Adiponectin Levels in Prepubertal Children with Prader-Willi Syndrome before and during Growth Hormone Therapy

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Context: Children with Prader-Willi syndrome (PWS) may have obesity and an abnormal body composition with a high body fat percentage, even if they have a normal body weight. Adiponectin has been inversely related to obesity and insulin resistance.

Objective: The objective of the study was to evaluate in prepubertal PWS children the following: 1) adiponectin levels, body composition, carbohydrate metabolism, and triglyceride levels; 2) associations between adiponectin and body composition, carbohydrate metabolism, and triglycerides; and 3) effects of GH treatment on these outcome measures.

Patients: Twenty prepubertal PWS children participated in the study.

Intervention: The subjects were randomized into a GH treatment group (n = 10, 1 mg/m²/day) and a non-GH-treated control group (n = 10).

Main Outcome Measures: At baseline, after 1 and 2 yr of GH treatment, fasting levels of adiponectin, glucose, insulin, and triglycerides were assessed. Body composition and fat distribution were measured by dual energy x-ray absorptiometry.

Results: PWS children had significantly higher median (interquartile range) adiponectin levels [17.1 mg/liter (13.9–23.2)] than healthy sex- and age-matched controls [11.8 mg/liter (9.7–12.5), P < 0.005]. Body fat percentage was significantly higher than 0.52 sd score [1.8 sd score (1.5–2.1), P < 0.001]. Adiponectin levels were inversely related to triglyceride levels (r = −0.52, P = 0.03). There was a tendency to an inverse relation with body fat percentage and body mass index, but no correlation with fasting insulin or glucose levels, the insulin to glucose ratio, or homeostasis model assessment index. During GH treatment, adiponectin levels increased significantly and did not change in randomized controls.

Conclusion: Adiponectin levels were increased, and inversely associated with triglyceride levels, in prepubertal, not overweight PWS children, although they had a relatively high body fat percentage. During GH treatment, adiponectin levels further increased, whereas no change was found in the controls, which is reassuring with respect to the development of insulin resistance during GH treatment.

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reported to be inversely related to adiponectin (23), we hypothesized that adiponectin levels would be low in PWS children, compared with healthy sex- and age matched children, due to their expected relatively high body fat percentage, although the counteraction of their low insulin levels might result in normal adiponectin levels. GH treatment might have a dual effect on adiponectin levels. GH is known to decrease body fat percentage, which would increase adiponectin levels. On the other hand, GH treatment has also been associated with lower insulin sensitivity (24). We hypothesized that the effects of GH on insulin sensitivity would be superior and therefore that adiponectin levels would decrease.

The aim of the present study was to evaluate the following: 1) serum adiponectin levels, measures of body composition and body fat distribution, carbohydrate metabolism, and triglyceride levels; 2) the associations between these parameters; and 3) the effects of GH treatment on these parameters, compared with randomized controls in prepubertal, generally not overweight PWS children.

**Patients and Methods**

**Patients**

Twenty PWS children were evaluated for the present study. These children were all enrolled in a randomized, controlled trial to investigate the effects of GH treatment on growth, body composition, activity level, and psychosocial development. They fulfilled the following inclusion criteria: 1) genetically confirmed diagnosis of PWS; 2) age between 4 and 9 yr at start of study; 3) prepubertal, defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys (25), both before and during the study. Most children were regularly seen by a dietitian and a physiotherapist. The caloric intake and activity level of all children were standardized at 3 months before study, and recommendations were given. Compliance to diet and exercise was evaluated by the research nurse, in close collaboration with the dietitian and, if indicated, the physiotherapist. All children were naive to GH treatment at the start of study. They were included irrespective of their GH status. After stratification for age and body mass index (BMI) [< 2 sd score (SDS) vs. > 2 SDS], children were randomized into either the GH group or the control group. For 2 yr, children of the GH group (n = 10) were treated with 1 mg Genotropin (somatropin) per square meter sc daily (during the first 4 wk they received 0.5 mg/m² with 1 mg Genotropin (somatropin) per square meter sc daily (during

**Methods**

**Anthropometry.** At baseline and after 1 and 2 yr of GH treatment or follow-up in the control group, standing height (or supine length, when appropriate) and weight were measured and BMI was calculated. Height and BMI were expressed as SDS, adjusting for age and sex, according to Dutch reference data for children (26, 27). We defined overweight as a BMI greater than +2 SDS. BMI, height SDS, and BMI SDS were calculated with Growthanalyser (version 3.0; www.growthanalyser.org).

**Body composition.** Dual energy x-ray absorptiometry (DXA) type Lunar Prodigy; GE Healthcare, Chalfont St. Giles, UK) was performed in all children, and total fat mass, fat percentage, lean body mass (LBM), and fat of the trunk region were measured. The trunk region consisted of chest, abdomen, and pelvis. The trunk fat to total fat ratio was considered as estimate for fat distribution. Fat mass, fat percentage, and LBM were transformed into SDS adjusting for sex and age, using Dutch reference values for children (28).

**Collection of blood.** Blood samples were collected in the morning after a 12-h overnight fast, immediately centrifuged, and stored at −20 C until assayed.

**Assays**

Each assay was performed in the same laboratory. Serum glucose and triglyceride levels were assessed on Abbott Architect clinical chemistry analyzer (Abbott Laboratories, Irving, TX). The intra- and interassay coefficients of variation (CVs) were, respectively, 0.7 and 0.8% for glucose and 0.5 and 0.6% for triglycerides. Serum insulin levels were measured by immunoradiometric assay (Medigenix, Biosource Europe, Fleury, Belgium). Fasting normal range was less than 15 mU/liter (manufacturer’s information). The intraassay CV was 2–4.7% and the interassay CV was 4.2–11.3%. The minimal detectable dose (MDD) was 1 mU/liter, and no cross-reactivity with proinsulin was observed. Assessment of insulin resistance was calculated using the homeostasis model assessment (HOMA) as previously described (29, 30). Serum adiponectin levels were assessed in duplicate by an ELISA (R&D Systems Inc., Minneapolis, MN). The intraassay CV was less than 7%, and the interassay CV was less than 7%. The MDD was 0.246 ng/ml, and no significant cross-reactivity or interference was observed. Adiponectin control values were obtained from 40 healthy prepubertal children with normal height and BMI between −2 SDS and +2 SDS, according to Dutch standards. The reference study was approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, The Netherlands, and written informed consent was obtained from the parents. Serum IGF-I and IGF-binding protein (IGFBP)-3 were measured using a specific RIA in one laboratory (31). The intraassay CV was 4% and the interassay CV was 6%. The MDD for IGF-I was 6.0 ng/ml and for IGFBP-3 0.002 mg/liter, and no significant cross-reactivity or interference was observed. For IGF-I and IGFBP-3, sex- and age-matched references were available obtained from a healthy Dutch population. This reference study was approved by the Medical Ethics Committee of Wilhelmina’s Children’s Hospital Utrecht (The Netherlands), and written informed consent was obtained from the parents. Because of the age and sex dependency, IGF-I and IGFBP-3 levels were transformed into SDS.

**Statistics**

Statistical analysis was performed by the Statistical Package for Social Sciences (version 11.0; SPSS, Chicago, IL). Most of our data were not Gaussian distributed. We therefore expressed data as median [interquartile range (iqr)] and we used nonparametric tests. Differences, compared with baseline within groups, were calculated using Wilcoxon signed rank test. Differences in changes, compared with baseline between the GH and control groups, were calculated with Mann Whitney U tests. Afterward we used Bonferroni’s correction for multiple testing for both inter- and intraindividual testing. Correlations were calculated by partial correlation coefficient, adjusting for age and sex. We compared the adiponectin levels of PWS children with reference data of healthy sex- and age-matched controls (n = 40) with Wilcoxon signed rank test.

**Results**

**Clinical characteristics**

Clinical characteristics of the study group are listed in Table 1. All children had a genetically confirmed diagnosis of PWS. Six children had a paternal deletion, five had a uniparental maternal disomy, two had an imprinting center mutation, and in seven PWS was confirmed by a positive methylation test, but the underlying genetic defect was yet unknown. There was no significant difference between age,
TABLE 1. General clinical characteristics in GH and control groups

<table>
<thead>
<tr>
<th></th>
<th>GH group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 yr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (male/female)</td>
<td>10 (5/5)</td>
<td>5.8</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>(5.1–7.1)</td>
<td>(4.9–7.8)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>−2.2</td>
<td>−2.8</td>
</tr>
<tr>
<td></td>
<td>(−3.1 to −1.8)</td>
<td>(−3.4 to −2.0)</td>
</tr>
<tr>
<td></td>
<td>−1.3 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>−1.7</td>
</tr>
<tr>
<td></td>
<td>(−1.7 to −0.8)</td>
<td>(−3.5 to −2.0)</td>
</tr>
<tr>
<td></td>
<td>−0.6 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>(−0.9 to −0.3)</td>
</tr>
<tr>
<td></td>
<td>(−0.9 to −0.3)</td>
<td>(−3.5 to −2.0)</td>
</tr>
<tr>
<td></td>
<td>(−2.8)</td>
<td>(−3.5 to −1.8)</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>16.9</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>(15.8–17.7)</td>
<td>(16.4–19.3)</td>
</tr>
<tr>
<td></td>
<td>16.1</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>16.3</td>
<td>18.5</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>(0.1–1.2)</td>
<td>(0.6–1.5)</td>
</tr>
<tr>
<td></td>
<td>(−0.2 to 0.8)</td>
<td>(−0.3 to 1.1)</td>
</tr>
</tbody>
</table>

Data are expressed as median (iqr).

<sup>a</sup> P < 0.05, compared with baseline.

<sup>b</sup> P < 0.01, change compared with baseline in group A vs. group B, corrected for multiple testing.

<sup>c</sup> P < 0.05, change compared with baseline in group A vs. group B, corrected for multiple testing.

height, or BMI of both groups at the start of the study. The median (iqr) BMI at start of study was 0.8 SDS (0.1–1.2) in the GH group and 1.1 SDS (0.6–1.5) in the control group. Only two children were considered overweight, at the start of the study, defined as BMI greater than 2 SDS (one patient in the GH group and one in the control group). Median (iqr) height SDS increased significantly during the first and the second year of GH treatment from −2.2 (−3.1 to −1.8) to −1.3 (−1.7 to 0.8) (P < 0.05) to −0.6 (−0.9 to −0.3) (P < 0.05) but remained low in the control group [from −2.8 (−3.4 to 7.8) to −2.8 (−3.5 to −2.0) to −3.0 (−3.5 to −1.8)]. BMI tended to decrease in the GH group during the first year, resulting in a significantly greater decrease in BMI SDS, compared with baseline, in the GH group than the control group after 1 yr of study (P < 0.05).

Adiponectin

Adiponectin levels are presented in Fig. 1 and Table 2. At baseline, adiponectin levels were significantly higher in the total group of PWS children [17.1 mg/liter (13.9–23.2)], compared with healthy age- and sex-matched controls [11.8 mg/liter (9.7–12.5), P < 0.005]. During GH treatment, median (iqr) adiponectin significantly increased from 15.9 μg/ml (13.3–23.9) to 24.7 μg/ml (15.0–25.9) (P < 0.05) to 24.6 μg/ml (15.4–28.2) (P < 0.05) after 1 and 2 yr, respectively. In the control group, adiponectin levels did not change significantly during the study [from 17.1 μg/ml (13.1–23.1) to 13.4 μg/ml (11.6–21.4) to 15.8 μg/ml (12.5–19.2)]. During 2 yr, the increase in adiponectin was significantly greater in the GH group than the control group (P < 0.05).

Body composition

Body composition of the study groups is presented in Table 3. Median LBM increased significantly, during 2 yr of GH treatment, compared with baseline from −2.2 SDS (−2.7 to −2.0) to −1.6 SDS (−1.9 to −1.4) (P < 0.05) to −1.2 SDS (−1.7 to −1.1) (P < 0.05). In the control group, LBM SDS decreased, compared with baseline, from −2.3 SDS (−2.8 to −1.8) to −2.5 (−3.0 to −1.8) to −2.8 SDS (−3.3 to −1.9) (P < 0.05 after 2 yr). Body fat percentage decreased during the first year of GH treatment from 1.7 SDS (1.6–2.0) to 1.4 SDS (0.9–1.7) (P < 0.05) and increased during the second year to 1.7 SDS (0.9–1.9). In contrast, in the control group, body fat percentage increased significantly over time from 1.8 SDS (1.5–2.4) to 2.1 SDS (1.8–2.2) to 2.1 SDS (1.9–2.4) (P < 0.05 after 2 yr). Trunk fat to total fat ratio tended to decrease, compared with baseline, during the first year of GH treatment (P = 0.06). After 24 months of GH treatment, the trunk fat to total fat ratio was not significantly lower than at baseline (P = 0.12).

Fasting insulin, glucose, and triglyceride levels

Median fasting insulin and glucose levels, the insulin glucose ratio, and the HOMA index as a measure of insulin resistance (HOMA-IR) were within normal range for the total

Fig. 1. Adiponectin levels in PWS children and controls. Boxplots represent adiponectin levels during study. The lower boundary is the 25th percentile, and the upper boundary the 75th percentile. The line in the box represents the median. Lines are drawn from the smallest to the largest observed value that is not an outlier. Open circles are outliers. Dark gray bars represent GH-treated PWS children, white bars non-GH-treated PWS children, and gray bars age- and sex-matched healthy controls. 1, P < 0.05, adiponectin levels, compared with baseline levels. Changes in adiponectin levels from baseline are significantly greater in the GH group, compared with the randomized PWS control group after 12 (P < 0.001) and 24 months (P < 0.001). PWS children had significantly higher adiponectin levels than healthy sex- and age-matched controls (P < 0.005). a, Healthy sex- and age-matched controls.
TABLE 3. Body composition in GH and control group

<table>
<thead>
<tr>
<th></th>
<th>GH group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 yr</td>
</tr>
<tr>
<td>LBM SDS</td>
<td>−2.2 (−2.7 to −2.0)</td>
<td>−1.6 (−1.9 to −1.4)</td>
</tr>
<tr>
<td>Fat mass SDS</td>
<td>0.8 (0.6 to 1.0)</td>
<td>0.5 (0.2 to 1.0)</td>
</tr>
<tr>
<td>Percent fat SDS</td>
<td>1.7 (1.6 to 2.0)</td>
<td>1.4 (0.9 to 1.7)</td>
</tr>
<tr>
<td>Trunk fat/total fat</td>
<td>0.44 (0.34 to 0.47)</td>
<td>0.40 (0.33 to 0.42)</td>
</tr>
</tbody>
</table>

Data are expressed as median (iqr).

a P < 0.05, compared with baseline corrected for multiple testing.

b P < 0.05, change compared with baseline in GH group vs. control group corrected for multiple testing.

c P < 0.001, change compared with baseline in GH group vs. control group corrected for multiple testing.

Table 3 shows the body composition data for GH and control groups. The changes in LBM SDS, Fat mass SDS, Percent fat SDS, and Trunk fat/total fat are presented. The data are expressed as median (iqr).
TABLE 4. Partial correlation coefficients (r) and P values between serum adiponectin levels and the various parameters, adjusted for age and sex at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>-0.52</td>
<td>0.03*</td>
</tr>
<tr>
<td>Body fat percent (SDS)</td>
<td>-0.46</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>-0.44</td>
<td>0.08</td>
</tr>
<tr>
<td>Trunk fat/total fat</td>
<td>-0.32</td>
<td>0.21</td>
</tr>
<tr>
<td>LBM (SDS)</td>
<td>-0.20</td>
<td>0.45</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.02</td>
<td>0.95</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.07</td>
<td>0.78</td>
</tr>
<tr>
<td>Insulin glucose ratio</td>
<td>-0.10</td>
<td>0.71</td>
</tr>
<tr>
<td>HOMA index</td>
<td>-0.10</td>
<td>0.71</td>
</tr>
</tbody>
</table>

* P < 0.05 is considered statistically significant.

Discussion

Our study demonstrates high serum adiponectin levels in young, prepubertal, generally not overweight children with PWS, compared with healthy sex- and age-matched controls. Serum adiponectin levels related inversely with triglyceride levels. There was a tendency to an inverse relation between adiponectin and body fat percentage and BMI but no significant association between serum adiponectin levels and fasting insulin and glucose levels, insulin to glucose ratio, and HOMA-IR. During GH treatment, serum adiponectin levels increased significantly, compared with no change in the control group. The increase in adiponectin levels was strongly, inversely correlated with the change in body fat percentage. Fasting insulin and glucose levels, as well as insulin to glucose ratio, increased significantly during the first year of GH treatment but returned to baseline levels during the second year. In the control group, fasting insulin, insulin to glucose ratio, and HOMA-IR significantly increased over 2 yr, compared with baseline.

This is the first report showing that young, prepubertal, not severely overweight PWS children have high serum adiponectin levels, compared with healthy sex- and age-matched controls. Because the reported prevalence of non-insulin-dependent diabetes mellitus in individuals with PWS is high (12), and body fat percentage in PWS is relatively high, we expected relatively low adiponectin levels. In previous studies in obese adults with PWS, serum adiponectin levels were high, compared with obese non-PWS controls (19–22), but compared with lean adult controls, adiponectin levels were low (19, 22) or similar (21). Previous studies showed that, in PWS children and adults, insulin sensitivity is high (6, 10), which might be due to a relatively low amount of visceral fat, compared with obese controls (11). We postulate that, in addition to a relatively low amount of visceral fat, high adiponectin levels might contribute to the increased insulin sensitivity in PWS children.

We found no significant association between adiponectin levels and BMI and body fat percentage, although there was a tendency toward an inverse relationship. In healthy subjects, a negative association has been described (13), but nowadays it is concluded that low adiponectin levels are more closely related to the degree of insulin resistance and hyperinsulinemia than to the degree of adipositas (16). Also Hoybye et al. (22) reported the absence of a relationship between adiponectin and BMI in adult PWS patients. Our study did not show an association between adiponectin levels and trunk to total fat mass as measured by DXA. This was in contrast to our expectations, because previous studies showed that adiponectin concentrations correlated stronger with central adiposity than with overall adiposity (32–34). The trunk to total fat mass ratio is, however, not a validated procedure for measuring visceral adipose tissue, which might explain the absence of the relation. It might also be that, particularly in PWS subjects, trunk to total fat is not a good measure for visceral fat. It has been shown in PWS women (11) that they had low visceral fat, as measured by magnetic resonance imaging, whereas measures of waist diameter, waist to hip ratio, waist to height ratio, or trunk/limb skinfold were similar to obese controls. This is most likely due to the fact that in PWS subjects, particularly the sc trunk fat, instead of the visceral fat, is high. These data strongly ask for more accurate measurements by magnetic resonance imaging of visceral fat vs. sc fat in PWS.

We found a significant inverse association between serum adiponectin and triglyceride levels in young PWS children. This is in line with findings in healthy obese and nonobese adolescents (35). It might well be that adiponectin modulates insulin sensitivity by diminishing the accumulation of triglycerides in the skeletal muscle tissue, as was found in animal studies (36).

We did not find a significant correlation between baseline adiponectin levels and insulin to glucose ratio or HOMA index, which is in contrast to previous findings in obese adults with PWS (20). This discrepancy might be explained by the fact that insulin resistance and overweight were not yet present in our young PWS children. In healthy children, adiponectin correlated with body weight, BMI, waist, fasting glucose, and 30-min insulin, but the correlations were positive in boys and negative in girls, indicating that plasma adiponectin is not a simple marker of central fat and insulin sensitivity in children (37). In our study, we did not find such sex differences (data not shown).

During the first year of GH treatment, adiponectin levels increased and remained at the same level during the second year. Also, body fat percentage, lean body mass, and IGF-I levels changed particularly during the first year. Based on these findings, the increase of adiponectin levels during GH treatment might reflect the decline in body fat percentage during GH treatment. The increase in LBM during GH treatment might also be related to the increased adiponectin levels. Because skeletal muscle is the primary tissue of insulin-stimulated glucose uptake, disposal, and storage (38), increased lean body mass might contribute to increased insulin sensitivity.

Insulin levels were normal at the start of the study. During the first year of GH treatment, insulin and glucose levels did increase, but levels returned to baseline after 2 yr. The high adiponectin levels in PWS children may contribute to the relatively low insulin levels because adiponectin is suggested to be protective against insulin resistance and diabetes mellitus (14, 15). The high IGF-I levels after 2 yr of treatment indicate that children were compliant to GH treatment.

In conclusion, adiponectin levels are increased in young, prepubertal, not severely overweight PWS children. This is in line with the relatively high insulin sensitivity in PWS.
Adiponectin levels are inversely related to serum triglyceride levels. There is a nonsignificant tendency to an inverse relation with body fat percentage and BMI and no relation with carbohydrate metabolism. GH treatment resulted in a significant increase in serum adiponectin levels, compared with the control group, which is particularly important because adiponectin is considered protective for cardiovascular disease and insulin resistance. Our results are therefore reassuring with respect to the development of insulin resistance during GH treatment in PWS children.

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Disclosure Statement: The multicenter study in children with PWS is an investigator-responsible study. Only the investigators decided on study design, data collection, data analysis and data interpretation, writing of the report, or the decision to submit the paper for publication. D.A.M.F., A.v.T., H.J.D., and A.C.S.H.-K. have nothing to declare.

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