CLINICAL STUDY

Serum adiponectin levels in adults with Prader–Willi syndrome are independent of anthropometrical parameters and do not change with GH treatment

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Abstract

Objective: Obesity and growth hormone (GH) deficiency are common in Prader–Willi syndrome (PWS) and these patients are at risk of metabolic diseases in adult life and of reduced life span. Low adiponectin values are associated with obesity and the metabolic syndrome. We therefore found it of interest to measure adiponectin levels in PWS.

Patients and methods: 17 adults, nine men and eight women, 17 to 32 years of age, with a mean body mass index (BMI) of 35 ± 3.2 kg/m² participated. All had clinical PWS. They were randomized to treatment with placebo or GH (Genotropin) for six months, and subsequently all received GH for 12 months. At baseline, serum total adiponectin levels in the PWS patients were compared with 25 lean and 34 obese controls. Body composition and various metabolic parameters, including adiponectin, were studied every six months in the PWS group.

Results: Serum adiponectin levels in PWS subjects were significantly lower ($P < 0.001$) compared with lean and significantly higher ($P < 0.001$) compared with obese controls. In PWS patients, no correlation was found between adiponectin and anthropometrical parameters or measures of insulin sensitivity (e.g. fasting insulin and insulin sensitivity as estimated by the homeostasis model assessment), or between adiponectin and IGF binding protein-1 or IGF-I. Adiponectin did not change during GH intervention.

Conclusion: In this study of adults with PWS serum total adiponectin levels were higher than in controls with simple obesity and were independent of anthropometrical parameters. In accordance with this the metabolic syndrome is not necessarily present in all PWS patients. Correction of GH deficiency had no effect on serum adiponectin levels.

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Introduction

Prader–Willi syndrome (PWS) is a genetic disorder characterized by mild mental retardation, short stature, abnormal body composition, muscular hypotonia and distinctive behavioural features (1). Excessive eating causes progressive obesity and individuals with PWS are at risk of lifelong morbidities including diabetes and cardiovascular disease, and of early death (1). PWS results from loss of one or more normally active paternal genes in the region q11–13 on chromosome 15 (1). It is believed that this leads to dysfunction of several hypothalamic centres. Thus, partial growth hormone (GH) deficiency (GHD) and hypogonadism are frequent clinical features (1). Epidemiological data suggest that adults with hypopituitarism have reduced life expectancy compared with healthy controls, with

an increase in mortality from cardiovascular diseases (2, 3). GHD has been proposed as an important causal factor. In adult PWS body composition and metabolic abnormalities (decreased total lean body mass and subnormal insulin-like growth factor-I (IGF-I) levels) resemble a state of partial GHD (1, 4, 5). Adiponectin is a novel adipocyte-derived protein (reviewed in 6, 7). It is decreased in patients with obesity, type 2 diabetes and cardiovascular diseases (8). Studies in animals and humans have shown that adiponectin increases insulin sensitivity and decreases development of atherosclerosis (9), probably through anti-inflammatory and anti-atherogenic effects (10). Levels of adiponectin in PWS have not been analysed previously. Because of the abnormal body composition and the partial GHD in PWS, we measured serum total adiponectin before and during GH treatment

in a group of young, obese PWS patients. Furthermore, serum adiponectin levels in PWS at baseline were compared with serum adiponectin levels in lean and obese controls.

Patients and methods

PWS patients

The PWS study group consisted of 17 consecutively recruited adults, nine men and eight women, between the ages of 17 and 32 years (mean 25 years). Informed consent to participate was obtained from the patients and their caretakers and the study was approved by the Committee for Medical Ethics at the Karolinska Institute and the Swedish Medical Product Agency. All patients fulfilled the criteria for the clinical diagnosis according to Holm *et al.* (11). Baseline characteristics have been described previously (4, 5). Most patients had short stature, obesity and hypogonadism; the mean body mass index (BMI) was 35 ± 2.3 kg/m². The activity of the GH-IGF-I axis was impaired. Stimulated GH secretion was reduced, mean peak value after arginine ($n = 16$) was 4.9 µg/l, range 0.4–16 µg/l, and mean peak value after insulin hypoglycaemia ($n = 8$) was 7.4 µg/l, range 1.1–21 µg/l. The PWS genotype was confirmed in 11 patients (4, 5). There were no statistically significant differences between the patients with positive and negative genotype with respect to BMI, body fat, serum lipids and glucose tolerance. Three patients had hypertension; one was treated with metoprolol and three with enalapril. One male aged 30 years had chronic oedema. All patients were reported to have behavioural problems, and had been on a strict diet of 1000 kcal/day for several years.

Controls

The lean controls consisted of 25 adults, 12 men and 13 women. The mean age was 40.1 ± 2.1 years, and the mean BMI value was 23.6 ± 2.6 kg/m². The obese controls consisted of 34 adults, 13 men and 21 women. The mean age was 44.3 ± 1.6 years and the mean BMI value was 38.9 ± 0.5 kg/m². Measurements of fat mass percentage and waist-hip ratio (WHR) were not performed in the control groups. The controls were participating in a weight reduction study approved by the Committee for Medical Ethics at Århus University.

Study design

Baseline serum adiponectin in the PWS group was compared with adiponectin levels in lean and obese controls. The PWS patients were then randomized to treatment with either placebo or GH (Genotropin, Pharmacia Corporation) 0.8 IU (0.26 mg) daily for one month, and then 1.6 IU (0.53 mg) daily for 5 months.

Subsequently all received 12 months of active GH treatment. Injections were administered subcutaneously in the evenings, and during the 12-month open label period GH doses were individually titrated to keep serum IGF-I concentrations within the normal age-related range. Body composition and metabolic parameters were evaluated at baseline and every 6 months during the study.

Anthropometry and body composition

Body mass index (BMI, kg/m²) and WHR were calculated. Waist and hip circumferences were measured in the standing position. Waist was measured halfway between the costal edge and iliac crest, and hip was measured as the greatest circumference around the buttocks. Body fat was determined by Dual Energy X-ray Absorptiometry, DXA (Hologic QDR 4500, Hologic, Inc., Waltham, MA, USA).

Assays

All assays were conducted on serum. Serum total adiponectin was determined by a novel in-house time-resolved immunofluorometric assay (TR-IFMA) based on two monoclonal antibodies and recombinant human adiponectin from R&D Systems (Abingdon, Oxon, UK). Adiponectin has a molecular mass of approximately 30 to 36 kDa depending on the degree of glycosylation, but the molecule is known to form a wide range of polymers. The predominant polymers include trimers, hexamers and highly congregated multimers of approximately 300 kDa (12). To investigate whether the two antibodies were able to recognize the different polymers of adiponectin, serum ($n = 6$ different samples) was subjected to SDS-PAGE (7.5%) under non-reducing conditions and immunoblotting according to Laemmli's method (13). These experiments showed that both antibodies were able to detect several adiponectin polymers in serum, including the major three molecular forms (data not shown). Assay standards were made by serial dilution of recombinant full length adiponectin (R&D Systems, catalogue # 1065-AC-50) in assay buffer, and ranged from 2 to 500 µg/l. Different approaches may be applied in order to break down adiponectin polymers prior to immunoassay (12, 14). In the present assay, sodium dodecyl sulphate (SDS) treatment was used: 10 µl serum were added to 50 µl assay buffer containing 2% (w/v) SDS, and incubated for 1 h at room temperature. All standards and unknown samples were analysed in duplicate, with the exception of non-specific binding (NSB), which was analysed in quadruplicate. The detection limit (NSB plus 3 S.D.) was estimated to be less than 1.5 µg/l. Within-assay coefficients of variation of standards and unknown samples averaged less than 5%. Between-assay coefficients of variation were estimated by repetitive analysis of a control sample diluted 1:2500, 1:500 and 1:50 respectively.

After 48 set-ups, between-assay coefficients of variation averaged 12.2% at 4.8 µg/l. The recovery of exogenously added adiponectin to serum was 101±1% (mean±S.E.M. based on 10 samples). Repetitive thawing and freezing of serum for up to seven cycles did not alter the immunoreactive levels of serum total adiponectin (data not shown). Finally, measurement in serum, heparin plasma and EDTA plasma yielded similar adiponectin levels (7.07±1.44, 7.42±1.57 and 6.89±1.40 mg/l respectively; mean±S.E.M. based on six samples).

Serum cholesterol and triglycerides were measured with colorimetric methods (Vitos 900, Johnson and Johnson, Orthoclinical Diagnostics, Rochester, NY, USA). The normal value for total cholesterol was <5 mmol/l and for triglycerides it was <2.0 mmol/l.

Leptin was measured with a Human Leptin RIA kit (LINCO Research, Inc., St Charles, MI, USA). Mean normal leptin values with BMI values between 18 and 25 kg/m² were for men 3.8±1.8 µg/l and for women 7.4±3.7 µg/l.

Insulin was measured by a fluoroimmunoassay (Auto-delfia Insulin Wallac Oy, Turku, Finland). The reference value for fasting serum insulin was <144 pmol/l. The homeostasis model assessment (HOMA) index = insulin × glucose/22.5 using a single fasting sample was calculated as an estimation of insulin resistance (15, 16). We used 2.77 as a threshold for insulin resistance, as suggested in the Bruneck study (17).

IGF-I was determined by radioimmunoassay (RIA) (18), and the normal range in adults was established in healthy subjects (19). IGF binding protein-1 (IGFBP-1) was analysed by the method described by Pova and co-workers (20).

Statistics

Results are presented as means±S.E.M. or median and range. When values were normally distributed, comparisons between groups were carried out with unpaired Student's *t*-test; otherwise the Mann–Whitney rank sum test was used. Linear regression analysis was performed using Spearman's correlation on ranks. Changes over time were analysed by Kruskal–Wallis one-way analysis of variance on ranks. The cut-off point for significance of differences (*P* value) was <0.05.

Results

Baseline total adiponectin levels in PWS adults were significantly lower (*P* < 0.001) compared with the levels in lean controls but significantly higher (*P* < 0.001) as compared with obese controls (Table 1). In the Prader–Willi group we did not find any difference in serum total adiponectin levels between men and women (*P* = 0.880), neither were there differences between the PWS patients with and without the positive genotype (*P* = 0.312). Anthropometric data, circulating lipids,

Table 1 Serum adiponectin levels (median and range) and BMI values (mean±S.E.M.) in Prader–Willi patients, and in obese and lean controls.

	Serum adiponectin (mg/l)	BMI (kg/m ²)
Prader–Willi patients	9.2 (7.4–21.4)	35.0±2.3
Lean controls	12.6 (7.0–27.6)	23.6±2.6
Obese controls	6.9 (4.2–11.9)	38.9±0.5

leptin, insulin, IGF-I, IGFBP-1 as well as results from HOMA calculations in the PWS patients can be seen in Table 2. There were no correlations between serum adiponectin levels and body weight, BMI, WHR, % body fat, triglycerides, total-, low-density lipoprotein- or high-density lipoprotein-cholesterol, leptin, insulin, HOMA index, IGF-I or IGFBP-1 (Table 2). There was a significant negative correlation between log adiponectin and BMI in the control subjects (*r* = −0.67, *P* < 0.001), while no such correlation could be demonstrated in the PWS patients (*r* = 0.30, *P* = 0.239) (Fig. 1). Serum adiponectin levels were similar in the group randomized to GH treatment and the group randomized to placebo treatment (*P* = 0.925). During the six-months placebo-controlled period we found no difference in adiponectin between the two groups. We subsequently analysed the effects of 12-months GH treatment in the entire group of 17 PWS patients and found median adiponectin values increased from 9.2 mg/l (range 7.4–21.4 mg/l) at baseline to 9.8 mg/l (5.7–27.0 mg/l) at 6 months and to 11.2 mg/l (5.8–30.1 mg/l) at 12 months – the difference, however, did not reach statistical significance.

Discussion

This is, to our knowledge, the first study to analyse serum total adiponectin in PWS adults. Adiponectin

Table 2 Characteristics of 17 adults with Prader–Willi syndrome (expressed as median and range), Spearman's correlation coefficient (*r*) and *P* values between serum adiponectin and the different parameters in 17 adults with Prader–Willi syndrome.

Parameter	Median (range)	<i>r</i>	<i>P</i> value*
Body weight (kg)	83.6 (48.2–165)	0.03	0.90
BMI (kg/m ²)	34.4 (20.4–57.8)	0.09	0.75
Waist/hip ratio	0.88 (0.79–1.01)	−0.05	0.87
% Body fat	53 (36.1–59.1)	0.36	0.21
Triglycerides (mmol/l)	1.2 (0.9–2.3)	−0.39	0.13
Total cholesterol (mmol/l)	4.4 (3.3–5.7)	0.04	0.88
Leptin (µg/l)	56.4 (2.1–86.3)	0.17	0.51
Insulin (pmol/l)	93 (36–151)	−0.14	0.60
HOMA index	2.86 (0.93–4.6)	−0.33	0.21
IGF-I (µg/l)	146 (87–284)	−0.12	0.66
IGFBP-1 (µg/l)	10 (2.7–30)	−0.12	0.65

* *P* value < 0.05 is significant.

Homeostasis model assessment (HOMA) index = insulin × glucose/22.5 using single fasting sample.

Figure 1 Correlation between log serum total adiponectin and BMI in 17 adults with Prader-Willi syndrome (solid circles) ($r = 0.30$, $P = 0.2396$) and 25 lean and 34 obese controls (open circles) ($r = -0.67$, $P < 0.0001$).

levels in PWS adults were significantly higher compared with obese controls and significantly lower compared with lean controls. In patients without PWS, negative correlations between adiponectin and body weight, BMI, WHR, % body fat, insulin or HOMA index have been demonstrated (7, 8). These relationships could not be found in our PWS cohort. Neither did we find correlations between adiponectin and IGFBP-1 or IGF-I. Serum total adiponectin did not change during GH treatment. The metabolic syndrome is associated with central adiposity and insulin resistance (21), as well as decreased adiponectin levels (8). Recently, Goldstone *et al.* (22) demonstrated that women with PWS had lower amounts of visceral fat and were more insulin sensitive compared with women with simple obesity. Although some discrepancies still exist, adiponectin mRNA expression has been demonstrated to be decreased in omental fat compared with subcutaneous fat (23), suggesting that the differences observed in the present study merely reflect the fact that PWS patients albeit obese do not have the metabolic syndrome. This is completely compatible with the non-suppressed adiponectin levels found in the PWS patients in our study. The difference in age between PWS subjects and controls might be a confounding factor, as visceral fat is believed to increase with age. This cannot be substantiated further from our study since anthropometric data were unfortunately not available in our controls. As a reflection of the increased amount of subcutaneous fat, the leptin levels were high and, like Gavrilu *et al.* (24), we did not find a correlation between circulating adiponectin and leptin levels. Insulin reduces adiponectin levels *in vitro* (25) and *in vivo* (26). We have previously shown that in this adult PWS cohort insulin levels are low in relation to body weight, BMI, WHR and % body fat (4), and the insulin levels might explain the relatively high adiponectin levels in these patients. Insulin is the major regulator of IGFBP-1 production

in the liver. As the patients were not hyperinsulinaemic, the IGFBP-1 levels were not suppressed. Also the patients had normal triglycerides and cholesterol levels despite the obesity. Altogether, these findings are consistent with the suggestion that PWS patients as a group do not have the metabolic syndrome. Furthermore, this is supported by the findings shown in Fig. 1, demonstrating that the PWS patients do not show the negative correlation between adiponectin and BMI – indicating that the metabolic features of the PWS-associated obesity are qualitatively different from simple obesity. Approximately half of the patients had a HOMA index indicating insulin resistance, but the HOMA index is based on mathematical approximations, which might not apply to this group of patients. As reviewed by Diez and Iglesias (7) a number of studies have shown that women have higher adiponectin levels than men. However, we did not find this gender difference in our PWS patients, probably because of the well-known hypogonadism in this syndrome. The activity of the GH/IGF-I axis was reduced in our patients (4, 5). When all received GH treatment, a mean reduction in body fat of 2.5% ($P < 0.01$) concomitant with a mean increase in lean body mass of 2.2 kg ($P < 0.05$) was seen (27). GH incubation *in vitro* was found to have no effect on adiponectin mRNA levels in 3T3-L1 adipocytes (25) whereas *in vivo* GH treatment of GHD subjects was found to increase adiponectin levels in women but not in men (28). In the present study, GH intervention increased adiponectin by 22%; however, the increment was not large enough to reach statistical significance, probably due to the small number of subjects.

In conclusion, we have shown that despite being obese serum total adiponectin levels were higher in PWS adults than in controls with simple obesity. We interpret this as a result of the PWS patients not having the metabolic syndrome with insulin resistance. Administration of GH to this population did not elicit any significant changes in adiponectin although levels were numerically higher.

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