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Increased Serum Resistin in Adults with Prader-Willi Syndrome Is Related to Obesity and Not to Insulin Resistance

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Context: Determinants of insulin resistance in Prader-Willi syndrome (PWS) are not completely understood. The discovery of several adipokines with relevant effects on insulin resistance and cardiovascular complications of metabolic syndrome offered new tools of investigation of insulin resistance in PWS.

Objective: The purpose of this study was to measure serum resistin and mRNA in adipose tissue of patients with PWS, those with simple obesity, and healthy controls and correlate resistin levels with anthropometric and biochemical features.

Design: Twenty-eight adult PWS patients, 29 obese patients, and 25 healthy controls were studied. Anthropometric variables were measured and fasting serum and plasma were collected for measurement of resistin, adiponectin, leptin, lipid profile, glucose, and insulin.

Results: Serum resistin and resistin mRNA expression in adipose tissue was significantly higher in PWS patients, compared with both healthy lean controls and obese patients. Moreover, on regression analysis resistin was significantly correlated with body mass index, whereas no significant association was found between resistin and homeostasis model assessment index. A weak association between resistin and adiponectin was found in the PWS group only. However, on multivariate analysis only the correlation between resistin and body mass index remained significant.

Conclusions: These results support a link between circulating resistin and obesity in humans but do not support a role for resistin in human insulin resistance. **(*J Clin Endocrinol Metab* 90: 4335–4340, 2005)**

PRADER-WILLI SYNDROME (PWS) is a genetic disorder whose clinical picture includes short stature, mild mental retardation, hyperphagia, and development of massive obesity during childhood and adult age and peculiar behavior abnormalities (1). Individuals with PWS have reduced life expectancy, compared with healthy controls, due to comorbidities that include diabetes and cardiovascular disease (2, 3). PWS results from genetic loss of one or more normally active paternal genes in the region 15q11-q13 (4, 5). Genetic damage is responsible for a major defect of hypothalamic centers leading to hyperphagia, hypogonadotropic hypogonadism, and deficient GH secretion. Excessive adiposity is due to both an increase of adipose tissue and to reduced lean body mass, compared with weight-matched obese non-PWS patients (6). Insulin resistance is a common feature of PWS, and type 2 diabetes often develops during childhood and adulthood.

Determinants of insulin resistance in PWS are not completely clear. High free fatty acids levels and hyperinsulinemia have been proposed to produce insulin resistance, but more recently a number of adipokines have been identified that are produced by adipose tissue and are involved in the pathogenesis of insulin resistance. Adiponectin was reported to be reduced in PWS, and this could explain the link between excessive fat deposition and insulin resistance and obesity complications (7–9).

Resistin was cloned in 2001 as a thiazolidinedione-regulated cytokine expressed in adipose tissue (10). Its effects on insulin action has been extensively investigated in mice (11–16), whereas in humans its role in insulin resistance and obesity complications is still controversial (17). In mice resistin has relevant effects on hepatic glucose and lipid metabolism (13, 18) and seems to be a major determinant of hepatic insulin resistance induced by a high-fat diet (19). However, data on humans are conflicting because resistin levels have been reported to be elevated in obesity and diabetes (20–22), whereas other authors reported no changes of resistin levels in these conditions (23–26).

The aim of this study was to determine circulating resistin and adipose tissue resistin expression in patients affected by PWS and simple obesity and correlate resistin levels with insulin sensitivity and other biochemical and anthropometric variables.

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Abbreviations: BMI, Body mass index; HOMA, homeostasis model assessment; PWS, Prader-Willi syndrome.

Subjects and Methods

We studied 28 patients with PWS whose diagnosis was based on the clinical picture and confirmed by genetic analysis according to Holm *et al.* (27). Mean age was 26.5 ± 1.3 yr. Genetic tests revealed that 18 patients were affected by a deletion of 15q11-q13 region, seven patients had a

TABLE 1. Anthropometric and clinical characteristics of PWS patients, obese and lean control subjects

	Lean (n = 25)	Obese (n = 29)	PWS (n = 28)
M/F	12/13	15/14	14/14
Age (yr)	27.1 ± 1.0	27.0 ± 1.4	26.5 ± 1.3
Body weight (kg)	63.4 ± 2.7	110.4 ± 5.4[a]	93.5 ± 4.9[a,b]
BMI (kg/m^2)	22.1 ± 0.6	38.3 ± 1.8[a]	41.2 ± 2.3[a]
Height (cm)	169.1 ± 2.8	170.0 ± 1.9	151.1 ± 1.7[a,b]
Waist (cm)	74 ± 2	106 ± 3[a]	107 ± 4[a]
Hip (cm)	94 ± 2	120 ± 3[a]	121 ± 4[a]
Waist to hip ratio	0.78 ± 0.02	0.89 ± 0.02[a]	0.88 ± 0.02[a]

Data are mean ± SEM. Statistical analysis was performed by ANOVA. M, Male; F, female.

[a] $P < 0.001$ *vs.* lean.

[b] $P < 0.05$ or less *vs.* obese.

uniparental disomy and one patient had a mutation of the imprinting center. Eight PWS patients had type 2 diabetes (four were taking insulin, two oral antidiabetic drugs, and two patients were on diet only). Nineteen patients had GH deficiency (nine were taking GH replacement therapy), four had normal GH secretion, and five patients had no evaluation of GH secretion or a borderline GH response to GHRH + arginine stimulation test. Liver ultrasound scan was performed in 17 patients, and fatty liver was reported in all patients examined.

The protocol was approved by the Ethics Committee of Padua University Hospital, and written informed consent to participate to the study was obtained from patients and their parents. Anthropometric and biochemical characteristics of PWS patients and control groups are illustrated in Tables 1 and 2. A group of lean subjects (n = 25) and a group of obese subjects (n = 29) with comparable age and sex were used as control. In all subjects anthropometric parameters were measured. Waist was measured in standing position halfway between costal edge and iliac crest whereas hip was measured as the greatest circumference around the buttocks. Body mass index (BMI) and waist to hip ratio were calculated. In all subjects a blood sample was collected between 0900 and 1000 h after overnight fasting for biochemical and hormonal determinations. Resistin was measured by ELISA using a commercially available kit (BioVendor, Heidelberg, Germany). Assay sensitivity was 1 ng/ml and interassay and intraassay coefficients of variation were less than 10% and less than 5%, respectively. The assay was linear between 1 and 20 ng/ml. All patients' samples were within this range and no dilution of samples was needed. No cross-reactivity was detected against human resistin-like molecule-β, leptin and leptin receptor, adiponectin, TNFα, IL-6, and agouti-related protein. Insulin and leptin were assayed by RIA (Linco Research, Inc., St. Charles, MO). Glucose was measured by the glucose oxidase method (glucose analyzer II, Beckmann, Inc., Palo Alto, CA). Lipids were measured by enzymatic automated methods.

Antibodies production and immunohistochemistry

Two synthetic peptides corresponding to amino acids 22–37 (CSMEEAINERIQEVAG) and 51–62 (CQSVTSRGDLAT) of the human resistin protein were synthesized by automatic solid-phase method on 4-hydroxymethyl-copolystirene-1% divinylbenzene-resin (Applied Biosystems, Foster City, CA) and fast 9-fluorenilmethoxycarbonyl chemistry using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and 1-hydroxybenzotriazole as coupling reagents (28) with a 431 A peptide synthesizer (Applied Biosystems) as improved in our laboratories (29). Antiresistin antibodies were raised in New Zealand rabbits against the synthetic peptides. Antisera were purified using an immobilized peptide affinity resin (Sulfo Link Coupling Gel, Pierce, Northumberland , UK) according to manufacturer's instructions.

Subcutaneous adipose tissue obtained by percutaneous biopsies from normal, obese, and PWS subjects were fixed in formalin and embedded in paraffin. Six micrometer sections were prepared for immunohistochemistry. Sections were washed three times with PBS, and endogenous peroxidase was blocked by a 10-min incubation with 3% H_2O_2. For resistin immunohistochemistry, sections were incubated for 30 min with purified antiresistin antibodies diluted 1:1000. The sections were then exposed to a secondary biotinylated IgG (ScyTek Laboratory, Logan, UT) and visualized by incubation for 20 sec with a peroxidase substrate solution containing 3,3′-diaminobenzidine tetrahydrochloride. Slides were then washed, counterstained with hematoxylin, shed in water and alcohol, and mounted in synthetic resin. All passages were performed at room temperature. Negative control studies were performed by adsorbing resistin antibodies (1:1000) by preincubation with the synthetic peptides at a final concentration of 0.3 mM for 60 min.

Quantification of resistin mRNA expression by real-time RT-PCR in adipose tissue

Resistin mRNA expression was assessed by quantitative RT-PCR in sc adipose tissue obtained by percutaneous needle biopsies of the gluteal region in lean (n = 8), obese (n = 13), and PWS (n = 8) subjects. Total RNA was extracted with RNEasy lipid tissue mini kit (QIAGEN GmbH, Hilden, Germany) following the supplier instructions. Five hundred nanograms of RNA was treated with DNase treatment and removal reagents (Ambion, Inc., Austin, TX) and reverse transcribed for 1 h at 37 C in a 50-μl reaction containing 1× reverse transcription buffer, 150 ng random hexamers, 0.5 mM deoxynucleotide triphosphates, 20 U RNAsin ribonuclease inhibitor, and 200 U Muloney murine leukemia virus reverse transcription (Promega Corp., Madison, WI). Oligonucleotide primers and probe for human resistin were designed using Omiga TM 2.0 program (Oxford Molecular Ltd., San Diego, CA) (30). PCR was carried out on DNA Engine Opticon TM 2 continuous fluorescence detection system (MJ Research, Waltham, MA), and all reactions were performed on at least two occasions. Standard curve for resistin amplification was constructed using 50 ng of RNA reverse-transcribed from peripheral blood mononuclear cells of healthy subject serially diluted

TABLE 2. Biochemical and hormonal parameters of PWS patients and lean and obese controls

	Lean	Obese	PWS
Fasting glucose (mmol/liter)	4.2 ± 0.3	5.2 ± 0.1[a]	5.6 ± 0.8[a]
Fasting insulin (mIU/liter)	8.0 ± 4	14.2 ± 1.4[a]	10.7 ± 1.1
HOMA	1.46 ± 0.10	3.14 ± 0.24[a]	2.13 ± 0.26[a,b]
AST (U/liter)	25 ± 1	26 ± 4	25 ± 3
ALT (U/liter)	24 ± 6	31 ± 8	25 ± 5
γGT (U/liter)	10 ± 2	24 ± 10	30 ± 12[a]
Fasting lipids			
Total cholesterol (mmol/liter)	4.82 ± 0.52	4.66 ± 0.20	4.91 ± 0.41
HDL cholesterol (mmol/liter)	1.50 ± 0.10	1.20 ± 0.07	1.39 ± 0.07
LDL cholesterol (mmol/liter)	3.16 ± 0.60	3.08 ± 0.22	3.18 ± 0.36
Triglycerides (mmol/liter)	0.74 ± 0.16	1.12 ± 0.13	0.89 ± 0.18
Leptin (ng/ml)	11.9 ± 1.5	59.0 ± 9.2[a]	61.7 ± 9.7[a]
Adiponectin (ng/ml)	17.83 ± 0.73	13.53 ± 0.47[a]	16.70 ± 0.79[b]

AST, Aspartate aminotransferase; ALT, aminotransferase; γGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] $P < 0.01$ or less, compared with lean.

[b] $P < 0.01$ or less, compared with obese.

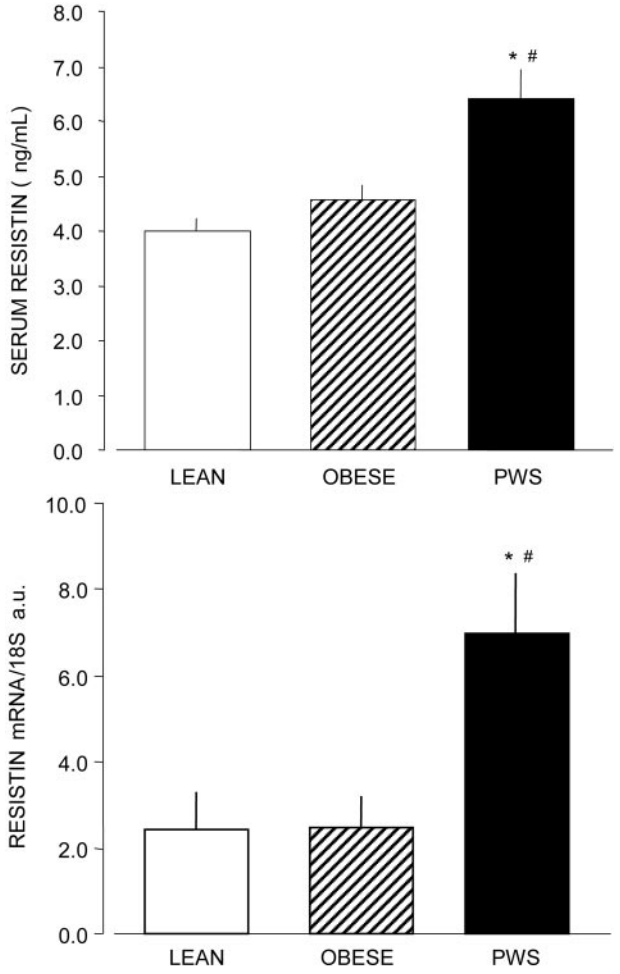

FIG. 1. Serum resistin (*upper panel*) and resistin mRNA quantification in sc adipose tissue (*lower panel*) of lean controls, simple obese patients, and PWS patients. Data are expressed as mean ± SEM. *, P = 0.01 or less *vs.* lean controls; #, $P \leq 0.003$ *vs.* obese group.

(1:3) and for reference with 50 ng of RNA reverse transcribed from adipose tissue of normal subject serially diluted (1:10) by plotting values for log cDNA quantity (in arbitrary units) *vs.* cycle threshold (Ct). For each sample, results were normalized by rRNA18S content. Each sample was assayed in triplicate and a no template control was included in every reaction.

Statistical analysis

Results are expressed as mean ± SE. Different groups were compared by ANOVA. Correlations between different variables were analyzed by linear regression analysis and multivariate analysis using the MDAS 2.0 (medical data analysis system) software package (EsKay Software, Pittsburgh, PA). $P < 0.05$ was considered significant.

Results

Patients with PWS were compared with a lean control group and an obese group with similar age and gender distribution (Table 1).

Serum resistin was significantly increased in PWS compared with both the lean and obese groups, whereas no difference was found between the lean and obese groups (Fig. 1). Circulating adiponectin was significantly reduced in the obese group, compared with lean controls (Table 2). Serum adiponectin in PWS was comparable with the lean group and was higher than the obese patients (Table 2). Adiponectin was significantly lower in the subgroup of diabetic PWS, compared with nondiabetic PWS (13.80 ± 1.25 *vs.* 17.52 ± 0.90 ng/ml, $P < 0.05$). Serum leptin was similar in PWS and obese subjects, whereas both groups had circulating leptin significantly higher than lean subjects. When subgroups of PWS were analyzed, serum resistin was found to be similar in diabetic and nondiabetic PWS patients (7.02 ± 1.25 *vs.* 5.92 ± 0.58 ng/ml, P = NS) and patients with and without GH deficiency (5.67 ± 0.57 *vs.* 7.39 ± 1.13 ng/ml, P = NS). When GH-deficient patients were divided according to GH replacement therapy, serum resistin was slightly lower in the patients taking GH replacement (4.57 ± 0.42 *vs.* 6.55 ± 0.89 ng/ml, $P = 0.08$), but the difference failed to reach statistical significance.

Immunohistochemistry of sc adipose tissue showed that all adipocytes were resistin positive in PWS and lean and obese patients (Fig. 2). Quantitative PCR revealed that resistin expression in sc adipose tissue was significantly increased in PWS, compared with both the lean controls (6.98 ± 1.39 *vs.* 2.44 ± 0.85 AU; $P = 0.01$) and obese patients (6.98 ±

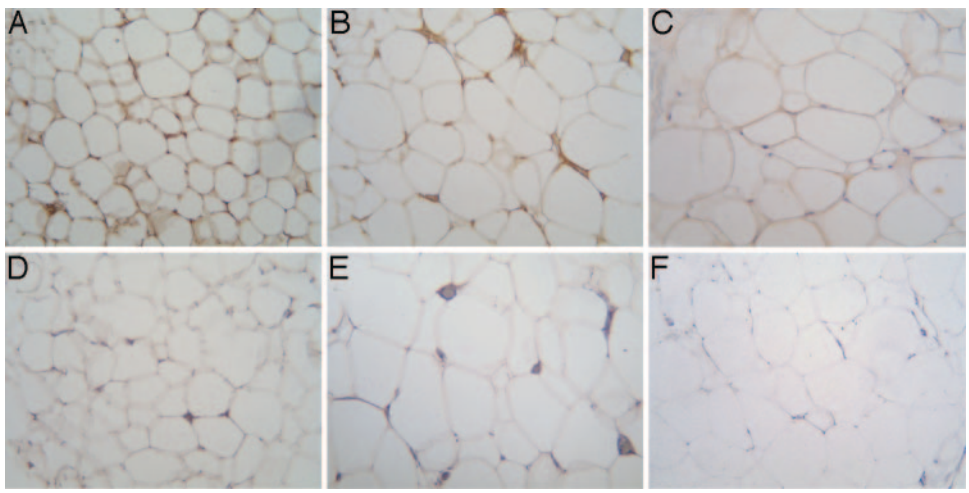

FIG. 2. Localization of immunoreactive-resistin in sections of sc adipose tissue of lean (A), obese (B), and PWS (C) as assessed by immunohistochemistry. Negative controls were carried out by adsorbing antibodies with specific peptides before the assay in lean (D), obese (E), and PWS (F) subjects. Magnification, ×20.

TABLE 3. Simple regression analysis between serum resistin and different parameters in adult PWS patients, in the obese group, and in normal-weight controls

Parameter	PWS		Obese		Lean		Overall	
	r	*P*	r	*P*	r	*P*	r	*P*
Age (yr)	0.12	0.54	0.18	0.35	0.17	0.44	0.11	0.33
BMI (kg/m^2)	0.37	0.04	0.19	0.31	−0.01	0.95	0.43	0.001
Leptin (ng/ml)	0.17	0.39	0.22	0.36	−0.33	0.18	0.26	0.03
Adiponectin (ng/ml)	−0.43	0.02	0.38	0.06	0.32	0.14	0.13	0.24
Insulin (mU/liter)	0.01	0.96	0.005	0.98	0.21	0.45	−0.03	0.79
HOMA-index	0.14	0.53	0.06	0.73	0.34	0.10	0.12	0.31
Triglycerides (mmol/liter)	0.12	0.72	−0.05	0.84	−0.12	0.81	0.001	0.99
Total cholesterol (mmol/liter)	0.17	0.62	−0.33	0.19	−0.64	0.17	−0.16	0.35
HDL cholesterol (mmol/liter)	0.27	0.43	0.004	0.98	−0.09	0.86	0.06	0.73
LDL cholesterol (mmol/liter)	0.12	0.73	−0.30	0.23	−0.08	0.29	−0.19	0.28

r, Coefficient of correlation; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

1.39 *vs.* 2.48 ± 0.74 AU; $P = 0.005$). No differences were found between lean and obese subjects (Fig. 1).

Simple correlation analyses showed a significant positive correlation between resistin and BMI in PWS, whereas no association was found among resistin and insulin, homeostasis model assessment (HOMA), and age (Table 3 and Fig. 3). However, a significant correlation also was found between resistin and adiponectin in PWS only. However, when multiple regression analysis was performed in all subjects, only BMI retained a significant correlation with resistin when age, HOMA, BMI, and adiponectin were included in the statistical model (Table 4).

Finally, a significant negative correlation between adiponectin and BMI was found in both the PWS group ($r^2 = 0.16$, $P = 0.03$) and lean and obese populations ($r^2 = 0.20$; $P = 0.002$) (Fig. 4).

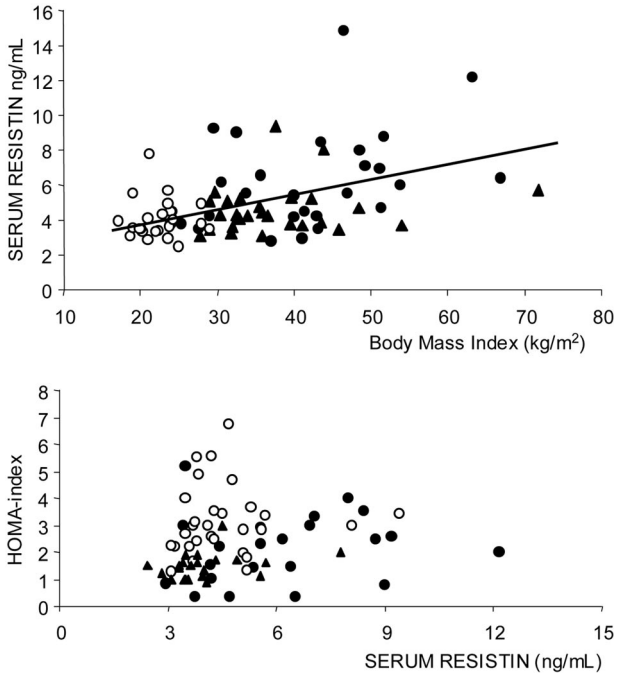

FIG. 3. Linear regression between serum resistin and BMI (*upper panel*) and between serum resistin and HOMA index (*lower panel*). *Solid circles*, PWS; *open circles*, lean subjects; *triangles*, obese subjects. y = 0.0797x + 2.2345; $r^2 = 0.19$; $P = 0.001$.

Discussion

This is, to our knowledge, the first study to analyze serum resistin and adipose tissue expression in adult PWS patients. Resistin levels were found to be significantly higher in PWS patients, compared with lean controls and an obese group with similar degree of adiposity. The increase of resistin levels was not associated to increased insulin or HOMA index and were significantly associated to the degree of obesity.

Factors that determine the evolution to diabetes and cardiovascular disease in these patients are not fully clear. The discovery in recent years of several proteins selectively produced in adipose tissue, the so-called adipokines, have provided new clues to the understanding of the mechanisms of insulin resistance and cardiovascular disease.

In our study higher circulating resistin levels were found in PWS, compared with both the lean and obese groups. Moreover, resistin was positively correlated to BMI and not to insulin resistance as assessed by the HOMA index. The relationship among resistin, BMI, and insulin resistance in humans is still controversial (26). Several studies have reported an association among these factors, whereas other authors failed to find any significant correlation.

Serum resistin was found by some authors to be increased in obesity and positively correlated with BMI or body fat (20, 22, 31–33), whereas this observation was not confirmed by other studies (21, 23, 24, 34, 35). On the other hand, resistin was found to be related to insulin resistance and increased in type 2 diabetes (20, 21, 34, 35), but these data were not confirmed by other studies (23, 24, 31, 32, 36, 37). Our results support an association between resistin and BMI in the PWS patients, whereas no association was found with the HOMA index of insulin resistance. Moreover, no difference was found between diabetic and nondiabetic PWS patients. It is noteworthy that Bajaj *et al.* (36) recently reported that pio-

TABLE 4. Overall multiple regression analysis between resistin (dependent variable) and BMI, age, adiponectin, and HOMA (independent variables)

Independent variable	B	*P*
BMI	0.083	0.001
Age	0.051	NS
Adiponectin	−0.018	NS
HOMA	−0.085	NS

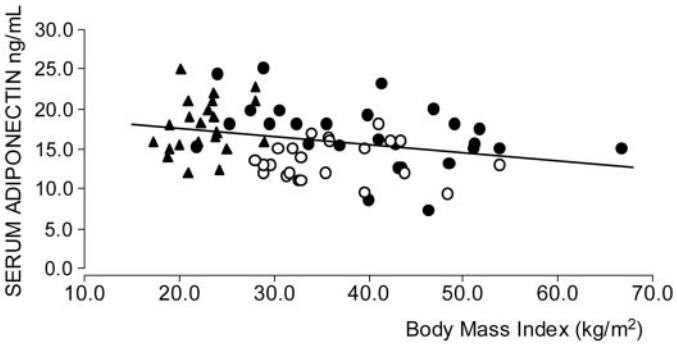

FIG. 4. Linear regression between serum adiponectin and BMI. *Solid circles*, PWS; *open circles*, obese subjects; *triangles*, lean subjects. y = −0.1074x + 19,637; r = 0.30; $P = 0.01$.

glitazone treatment in type 2 diabetic patients causes a reduction of circulating resistin and that plasma resistin was associated with hepatic fat content and hepatic insulin resistance and not to peripheral insulin resistance measured by the euglycemic hyperinsulinemic clamp. Most of the PWS patients in our study have a fatty liver at ultrasound scans, and therefore, one could speculate that higher resistin levels in these patients may be related to increased hepatic fat and hepatic insulin resistance. This is in agreement with experiments in rodents that clearly showed a major role of resistin in hepatic glucose metabolism and the pathogenesis of diet-induced hepatic insulin resistance (13, 19). However, the hypothesis of a link between resistin and hepatic glucose metabolism needs further confirmations in humans. The inconstant link among serum resistin, obesity, and insulin resistance could also be explained by genetic factors. In fact, it has been reported that genetic polymorphisms in the promoter region of resistin gene may be independent predictors of circulating resistin concentrations in humans (38, 39). However, it is unlikely that these polymorphisms may be responsible for resistin levels in PWS.

A possible confounding factor in evaluating adipokines in PWS may be represented by hormonal defects that are present in these patients. However, GH treatment was found not to modify circulating resistin in GH-deficient adults and circulating resistin is normal in acromegalic patients (40, 41). In the PWS group of this study, we found no difference in GH-deficient patients, compared with those with normal GH secretion. Moreover, GH treatment did not modify significantly resistin levels. It is therefore unlikely that GH deficiency could play a role in altered resistin levels in PWS.

Because resistin is produced in not only adipose tissue but also inflammatory and immune cells (42), we evaluated resistin expression in sc adipose tissue by immunohistochemistry and quantitative RT-PCR. Our results confirmed that resistin protein is present in adipocytes of PWS patients as well as lean and obese subjects and that resistin mRNA is higher in sc adipose tissue of PWS, thus confirming that adipose tissue overproduction is responsible for increased serum resistin. However, it cannot be excluded that infiltration of inflammatory cells in adipose tissue could be responsible for the increased resistin levels in PWS (43). In fact, it has been reported that macrophage accumulation in adipose tissue is increased in obesity (44), and then one could put forward the hypothesis that activation of macrophages could directly or indirectly be responsible for the increased resistin expression in adipose tissue.

Adiponectin, another adipokine that was proposed to be protective in the evolution of atherosclerosis, probably because of its antiinflammatory action (45–49), has received attention also in PWS as a possible clue to understanding cardiovascular complication. Adiponectin has also been related to insulin resistance, and in particular, its effect was demonstrated, in rodents, on hepatic glucose production (18, 19). In our study serum adiponectin was significantly higher in PWS patients, compared with the obese group. These is in agreement with a previous report by Hoybye *et al.* (7). However, in contrast to their data, a weak but significant negative correlation was found between adiponectin and BMI. This discrepancy probably could be explained by the greater number of PWS patients enrolled in our study. Our data support that the negative correlation between increasing BMI and circulating adiponectin is present in not only the lean and obese population but also PWS patients. Therefore, it is not possible to rule out the hypothesis that lower serum adiponectin may be involved in cardiovascular complications of obese PWS patients. Moreover, recently Goldstone *et al.* (50) demonstrated that adult women with PWS are more insulin sensitive and have lower amounts of visceral adipose tissue, compared with women with nongenetic obesity. This fact could explain why we found insulin and HOMA values similar in PWS and lean controls that were significantly lower than in simple obese controls. However, when the diabetic PWS subjects were considered, a lower adiponectin concentration was found, thus suggesting that the role of adiponectin in insulin resistance in diabetic PWS patient cannot be definitively ruled out.

In conclusion, we report that circulating resistin is increased in PWS and its concentration is related to the degree of obesity. The lack of correlation between resistin and insulin resistance does not support a role for resistin in the pathogenesis of insulin resistance in obesity associated with PWS.

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