H., Ford, E.S., Bowman, B.A. *et al.* (2003) Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA*, **289**, 76–79.

38 Angulo, M.A., Butler, M.G. & Cataletto, M.E. (2015) Prader–Willi syndrome: a review of clinical, genetic, and endocrine findings. *Journal of Endocrinological Investigation*, **38**, 1249–1263.