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Altered distribution of adiponectin isoforms in children with Prader–Willi syndrome (PWS): association with insulin sensitivity and circulating satiety peptide hormones

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Summary

Objective—Prader–Willi syndrome (PWS) is a genetic syndrome characterized by relative hypoinsulinaemia and normal or increased insulin sensitivity despite profound obesity. We hypothesized that this increased insulin sensitivity is mediated by increased levels of total and high molecular weight adiponectin and associated with changes in levels of satiety hormones.

Design, patients and measurements—We measured total adiponectin and its isoforms [high molecular weight (HMW), middle molecular weight (MMW) and low molecular weight (LMW) adiponectin] and satiety hormones in 14 children with PWS [median age 11.35 years, body mass index (BMI) Z-score 2.15] and 14 BMI-matched controls (median age 11.97 years, BMI Z-score 2.34).

Results—Despite comparable BMI Z-scores and leptin levels, the PWS children exhibited lower fasting insulin and HOMA-IR (homeostasis model assessment of insulin resistance) scores compared to obese controls. For any given BMI Z-score, the PWS children showed higher concentrations of fasting total and HMW adiponectin and higher HMW/total adiponectin ratios. The HMW/total adiponectin ratio was preserved in children with PWS at high degrees of obesity. In PWS children, fasting plasma total adiponectin, HMW adiponectin and HMW/total adiponectin ratio correlated negatively with age ($P < 0.05$), HOMA-IR ($P < 0.01$), BMI Z-score ($P < 0.05$), insulin ($P < 0.01$) and leptin ($P < 0.05$). In addition to higher fasting ghrelin concentrations, the PWS children showed significantly higher fasting levels of total peptide YY (PYY) and gastric inhibitory polypeptide (GIP) compared to obese controls.

Conclusions—Relative to controls of similar age and BMI Z-score, the PWS children had significantly higher levels of total and HMW adiponectin, and increased ratios of HMW/total

adiponectin. These findings may explain in part the heightened insulin sensitivity of PWS children relative to BMI-matched controls.

Introduction

Prader–Willi syndrome (PWS) is a genetic syndrome of human obesity involving disruption of the normal neuroendocrine control of energy balance. It is characterized in infancy by failure to thrive, decreased arousal, poor suck, and hypotonia. By age 1–5 years, the child with PWS develops insatiable hunger, progressive obesity and short stature. Motor and cognitive development are also delayed, in association with behavioural difficulties and sleep disturbances.

Despite their profound obesity, a state of relative hypoinsulinaemia occurs in PWS individuals. For example, fasting insulin concentrations are lower in PWS compared to body mass index (BMI)-matched children,^{1–3} and insulin sensitivity is increased.³ Compared to BMI-matched controls, children with PWS have a decreased insulin response to mixed meals and to oral and intravenous glucose loading. Additionally, the first- and second-phase insulin secretion responses are significantly lower in PWS adults compared to obese controls during an intravenous glucose tolerance test (IVGTT). Finally, normal or increased sensitivity to exogenous insulin has been observed in PWS individuals.^{4–6} These findings suggest that subjects with PWS may be protected from obesity-associated insulin resistance.^{3,7–9}

Type 2 diabetes and other states of insulin resistance are associated with low levels of adiponectin, a protein that is exclusively expressed and secreted by adipose tissue.^{10–12} Adiponectin is the most abundant adipose-derived protein and circulates in plasma in a variety of multimeric forms. It combines through its collagen domain to create three major oligomeric structures: a low molecular weight (LMW) trimer, a middle molecular weight (MMW) hexamer and a high molecular weight (HMW) 12- to 18-mer.^{13–15}

The higher-order multimeric complexes are thought to mediate the biological activities of adiponectin.^{14,15} Several lines of evidence suggest that the HMW adiponectin form has a dominant role in modulation of insulin sensitivity and protection against diabetes. First, certain rare human adiponectin mutations of the collagen domain (G84R and G90S) result in very low circulating HMW adiponectin levels and are closely associated with type 2 diabetes.¹⁶ Second, the improvement in insulin sensitivity associated with thiazolidinedione treatment correlates more strongly with increases in the ratio of HMW adiponectin levels to total adiponectin levels than to increases in total adiponectin levels alone.¹⁷ Third, in obese adults, the ratio of plasma HMW adiponectin to total adiponectin correlates more significantly with glucose and insulin levels than does total adiponectin levels.^{18,19} Thus, the distribution of circulating adiponectin isoforms plays a central role in adiponectin action and insulin sensitivity.

A few reports have implicated high total adiponectin levels in the altered insulin sensitivity found in adults and children with PWS.^{20–23} However, to our knowledge no study has compared the different adiponectin isoforms (HMW, MMW and LMW) in PWS children and BMI-matched controls. We hypothesized that the increased insulin sensitivity found in children with PWS is mediated by increased levels of total and HMW adiponectin. To test this hypothesis, we compared the isoform species of adiponectin in children with PWS with those of children of similar age and BMI Z-score and examined the relationships among fasting plasma adiponectin, glucose, insulin and homeostasis model assessment of insulin resistance (HOMA-IR) score in both groups. Because adiponectin acts centrally in the brain to regulate energy expenditure, food intake and body weight,²⁴ and because changes in insulin sensitivity are associated with changes in food intake and satiety, we hypothesized that differences in adiponectin expression in PWS and BMI-matched controls might be associated with changes

in satiety factors such as leptin, ghrelin, total peptide YY (PYY), neuropeptide Y (NPY), pancreatic polypeptide (PP) and gastric inhibitory polypeptide (GIP).

Subjects and methods

Subjects

Fourteen children with PWS [median age 11.35 years, body mass index (BMI) Z-score 2.15] and 14 BMI-matched control children without PWS (median age 11.97 years, BMI Z-score 2.34) were studied. BMI Z-scores were calculated using EpiInfo Version 3.3.2 (Centers for Disease Control and Prevention, CDC, Atlanta, GA). Subjects with chronic secondary illness, such as diabetes mellitus, liver or kidney disease, or active malignancy or those taking investigational drugs were excluded. Nine PWS subjects were taking a stable dose of GH at the time of study. Referring physicians made the initial decisions whether or not to treat with GH; the average GH dose of each subject was 0.02 mg/kg/day.

All PWS subjects had free T4 and TSH levels in the normal range (either endogenous or on replacement). One PWS subject was on thyroid replacement to treat central hypothyroidism. Four subjects with PWS had uniparental disomy; all others had a deletion in chromosome 15.²⁵ Control subjects were recruited from the insulin resistance and obesity clinics at Duke University Medical Center. Table 1 shows the subjects' clinical characteristics. This study was approved by the Institutional Review Board of Duke University Medical Center. A parent or legal guardian of each child gave consent and children ≥ 12 years gave assent before entry into the study.

Experimental design

All subjects were admitted to the General Clinical Research Center at Duke University Medical Center. After an observed 10-h overnight fast, each subject had an initial fasting blood sample for determination of circulating levels of serum ghrelin and plasma glucose, insulin, total adiponectin, HMW, MMW and LMW adiponectin isoforms and IGF-I, leptin, PYY, NPY, GIP and PP. Each subject had a fasting blood sample taken on two separate days; one sample was obtained fasting prior to a high carbohydrate meal tolerance test and a second sample was obtained fasting prior to a high fat meal tolerance test as part of a larger yet unpublished study. The average of these two fasting baseline measurements is the value that is reported in this analysis. The median length of the washout period between these two test meals was 35 days. There were no significant differences between these two baseline measures and comparable results were obtained when only a single fasting measure was used.

Blood sample analysis

Following an overnight fast, blood samples were collected between 0800 and 1000 h into red-top Vacutainer tubes (for serum) or purple Vacutainer tubes (for plasma). Aprotinin (500 KIU/ml of blood, Roche, Indianapolis, IN) was added to each vacutainer. Serum samples were allowed to clot on ice for 30 min and each sample was centrifuged and the serum or plasma was removed and stored at -70°C until assay. All analytes were measured in duplicate.

Hormonal assays: adiponectin analysis

Fasting plasma total adiponectin concentrations and the HMW isoform of adiponectin were measured by enzyme immunoassay (EIA) (Multimeric adiponectin EIA, Alpco Diagnostics, Salem, NH).²⁶ This assay measures total adiponectin and the combined MMW + LMW isoforms (calculated by the difference between total and HMW adiponectin fractions). The intra-assay coefficient of variation (CV) was 5.4% for total adiponectin and 5.0% for HMW

adiponectin while the interassay CV was 5.0% for total adiponectin and 5.7% for HMW adiponectin. The sensitivity was 0.075 ng/ml for both total and HMW adiponectin.

Additional hormone assays

Fasting serum samples were assayed for immunoreactive total ghrelin concentration using a commercial radioimmunoassay (RIA; Linco, St Charles, MO). The intra- and interassay CVs were < 10% and < 18%, respectively. The lower and upper limits of detection for this assay were 100 and 6000 pg/ml, respectively. The assay measures both active (octanoylated) and inactive ghrelin, and shows no significant cross-reactivity.

Plasma leptin concentrations were determined using a commercially available RIA (Linco) with a detection limit of 0.5 ng/ml (upper limit 100 ng/ml), and intra-assay and interassay CVs of each < 10%.

Plasma insulin concentrations were measured by RIA (Linco). The intra- and interassay CVs were each < 10%. The normal range of fasting plasma insulin is 2–100 μ U/ml. HOMA was calculated using the formula: fasting glucose (mg/dl) \times fasting insulin (μ IU/ml) divided by 405.²⁷ Plasma IGF-I was measured by immunoradiometric assay (IRMA; Diagnostic Services Laboratory); the sensitivity as 2.0 ng/ml and intra- and interassay CVs were 3.9–7.0% and 3.8–7.4%, respectively.

Plasma NPY and PP were measured by RIA (Alpco Diagnostics). For the NPY assay, the intra- and interassay CVs were 2.6–3.9% and 10.5–12.7%, respectively, with a sensitivity of 6 pM. For the PP assay, the intra- and interassay CVs were 1.8–2.6% and 2.0–3.5%, respectively, with a sensitivity of 3 pM. Plasma GIP was measured by enzyme-linked immunosorbent assay (ELISA; Linco); the sensitivity was 8.2 pg/ml and intra- and interassay CVs were 3.0–8.8% and 1.8–6.1%, respectively. Plasma total PYY was measured by RIA (Linco); the sensitivity was 10 pg/ml and intra- and interassay CVs were 2.9–9.4% and 5.5–8.5%, respectively.

Statistical analysis

Because of the non-normal distribution of many variables, all values are summarized using the median and interquartile range. Between-group comparisons were performed using the nonparametric Wilcoxon rank-sum test. Spearman rank correlation coefficients were used to measure monotonic associations between variables. Simple linear regression was used to characterize the relationships between adiponectin isoforms and BMI Z-scores within and between the groups. Statistical analyses were performed using SAS 9.1 for Windows (SAS Institute, Cary, NC) and SigmaStat (SPSS, Inc., Chicago, IL). Two-sided *P*-values at the standard 0.05 level were used to determine statistical significance.

Results

Baseline characteristics of the study participants are illustrated in Table 1. PWS and BMI-matched control subjects were of similar age, gender and BMI Z-score and had comparable fasting plasma glucose and leptin concentrations.

Fasting insulin, HOMA-IR and adiponectin

Despite comparable BMI Z-score and leptin levels, fasting insulin levels were significantly lower in PWS children (*P* = 0.029) compared with BMI-matched controls. Similarly, the HOMA-IR was significantly lower in PWS children (*P* = 0.029) compared to BMI-matched controls. Total plasma adiponectin levels were significantly higher in the PWS subjects compared to the BMI-matched controls (*P* = 0.012), consistent with higher insulin sensitivity in PWS subjects (Table 1).

Adiponectin isoforms

Analysis of the isoforms of adiponectin revealed significantly higher levels of HMW adiponectin in PWS children compared to BMI-matched controls ($P = 0.013$). The HMW/total adiponectin ratio was also significantly increased in PWS children compared to BMI-matched controls ($P = 0.033$); No significant differences were seen in the LMW + MMW molecular isoforms of adiponectin (Table 1).

Total adiponectin, HMW adiponectin and the ratio of HMW/total adiponectin decreased with increasing BMI Z-score in children with PWS as well as in controls (Fig. 1a–c). However, total and HMW adiponectin and the ratio of HMW/total adiponectin were higher in children with PWS than in controls at any given BMI Z-score. Of note, the ratio of HMW/total adiponectin appeared to decline more precipitously with increasing BMI Z-score in obese controls (OC) than in children with PWS, although the differences in the slopes of the two regression lines were not statistically significant [slopes of regression curves -3002.1 (PWS), -3526.3 (OC); P -value for difference in slopes = 0.087]; thus, a relative excess of HMW adiponectin may be preserved even as BMI Z-score increases in children with PWS. In BMI-matched controls, HOMA-IR correlates weakly with, and is of similar magnitude to, total adiponectin ($r^2 = 0.08$), HMW adiponectin ($r^2 = 0.15$) and HMW/total adiponectin ratio ($r^2 = 0.13$). In PWS children, all three measures [total ($r^2 = 0.48$), HMW ($r^2 = 0.42$) and HMW/total adiponectin ratio ($r^2 = 0.63$)] correlated more strongly with HOMA-IR (Fig. 2a–c).

Ghrelin, satiety peptides and IGF-I

Consistent with previous findings, fasting serum ghrelin levels were higher in PWS children compared to BMI-matched controls ($P < 0.001$). To determine whether PWS is associated with dysregulation of other gut satiety peptides, we compared the fasting levels of total PYY and GIP in PWS with those in BMI-matched controls. Fasting total PYY ($P = 0.016$) and GIP ($P = 0.031$) were significantly higher in PWS children than in BMI-matched controls. Thus, we found that PWS is accompanied by high levels of total PYY and GIP, as well as ghrelin. No significant differences were seen in fasting IGF-I SDS, NPY or PP concentrations in PWS children compared with BMI-matched controls. It should be noted, however, that those PWS subjects on GH therapy had higher IGF-I SDS values compared to those PWS subjects not on treatment ($P = 0.003$).

Correlations of adiponectin to auxological and metabolic parameters

We analysed the correlations of total adiponectin, HMW adiponectin, HMW/total adiponectin ratio and LMW + MMW adiponectin to age, HOMA, BMI, BMI Z-score, and fasting ghrelin, glucose, insulin, leptin, PYY, NPY, PP, IGF-I and GIP in both BMI-matched control and PWS children (Table 2). In PWS children, fasting plasma total adiponectin, HMW adiponectin and HMW/total adiponectin ratio correlated negatively with age ($P < 0.05$), HOMA-IR ($P < 0.01$), BMI and BMI Z-score ($P < 0.05$), insulin ($P < 0.01$) and leptin ($P < 0.05$). Notably, the LMW + MMW adiponectin isoforms did not correlate significantly with any parameters. In BMI-matched control children, fewer and less powerful significant correlations were detected. The absence of significant negative correlations between adiponectin and BMI, insulin and HOMA-IR in BMI-matched controls might be explained in part by the relatively small sample size.

There were negative correlations between total adiponectin, HMW adiponectin and LMW + MMW adiponectin isoforms and age ($P < 0.05$), and a negative correlation between HMW adiponectin and BMI ($P < 0.05$). HMW adiponectin correlated positively with plasma ghrelin ($P < 0.05$), but total adiponectin did not. There was a positive correlation between total adiponectin and HMW adiponectin and NPY ($P < 0.05$). Other than a negative correlation with age, no significant correlations were found between the LMW + MMW adiponectin isoforms

and any other parameters. In the PWS group only, HMW/total adiponectin ratio correlated positively with IGF-I.

Discussion

Previous studies,^{20–23,28} with one exception,²⁹ have reported an increase in total adiponectin in adults and children with PWS relative to obese controls. However, other investigators have not characterized the distribution of adiponectin isoforms in PWS. This is important because the isoform distribution plays a crucial role in adiponectin action. Our study reveals novel findings of elevations in the HMW isoform of adiponectin in children with PWS. Additionally, we found an increased ratio of HMW/total adiponectin in the PWS children, but no changes in the LMW + MMW fraction of adiponectin compared to BMI-matched controls. For any given BMI Z-score, the PWS children had higher levels of total and HMW adiponectin and HMW/total adiponectin ratios compared to controls.

The preferential increase in the HMW form of adiponectin in children with PWS is of considerable interest because this isoform is thought to correlate better with insulin sensitivity than total adiponectin. Thus, the relative excess of higher total and HMW adiponectin in children with PWS may explain the increased insulin sensitivity in these children. Plasma adiponectin levels are inversely correlated with degree of adiposity and correlated positively with insulin sensitivity in both healthy subjects and subjects with diabetes. Furthermore, a lower concentration of plasma adiponectin has been found in obese adolescents with high visceral adipose tissue (VAT) compared to those with lower VAT.³⁰ Negative correlations between concentrations of adiponectin and glucose, insulin and triglyceride levels, BMI, percentage body fat and HOMA-IR have been demonstrated in non-PWS subjects.³¹ These relationships between adiponectin and insulin, BMI and HOMA-IR were also found in the PWS children studied here.

In theory, high levels of adiponectin may be a consequence, rather than a cause, of increased insulin sensitivity and hypoinsulinaemia because insulin reduces adiponectin concentrations both *in vivo* and *in vitro*.^{32,33} It is therefore possible that higher adiponectin concentrations in PWS reflect their lower insulin levels relative to BMI-matched controls. Of note, we did find significant negative correlations between adiponectin and insulin, and between adiponectin and HOMA-IR in the PWS children. Given the cross-sectional nature of this study, we cannot determine the causal direction of these relationships.

The cause of the relative hyperadiponectinaemia in PWS is not fully understood at present. Elevated levels of adiponectin in PWS subjects may be the result of the proportionally lower levels of visceral fat in these PWS patients compared with BMI-matched controls,⁸ as lower levels of adiponectin expression have been found in omental or visceral fat vs. subcutaneous fat.^{34–36} It is also possible that post-transcriptional processing of adiponectin may be different between visceral fat and subcutaneous fat, leading to higher levels of HMW adiponectin in PWS. Finally, because adiponectin might cross the blood–brain barrier and bind to adiponectin receptors 1 and 2 localized in the central nervous system,²⁴ it is possible that PWS involves central dysregulation of adiponectin through unknown mechanisms.

A number of lines of evidence suggest that hyperadiponectinaemia of PWS is not explained simply by GH deficiency or therapy. First, plasma IGF-I levels were not significantly different between our PWS and BMI-matched controls and there was no correlation between fasting total or HMW adiponectin and IGF-I in the PWS children. In addition, a previous study detected no change in adiponectin levels in response to GH treatment of adult subjects with PWS.²² Furthermore, a recent report concludes that 12 months of GH treatment of 34 GH-deficient children did not change adiponectin levels.³⁷ These findings suggest that GH treatment of a

subset of PWS subjects does not explain the differences in adiponectin isoforms that we detected in this study. By contrast, one recent report found an increase in total adiponectin levels in children with PWS during initiation of GH treatment.²³ We cannot therefore exclude the possibility that GH deficiency or therapy might have had effects on adiponectin that were not reflected in differences in plasma IGF-I levels. One limitation of our study is the inability to perform a sub-analysis of PWS subjects on GH *vs.* nontreated. A limited sample size and differences in age, BMI Z-score, gender and pubertal status among the GH- and non-GH-treated PWS subjects made it impossible to analyse the effects of GH therapy on adiponectin levels or the distribution of adiponectin isoforms. Differences between GH-treated and nontreated PWS subjects in adiponectin levels need to be studied further in larger, prospective studies.

Because adiponectin acts centrally in the brain to regulate energy expenditure, food intake and body weight, and because differences in insulin sensitivity can lead to changes in food intake and satiety, we also examined the relationship between adiponectin and a number of hormones that regulate satiety and appetite.

Ghrelin is an orexigenic hormone produced primarily in the stomach. Markedly elevated ghrelin concentrations have been reported in previous studies of both children and adults with PWS.^{38–41} Two other papers found no difference in ghrelin levels among PWS *vs.* control subjects; one paper examined ghrelin levels in a small number of PWS ($n = 9$) young infants (17–60 months) compared to healthy controls of equivalent age, sex and BMI ($n = 8$) and found no difference between groups,⁴² while another found no difference in ghrelin levels in children with PWS compared to controls.⁴³ Because the human ghrelin gene on chromosome 3p26–25 is not located within the known deleted gene sequence that causes PWS, the cause of the elevation in ghrelin concentrations in PWS is unclear.

The increased concentrations of ghrelin found in children with PWS are postulated to cause stimulation of food intake through the hypothalamic NPY/agouti-related protein (AGRP) signalling pathways. However, the regulation of other gut satiety peptides such as PYY, GIP, PP and circulating NPY in PWS has been less well characterized. In this study, we found higher fasting levels of total PYY (0.014) and GIP ($P = 0.031$), as well as ghrelin. Plasma NPY and PP in children with PWS were comparable to those in BMI-matched controls. These findings contrast with those of other investigators. Zipf *et al.* reported lower concentrations of both fasting GIP and PP in PWS children compared to lean or obese adults.⁴⁴ In that study, the mean age of the PWS children was significantly less than that of the obese or lean adult controls; thus, the confounding effect of age might have given different results from ours. Butler *et al.* reported that PYY levels were lower in 12 infants and children with PWS compared with values for normal children reported in the literature. However, as control samples were not run concurrently with the measured PWS samples in the same assay, these findings might not be valid.⁴⁵ On the contrary, Goldstone *et al.* reported that fasting PYY in PWS adults was not significantly different from that of obese or hypothalamic obese controls.⁴⁶ Finally, Gimenez-Palop *et al.* reported similar fasting PYY concentrations in subjects with PWS compared to obese controls, but lower levels in PWS compared to lean controls.⁴¹ Given the variability of results for levels of satiety factors in PWS, further studies are warranted. Because PYY suppresses food intake in humans and rodents,⁴⁷ we speculate that the elevated total PYY levels in children with PWS that we found in our study may represent a compensatory response to the high ghrelin levels. Alternatively, PWS children might oversecrete ghrelin, PYY and GIP in response to disordered autonomic or vagal efferent input to the GI tract.

In summary, we find that despite similar degrees of obesity, the PWS children had significantly higher levels of total and HMW adiponectin and increased ratios of HMW to total adiponectin relative to controls of similar age and BMI Z-score. The findings of high total and HMW adiponectin and low insulin levels in children with PWS support a link between altered

processing or secretion of adiponectin forms and the increased insulin sensitivity demonstrated here and in previous reports of children and adults with PWS. Our findings provide a possible mechanism that might explain the relative paucity of metabolic complications in obese PWS children and adults compared to those of equal adiposity.^{7–9,48} The study of PWS, a unique model of obesity, may provide further insight into the mechanisms that control the relative distribution of the different molecular weight forms of circulating adiponectin. This might, in turn, lead to novel treatment strategies for the amelioration of obesity-related insulin resistance, type 2 diabetes and the metabolic syndrome.

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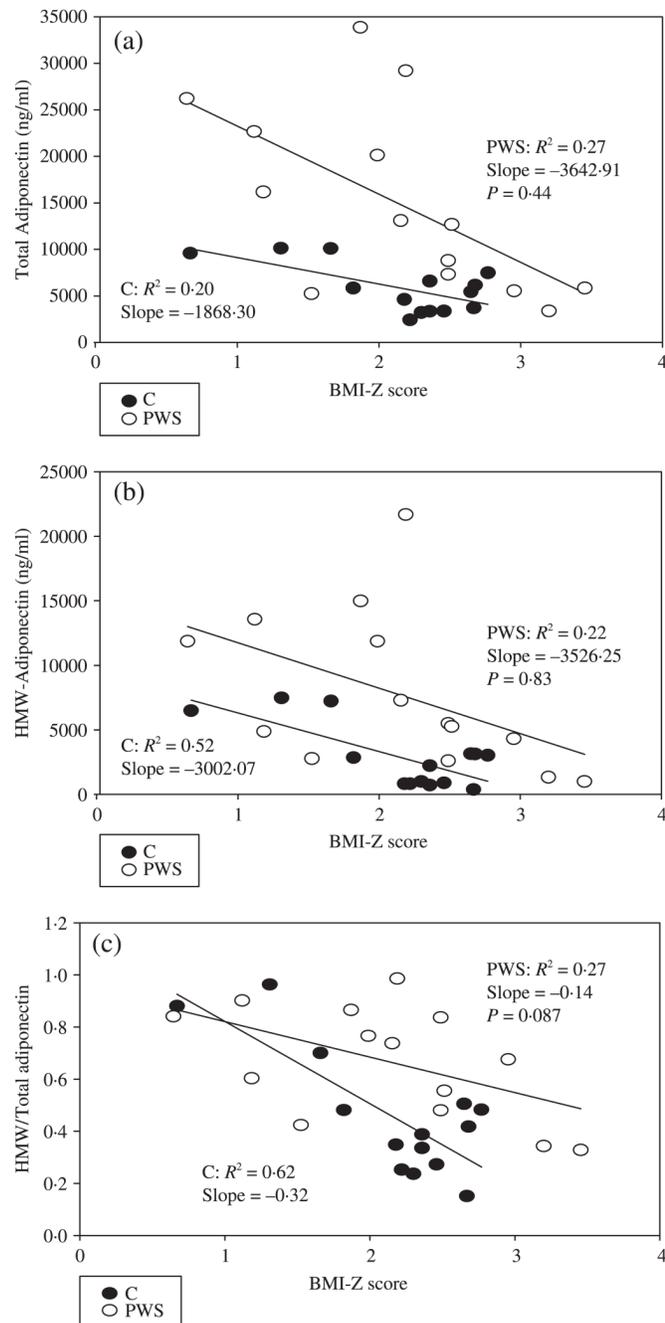


Fig. 1. Relationship between (a) total adiponectin, (b) high molecular weight (HMW) adiponectin and (c) HMW/total adiponectin ratio and BMI Z-score in children with Prader-Willi syndrome (PWS) and BMI-matched controls (C).

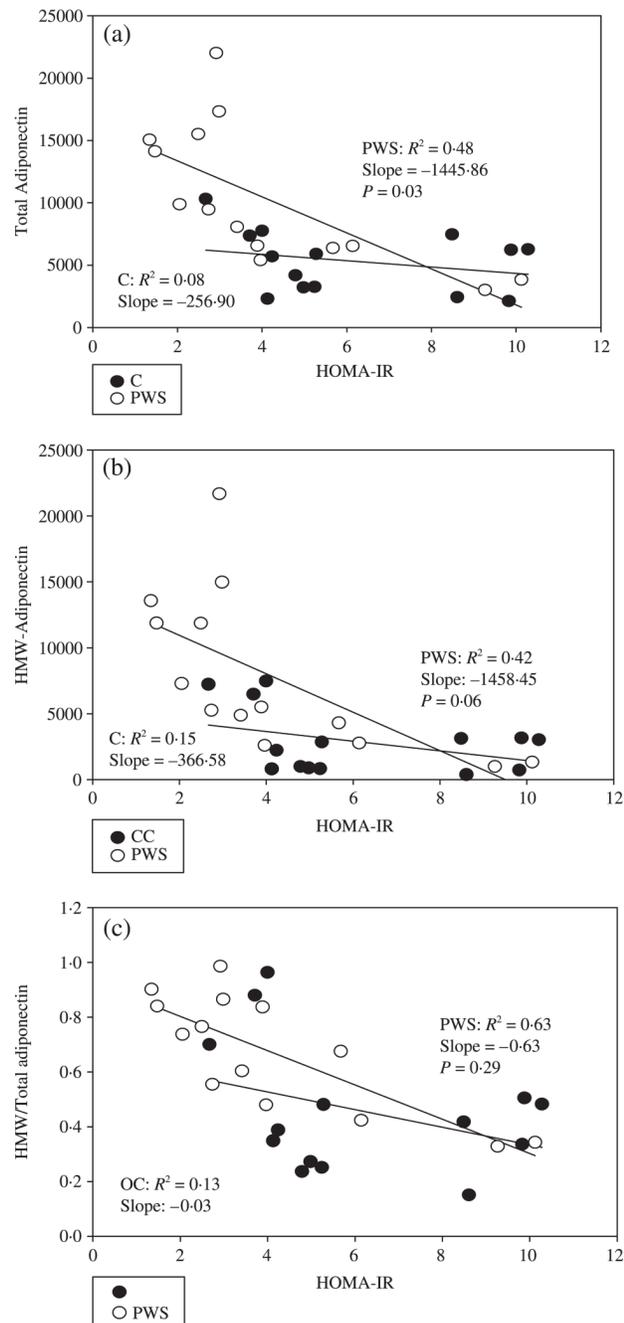


Fig. 2. Relationship between (a) total adiponectin, (b) high molecular weight (HMW) adiponectin and (c) HMW/total adiponectin ratio and HOMA-IR in children with Prader-Willi syndrome (PWS) and BMI-matched controls (C).

Table 1
Baseline characteristics of the study participants

	Prader–Willi syndrome (PWS)	BMI-matched control (C)	P-value*
Age (years)	11.35 (7.13–14.61)	11.97 (10.56–14.17)	0.505
Males/females	5/9	6/8	
BMI Z-score	2.15 (1.465–2.60)	2.335 (1.78–2.65)	0.698
	<i>Average baseline</i>	<i>Average baseline</i>	
Glucose (mmol/l)	4.87 (4.54–5.63)	5.27 (4.94–5.52)	0.448
Leptin (nM)	1.97 (0.81–2.67)	2.01 (0.976–3.41)	0.800
Insulin (pmol/l)	93.69 (70.64–204.02)	161.13 (119.26–241.01)	0.029
HOMA-IR	3.199 (2.494–5.676)	5.109 (4.130–8.611)	0.029
Total adiponectin (ng/ml)	8769.000 (6363.500–15 045.500)	5798.000 (3216.500–7352.500)	0.012
Adiponectin HMW: total ratio	0.706 (0.480–0.840)	0.403 (0.273–0.505)	0.033
Adiponectin HMW (ng/ml)	5370.500 (2763.000–11 867.500)	2523.250 (819.000–3147.000)	0.013
Adiponectin LMW/MMW (ng/ml)	2557.500 (2065.500–3503.000)	2746.500 (1501.500–3191.000)	0.765
Ghrelin (pM)	434.66 (339.13–479.32)	248.55 (213.05–263.34)	< 0.001
IGF-I SDS	2.36 (–0.81 to 3.50)	0.15 (–0.60 to 0.84)	0.11
GIP (pM)	7.86 (5.88–9.65)	5.60 (4.81–6.05)	0.031
PYY (pM)	31.72 (29.32–35.03)	26.23 (24.13–29.50)	0.016
NPY (pM)	49.987 (36.062–62.088)	49.858 (43.007–52.470)	0.909
Pancreatic polypeptide (pM)	4.93 (3.32–6.44)	5.02 (4.49–6.04)	0.872

* Wilcoxon sign rank test for comparison of PWS vs. OC. All data reported as medians (interquartile range).

Average baseline = (BL1 + BL2)/2 for each individual subject and then averaged overall. No significant differences detected between BL1 and BL2 values.

Table 2
Correlations between adiponectin fractions and other variables in PWS children and BMI-matched controls

	Total adiponectin	HMW adiponectin	HMW/total adiponectin	LMW + MMW adiponectin
<i>PWS children</i>				
Age	-0.578*	-0.604*	-0.640*	-0.169
HOMA	-0.798**	-0.802**	-0.736**	-0.226
BMI	-0.656*	-0.663**	-0.641*	-0.236
BMI Z-score	-0.620*	-0.603*	-0.557*	-0.321
Ghrelin	0.495	0.495	0.481	0.095
Glucose	0.235	0.200	0.088	-0.277
Insulin	-0.780**	-0.793**	-0.719**	-0.187
Leptin	-0.565*	-0.591*	-0.609*	-0.081
PYY	-0.112	-0.143	-0.187	-0.024
NPY	0.305	0.385	0.530*	-0.152
PP	0.178	0.222	0.310	-0.051
GIP	-0.484	-0.478	-0.401	0.005
IGF-I	0.345	0.424	0.569*	-0.156
<i>Control children</i>				
Age	-0.587*	-0.552*	-0.244	-0.565*
HOMA	-0.314	-0.363	-0.305	0.240
BMI	-0.471	-0.546*	-0.447	0.145
BMI Z-score	-0.244	-0.352*	-0.411	0.515
Ghrelin	0.490	0.578*	0.407	-0.226
Glucose	-0.224	-0.262	-0.022	-0.251
Insulin	-0.270	-0.310	-0.314	0.218
Leptin	-0.341	-0.393	-0.336	0.248
PYY	0.332	0.248	0.007	0.468
NPY	0.560*	0.569*	0.473	0.213
PP	0.160	0.0286	-0.108	0.222
GIP	0.380	0.240	0.099	0.327
IGF-I	0.521	0.398	0.253	0.319

Spearman rank correlations reported.

* $P < 0.05$;

** $P < 0.01$.