Eur J Pediatr (2000) 159: 835–842

ORIGINAL PAPER

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Cardiovascular risk factors improve during 3 years of growth hormone therapy in Prader-Willi syndrome

Received: 25 January 2000 / Accepted: 9 May 2000

Abstract Cardiovascular risk factors in Prader-Willi syndrome (PWS, OMIM 176270) may be independently caused by overweight or hypothalamic growth hormone (GH) deficiency. The present observational study in 23 children with PWS, aged 0.3–14.6 years, focuses on the specific pattern, age-dependency and interrelation of cardiovascular risk factors, namely percentage fat mass and regional fat distribution, triglycerides (TG), lipoprotein cholesterols (LDL-C, HDL-C), lipoprotein (a) (Lp(a)), apolipoproteins A-I (Apo A-I) and B (Apo B), as well as on the longer-term effects of GH therapy (ca. 0.037 mg/kg per day for 3 years on average). We report that in children above 4 years, percentage body fat was increased in all and waist-to-hip-ratio (WHR) in 35%. Abnormal levels of LDL-C, Apo B, HDL-C and TG were found in 6, 7, 6 and 3 children, respectively. Lp(a) was above 300 mg/l in 5 patients and remained unchanged during GH therapy. However, percentage fat mass dropped to the upper normal range and WHR became normal in all patients receiving GH therapy, as did the ratio of LDL-C to HDL-C, subsequent to decreasing LDL-C and increasing HDL-C. Nevertheless, we could not find any significant correlation between parameters of total fat mass or fat distribution and serum lipid parameters, except for abdominal fat distribution (trunk-/leg-fat ratio) to TG before therapy.

Conclusion Several cardiovascular risk factors are already present in prepubertal children with Prader-Willi-syndrome and they are improved by growth hormone treatment, acting both on body composition and lipid metabolism.

Key words Prader-Willi syndrome · Growth hormone therapy · Cardiovascular risk factors · Body composition · Lipids

Abbreviations *Apo A-1* apolipoprotein A-1 · *Apo-B* apolipoprotein B · *DEXA* dual-energy X-ray absorptiometry · *GH* growth hormone · *HDL-C* high density lipoprotein cholesterol · *LDL-C* low density lipoprotein cholesterol · *Lp(a)* lipoprotein (a) · *PWS* Prader-Willi syndrome · *TC* total cholesterol · *TG* triglycerides · *WHR* waist/hip ratio

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This work was supported by Swiss National Science Foundation Grant 32.056063.98 and Swiss Academy of Medical Science Grant NF 455/98

Introduction

At present, the Prader-Willi syndrome (PWS) is the most frequent known genetic form of obesity. The complex pattern of abnormalities is related to a hypothalamic disorder, of which the precise mechanism remains unknown. Among the main features of PWS, namely polyphagia, obesity, short stature, hypogonadism, muscle hypotonia and mental retardation, obesity is the main cause of morbidity and mortality [12, 30], and premature coronary artery disease has been documented in single patients with PWS [37]. This study therefore investigated cardiovascular risk factors in PWS, which may originate from two pathogenic mechanisms: overweight and hypothalamic growth hormone (GH) deficiency.

In non-syndromal obesity, overweight is gradually linked to secondary conditions such as type 2 diabetes or hyperlipidaemia [5, 36, 42], which may develop as early as in childhood. Some 50% of obese children present at least one cardiovascular risk factor [23, 26]. It has to be questioned, however, whether there is the same pathogenic relationship between obesity, abdominal fat distribution, hyperinsulinism, hyperlipidaemia and arteriosclerotic disease in PWS as in non-syndromal obesity [5, 36, 39] because in PWS, a rather low insulin secretion has been observed [18, 33]. This relative hypoinsulinism has been explained by GH deficiency.

Hypothalamic GH deficiency has been shown in children with PWS by decreased insulin-like growth factor-1 and low levels of GH in stimulation tests [2, 3, 18, 32], as well as by the pattern of growth response to GH [17]. Moreover, the specific body composition of children with PWS, consisting of a reduced lean mass, as compared to healthy lean and obese controls, in the presence of an increased fat compartment [9, 19], has been attributed to GH deficiency. Apart from the reduction of the metabolically active compartment, GH deficiency enhances the cardiovascular risk, because in GH deficiency, the regulation of lipoprotein turnover is disturbed and hepatic low density lipoprotein (LDL) receptors are decreased [1, 4]. Several, for the most part controlled, studies have reported favourable lipolytic and anabolic effects of GH treatment in PWS, improving lipid metabolism [11] and body composition during 12 months [16, 17, 31, 32]. While it has also been shown in children with GH deficiency [28] or Turner syndrome [44], that GH decreases LDL-cholesterol (LDL-C) and increases high density lipoprotein cholesterol (HDL-C) during GH treatment, this favourable effect might be counteracted by an increase in lipoprotein (a) (Lp(a)), which independently is associated with a high risk for arteriosclerosis [34, 35]. In addition, the long-term effects of GH on lipid metabolism have not yet been studied in PWS patients.

The course of PWS is biphasic, the characteristic features during infancy being muscle hypotonia and underweight, with obesity developing only after the 2nd year of life [46]. It has not yet been reported, if the incidence of lipid metabolism disorders is increased already in young, still underweight patients. The present paper focuses on the specific pattern and interrelation of cardiovascular risk factors in children with PWS, namely fat mass, regional fat distribution as well as serum lipid and lipoprotein levels, and on the longer-term effects of, on average, 3 years of GH therapy on these parameters.

Patients and methods

A total of 23 children with PWS, documented by deletion or uniparental disomy of chromosome 15, were studied. Based on the age-related variations in manifestation, the children were divided into three groups, whereby group 1 consisted of the young, still underweight children, weight for height SDS < 0, group 2 of the prepubertal overweight children (weight for height SDS > 0) and group 3 of the pubertal overweight children (Tanner stages 2 and 3) (Table 1). Patients with a family history of metabolic diseases affecting lipid metabolism were excluded from this study (one patient, data not shown). Missing values are not due to drop-outs, but to shortage of serum (as for Lp(a) levels) or to uncompleted investigation intervals as a result of delayed start of therapy (missing values after 12 months).

As described earlier [17], all anthropometric measurements were performed 6-monthly according to Prader et al. [38]. Blood samples were taken between 8 and 9 a.m. after a 12 h overnight fast before and at intervals of 6–12 months during therapy, and in 16 children (Table 1), body composition was determined before therapy and after 6, 12, 24 and 36 months, by dual-energy X-ray absorptiometry (DEXA) (Hologic QDR-2000, Waltham, Mass., USA, software version 7.10B). Fat mass was expressed as percentage fat relating to normative data established by Boot et al. [7]. Before therapy, DEXA fat mass (kg) was separately assessed for trunk and legs as described elsewhere [8]. Waist and hip circumference (cm) were measured in all children with PWS according to WHO recommendations [45]. The waist/hip ratio (WHR) was used as a simple method for describing the distribution of subcutaneous and intra-abdominal fat and compared with North American percentiles (NHANES III) from ages 4 to 19 years [25]; 14 children with PWS of all groups, average age 7 years, could be compared with these references.

The children were treated with recombinant GH (Pharmacia, Dübendorf, Switzerland, 24 IU/m^2 per week, ca. 0.037 mg/kg per day) administered in daily subcutaneous injections over 3 years on average (Table 1). As insulin-like growth factor-1 levels were elevated under this regime [17], the children under 2 years of age (seven of group 1) were started on a lower dose of 18 IU/m^2 per week (ca. 0.025 mg/kg per day). No additional medication was administered (refer to Table 1 for exceptions). The study was approved by the Ethics Committee of the Children's University Hospital of Zurich and informed consent was obtained from the parents.

Determination of lipids and lipoproteins

Due to the staggered start of therapy and the long-term follow-up of the patients, the study extended over more than 5 years and some methods were subject to changes inbetween.

Total cholesterol (TC) and triglycerides (TG) were determined enzymatically (Boehringer Mannheim, Mannheim, Germany) on a Hitachi 717 or Hitachi 917 analyser. HDL-C was at first determined measuring cholesterol content of HDL enzymatically as described above after precipitation of very-low-density lipoprotein and LDL-C with phosphotungstic acid. More recently, HDL-C was directly measured by the HDL-C plus assay (Boehringer-Mannheim, Germany) on a Hitachi 917 analyser. The two methods yielded similar results. LDL-C could always be calculated according to the Friedewald equation [24] because TG levels were all below 4.5 mmol/l.

Table 1 Clinical data of the children with PWS

Patient	Sex	Age at the beginning of therapy (years)	Height (SDS)	Weight for height (SDS)	Observation period with GH therapy (months)
Group 1 Young underweight children					
1	M	0.30	−0.83	−0.91	18
2	M	0.50	−1.90	−1.82	30
3	F	1.10	−1.80	−2.23	30
4	F	0.80	−0.88	−2.01	36
6	M	0.60	−1.80	−1.77	36
7[a,b]	M	1.50	−1.52	−2.47	36
8[a]	M	1.80	−1.60	−0.64	36
9[a]	M	3.00	−1.88	−1.24	36
10[a]	M	4.10	−3.90	−2.78	36
$N = 9$	2 F,7 M				
Median (range)		1.10 (0.30–4.1)	−1.80 (−3.9–−0.83)	−1.82 (−2.78–−0.64)	36 (18–36)
Group 2 Prepubertal overweight children					
11	F	4.4	−1.50	3.28	12
12[a]	F	3.70	−1.90	4.14	36
13[a]	M	6.70	−0.60	3.00	18
14[a]	F	5.00	−0.80	3.16	36
15[a]	F	6.80	−2.08	6.38	36
16[a]	M	6.80	−1.40	4.37	36
17[a]	M	7.00	−1.60	4.34	36
18[a]	F	7.10	−2.43	0.84	36
19[a]	M	9.50	−2.10	3.76	36
$N = 9$	5 F,4 M				
Median (range)		6.80 (3.70–9.5)	−1.60 (−2.43–−0.6)	3.76 (0.84–6.38)	36 (12–36)
Group 3 Pubertal overweight children					
20	F	11.10	−0.40	5.31	12
21[a]	F	9.00	0.10	6.29	12
22[a,c]	F	13.30	−4.31	4.31	36
23[a,d]	M	13.50	−0.74	12.04	36
24[a,e]	F	14.60	−4.82	1.57	36
$N = 5$	4 F,1 M				
Median (range)		13.30 (9.0–14.6)	−0.74 (−4.82–0.1)	5.31 (1.57–12.0)	36 (12–36)

[a] Children examined by DEXA
[b] Substitution with L-thyroxine (50 μg/day)
[c] Substitution with ethinyloestradiol since age 14.5 years
[d] Testosterone therapy (100 mg i.m.) since age 14.8 years
[e] Combined oestradiol/progesterone therapy since age 16.6 years

Lp(a) levels were measured either by an immunoradiometric assay (Apo(a) RIA, Pharmacia, Uppsala, Sweden) or with an immunonephelometric assay system (BNA, Behringwerke, Marburg, Germany). The results of both assay systems were similar. Apolipoprotein A-1 (Apo A-1) and apolipoprotein B (Apo B) were determined with an immunonephelometric assay system (BNA, Behringwerke, Marburg, Germany).

Lipid levels were referred to normal ranges as published by Kwiterovich [29].

Statistical methods

All biochemical values before and during GH therapy are given as means and standard deviations, with the exception of Lp(a), as well as WHR, height and weight, which showed a skew distribution and are given as medians and ranges. All data were processed by GAS 3.3 of the Institute for Medical Informatics (IMI, Zurich, Switzerland). The changes induced by GH therapy after 6, 12, 24 and 36 months were tested by the non-parametric Wilcoxon signed ranks-test for paired samples. Bivariate linear regressions or partial correlations controlled for age and sex were given as Spearman correlation coefficients. P values of less than 0.05 were considered significant. Statistical calculations were performed with the Statistical Package for Social Sciences (SPSS 8).

Results

Total body fat

Before therapy, total body fat measured by DEXA and expressed as percentage fat was increased in all overweight patients (groups 2 and 3), and in one patient of group 1 in spite of underweight. The percentage of fat significantly decreased with therapy (combined group, $P < 0.001$ compared to values before therapy), the more, the higher the initial fat mass was (Fig. 1, group 3).

Body fat distribution

Before therapy, anthropometric parameters of fat distribution (waist and hip circumference) showed a good correlation ($r = 0.95$ and $r = 0.97$, respectively, $P < 0.001$) with regional fat mass measured by DEXA (trunk and leg fat). Since normal values for trunk and

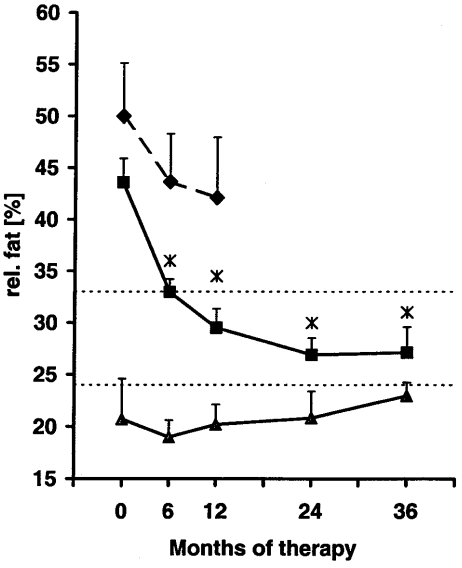

Fig. 1 Relative fat mass measured by DEXA in 16 children with PWS before and during 3 years of GH therapy. Mean and SEM of young underweight (group1, $n = 4$) (*filled triangles*), prepubertal overweight (group 2, $n = 8$) (*filled squares*), and pubertal overweight (group 3, $n = 4$) (*filled diamonds*, *dotted line*). *Significant difference ($P < 0.05$) compared to the basal value. The upper limit of the normal range of children above 4 years (7) is indicated by the *dotted horizontal lines*, lower: boys, upper: girls

leg fat matched for age and genetic background are lacking, we decided to assess body fat distribution by the WHR. The WHR results of 14 children older than 4 years could be compared to North American NHANES III data for healthy non-Hispanic white boys and girls. At the start, in this combined PWS group, WHR amounted to 0.92 (median, range 0.79–1.22), and significantly decreased to 0.88 (range 0.73–0.94) up to 36 months of GH therapy ($P < 0.01$), which, on average, compares to the age-dependant decrease in WHR observed in the healthy population. However, while before therapy, 5 out of 14 PWS patients above 4 years showed an elevated WHR (35.7% > 90th percentile) when compared to normative data, after 36 months of GH, WHR was normal in all of them.

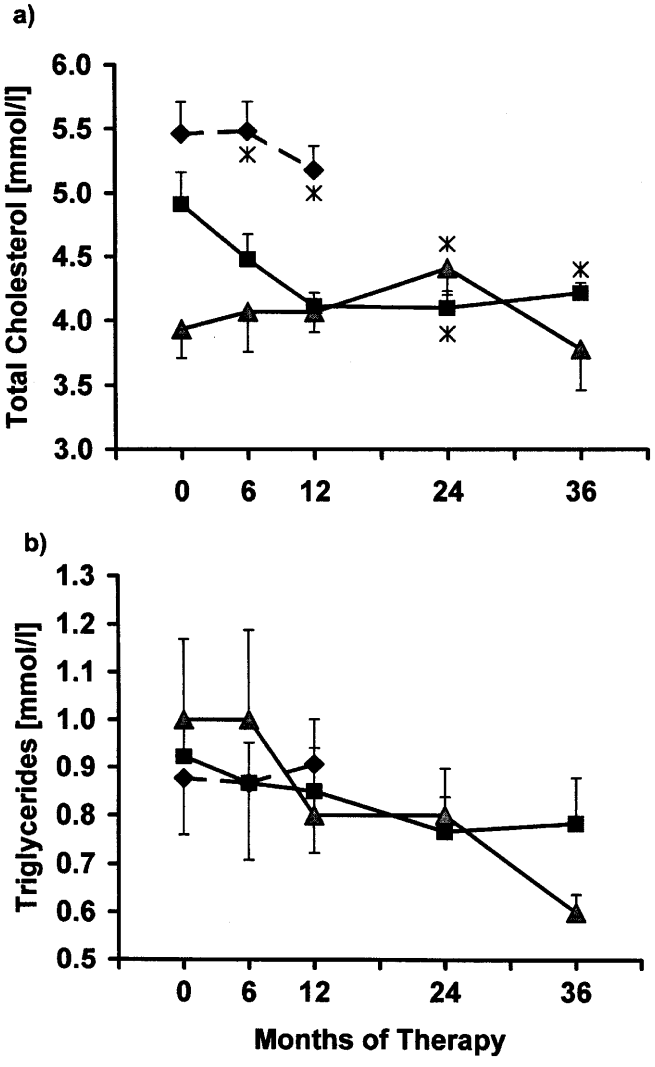

Fig. 2 TC (**a**) and TG (**b**) measured in 23 children with PWS before and during 3 years of GH therapy, mean and SEM of young underweight (group1, $n = 9$) (*filled triangles*), prepubertal overweight (group 2, $n = 9$) (*filled squares*), and pubertal overweight (group 3, $n = 5$) (*filled diamonds*, *dotted line*). *Significant difference ($P < 0.05$) compared to the basal value in group 2

Table 2 Prevalence of abnormal lipid levels (according to [29]) in 23 children with PWS before and at last visit (see Table 1) up to 36 months of GH therapy

Abnormal level	TC (mmol/l) > 5.2	LDL-C (mmol/l) > 3.4	HDL-C (mmol/l) < 1.0	LDL-/HDL-C (molar ratio) > 3	TG (mmol/l) > 1.55	Lp(a) (mg/l) > 300	Apo A-1 (g/l) < 1.1	Apo-B (g/l) > 1.1
Group	Before GH therapy							
1 Young underweight	0	0	4	3	2	1	1	1
2 Overweight prepubertal	4	4	2	3	1	2	0	3
3 Overweight pubertal	4	2	0	0	0	2	0	3
Prevalence (of all, $n = 23$)	35%	26%	26%	26%	13%	24%	4%	30%
Group	Under GH therapy							
1 Young underweight	1	1	0	0	1	1	0	1
2 Overweight prepubertal	0	0	0	0	0	2	0	0
3 Overweight pubertal	2	0	0	0	0	2	0	0
Prevalence (of all, $n = 23$)	13%	4%	–	–	4%	24%	–	4%

Total cholesterol (Fig. 2)

TC levels were lowest in group 1 (before therapy: 3.93 ± 0.67 mmol/l) and highest in group 3 (5.46 ± 0.55 mmol/l). In the combined group, TC was elevated in 8 out of 23 children before therapy: namely in four of nine prepubertal overweight (group 2) and in four pubertal overweight children (group 3), but in none of the nine young, underweight children (group 1). During GH treatment, the prevalence of elevated TC levels dropped (Table 2), and TC significantly decreased in group 2 at 12 and 24 months of therapy (from 4.91 ± 0.76 mmol/l before to 4.10 ± 0.35 mmol/l after 24 months, $P = 0.03$), but there were no significant changes of TC in the combined group.

Low density lipoprotein cholesterol (Fig. 3)

Basal LDL-C was lowest in group 1 (2.48 ± 0.67 mmol/l), and clearly higher in groups 2 and 3 (3.21 ± 0.76 and 3.48 ± 0.41 mmol/l, respectively). Levels elevated for age were found in six overweight children (Table 2). GH treatment induced a significant and steady decrease in LDL-C in both the prepubertal and pubertal overweight groups (e.g. group 2: 2.23 ± 0.3 mmol/l, $P = 0.03$), from the first 6 months of therapy onward, but there was no change in group 1. After 36 months, the formerly elevated LDL-C levels normalised whereas LDL-C rose to elevated levels in one patient of group 1.

High density lipoprotein cholesterol (Fig. 3)

As for TC and LDL-C, HDL-C was also highest in group 3 (1.6 ± 0.2 mmol/l) and lowest in group 1 (0.98 ± 0.22 mmol/l) before therapy; decreased levels were found in four children of group 1 and two of group 2. During GH therapy, HDL-C increased in each group, reaching significance in groups 1 and 2 after the 2nd year (1.27 ± 0.15 mmol/l, $P = 0.03$, and 1.43 ± 0.34 mmol/l, $P = 0.04$, respectively), and finally resulting in normal levels in all patients (Table 2).

Low density lipoprotein cholesterol/
high density lipoprotein cholesterol ratio (Fig. 3)

The initial LDL-/HDL-C was similarly high in all groups. Increased levels were found in three under- and three overweight children before therapy (Table 2). During the whole period of GH therapy, a similar decrease in the ratio was found in each of the three groups, being highly significant in group 2 and the whole age-mixed group, and resulting in normalisation of all increased LDL-/HDL-C ratios (Table 2).

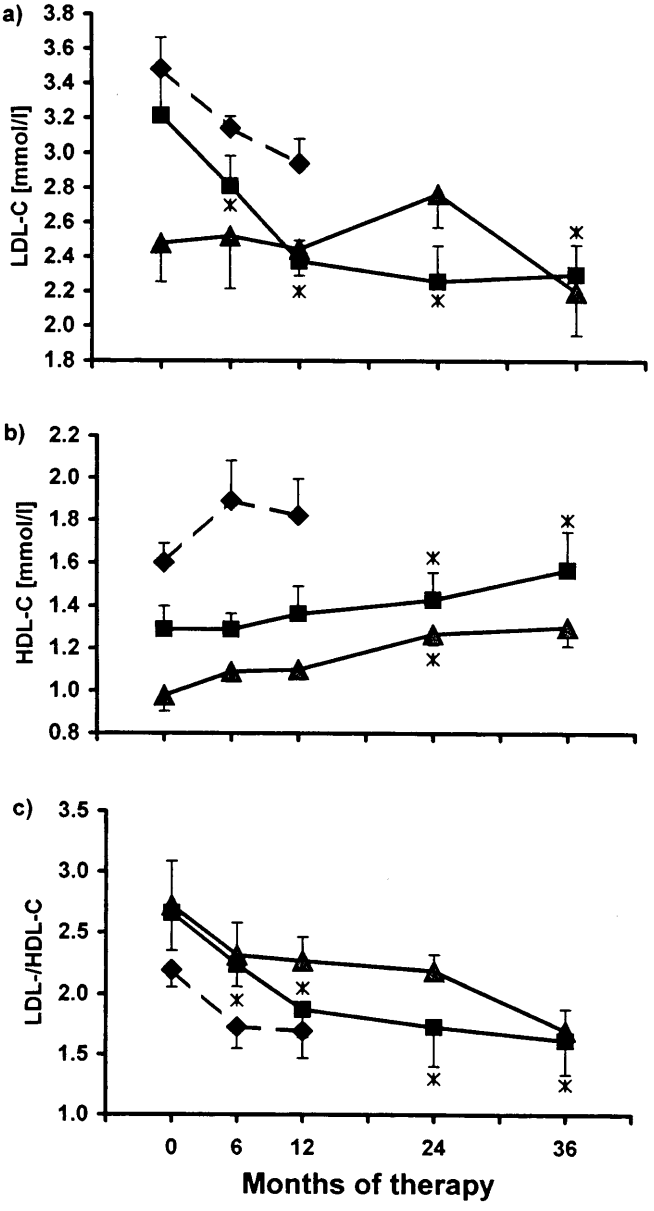

Fig. 3 LDL-C (**a**), HDL-C (**b**) and LDL-C/HDL-C molar ratio (**c**) measured in 23 children with PWS before and during 3 years of GH therapy Mean and SEM. See Fig. 2 for symbols

Apolipoprotein A-1

Apo A-1, besides Apo A-2 the main lipoprotein of HDL-C, did not change while on therapy levels in all groups remaining within the normal range (groups 1, 2 and 3, before therapy: 1.25 ± 0.23, 1.46 ± 0.15 and 1.77 ± 0.15 mmol/l, respectively) (Table 2).

Apolipoprotein B

Apo-B, the major protein of LDL, again showed a trend to lower levels in group 1 than in groups 2 and 3

(0.9 ± 0.23, 1.02 ± 0.22 and 1.10 ± 0.15 mmol/l, respectively) and decreased after 36 months to a highly significant degree during GH treatment in the combined group (0.8 ± 0.14 mmol/l, $P = 0.003$), the decrease being even more marked in the overweight prepubertal children (0.72 ± 0.09 mmol/l, $P = 0.03$). During GH therapy, the prevalence of increased values dropped as did that of LDL-C (Table 2).

Triglycerides (Fig. 2)

There was no significant difference in basal TG levels between the groups (combined group: 0.97 ± 0.37 mmol/l); high levels before therapy were observed in three infants (group1) and in one child of group 2 only (Table 2), in spite of obesity in groups 2 and 3. No signifcant changes in TG were observed during therapy.

Lipoprotein (a) (Fig. 4)

Levels of Lp(a) were widely scattered in all groups. High levels (> 300 mg/l) were found in one child of group 1 and in two children of groups 2 and 3 each (Table 2). A similar, biphasic development was seen in all groups receiving GH therapy: Lp(a) levels significantly increased during the first 6 months of therapy and then reverted to levels similar to those before therapy (Fig. 4). Finally the prevalence of increased Lp(a) was not changed by administration of GH (Table 2).

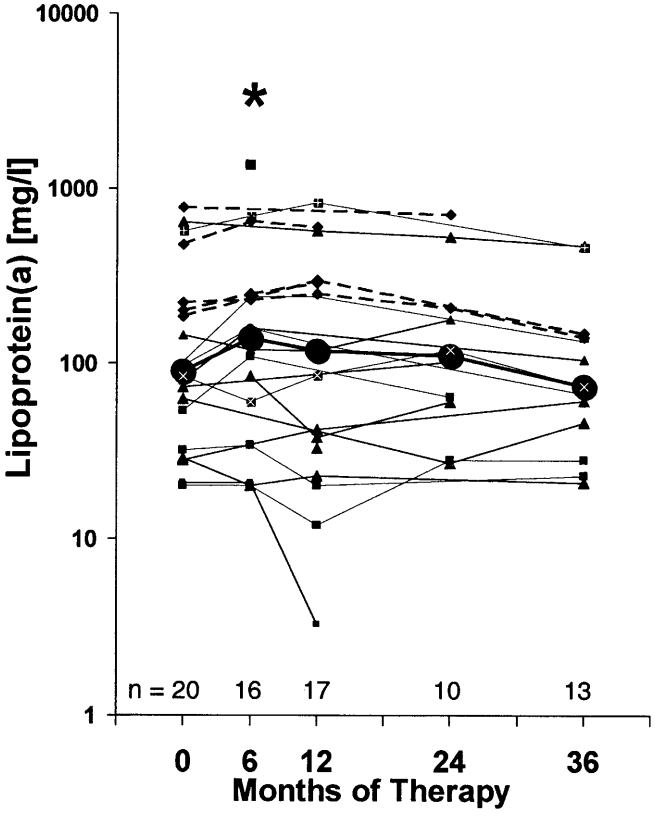

Fig. 4 Lp(a) measured in 21 children with PWS before and during 3 years of GH therapy. Median of the combined groups and individual courses, note the logarithmic scale. See Fig. 2 for symbols

Correlations of lipid parameters with body fat mass

While the pattern of decrease of body fat mass during GH therapy seemed to be related to that of lipid levels, we neither found significant bivariate correlations nor partial correlations, checking for age and sex, between parameters of fat mass (fat Z-scores, percentage fat) or fat distribution (trunk fat, trunk/leg fat ratio, waist circumference, WHR) with any lipid parameter (e.g. LDL-C, HDL-C, LDL-/HDL-C-ratio) before or during GH therapy. There was only a significant correlation of TG to trunk/leg fat ratio in the combined group ($r = 0.59$, $P = 0.03$) before therapy; the partial correlation remained significant after correction for age and sex ($r = 0.55$, $P = 0.03$).

Discussion

We have demonstrated that several cardiovascular risk factors are already present in prepubertal children with PWS; most importantly, the percentage of body fat is increased in all children above 4 years, and WHR in 33% of the same age group; LDL-cholesterol and Apo-B are elevated in 25% of these patients. In the children below 4 years, a high frequency of decreased HDL levels was found. During 3 years of GH therapy, relative fat mass decreased to the upper normal range, WHR normalised in all patients, and the ratio of LDL-C to HDL-C normalised in all patients of the three age groups. The normalisation of this ratio results from the decrease in LDL-C and Apo-B and from the increase in HDL-C, and is an index of the reduction of atherogenic risk during GH therapy. There is a discordance between the constancy of Apo A-1 levels during GH therapy and the increase in HDL-C. This could be caused by a change of the composition of HDL particles by GH.

The basal findings of an increased relative fat mass in PWS [15, 16, 19, 31, 32] and a high prevalence of a predominantly abdominal fat distribution [9] have been described before. We were now able to show that 3 years of GH therapy led to a significant decrease in both parameters, thus mimicking the pattern encountered in patients with GH deficiency on substitution [28]. This is also true for serum lipid profiles. While the means of all lipid parameters were within the normal range, the prevalence of abnormalities in lipid profiles (TC, Apo-B, HDL-C, LDL-C, and their ratio) was increased before GH therapy (ca. 25%), even higher than observed in children with GH deficiency (18%) [28], and decreased during therapy to levels encountered in the healthy age-matched population (2.5%–5%) [29].

In children and adolescents with non-syndromal obesity, fat mass [26], abdominal fat distribution [13, 23, 47] and, most importantly, visceral fat [8, 10, 36] are involved in the determination of serum lipid levels. However, in children with PWS, we could not find any consistent correlation between total body fat or trunk fat as well as WHR or trunk/leg fat ratio with TC, Apo-B, HDL-C, LDL-C, and their ratio, irrespective of the correlation method or therapy interval considered. We suggest that the changes in cholesterol profiles during GH therapy are less dependant on fat mass and distribution, but rather on the effects on lipid metabolism of GH itself [4], as this has been observed after onset of GH treatment in adult patients with GH deficiency [43]. There was only a significant correlation of TG to trunk/leg fat ratio, as in healthy obese children [13, 47]. In contrast to the usual findings in obesity [23, 27], TG are not elevated in children with PWS and the reduction in fat mass is not accompanied by a decrease in TG levels. This might reflect the absence of hyperinsulinism in the majority of children with PWS, before [18] and even during GH therapy [33]. Therefore, the pattern of obesity-related risk factors that we could track to childhood seems to be primarily linked to a hypothalamic GH deficiency and less to a ''metabolic syndrome'' combined with hyperinsulinism.

Beyond the direct effect of GH on the expression of hepatic LDL-receptors, which regulate the concentration of circulating LDL-C, there also is an indirect effect of GH: by stabilising lean body mass and enhancing of physical activity [11, 17, 21], it might contribute to the observed increase in HDL-C during treatment in the prepubertal children with PWS, since it is known that physical activity raises the levels of HDL2-C [6].

There is concern that GH increases the concentration of atherogenic Lp(a) particles [34, 35]. In contrast to these shorter term observations, in the present study, levels only rose transiently during GH therapy, and, in the long term, did not significantly increase with GH. Thus our data in PWS are in line with reports on long-term treatment in children with and without GH deficiency [28]. The high prevalence of elevated Lp(a) levels in our PWS patients, however, is remarkable. This again might be explained by the reduction of physical activity in PWS [16], higher Lp(a) levels being found in inactive children, as extrapolated from the Young Finns Study [41].

While it was beyond the scope of this study to analyse in detail the nutritional intake, the success of GH therapy with respect to metabolism [22] might crucially depend on restrictive control of food intake in these patients, taking into account the reduced energy requirements in PWS [14]. Nutritional habits were monitored by interview and dietary protocols for being balanced and not to change after onset of GH therapy beyond the growth-related requirements. Obviously, energy intake was about 20% to 40% below the age-dependant recommendations for healthy children [40].

GH treatment improves several atherogenic risk factors in PWS, acting both on body composition and lipid metabolism. We were able to confirm that the beneficial effects of GH therapy on cardiovascular risk factors are maintained over 3 years and that there is no long-term increase in adverse factors such as Lp(a). In addition to the growth response to GH therapy [20], the present data underline that there is a hypothalamic GH deficiency in PWS. Therefore GH therapy might be indicated in prepubertal obese children with PWS, provided diet, scoliosis and carbohydrate metabolism are carefully monitored.

Acknowledgements The authors thank Mrs. Claudia Weinmann for the research coordination and technical assistance, Professor Theo Gasser (Department of Biostatistics, Institute of Social and Preventive Medicine, University of Zurich), for his valuable criticism and Mrs. Karin Stutz for her help in translating and preparing the manuscript. Our special thanks go to the PWS Association of Switzerland and the parents and their children for their great commitment and efforts.

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