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Elevated Fasting Plasma Ghrelin in Prader-Willi Syndrome Adults Is Not Solely Explained by Their Reduced Visceral Adiposity and Insulin Resistance

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Plasma ghrelin is elevated in Prader-Willi syndrome (PWS). This might contribute to obesity or GH deficiency in such patients. Visceral adiposity and insulin resistance are reduced in PWS, which might lead to hyperghrelinemia. We measured fasting plasma ghrelin in control female (n = 39), PWS female (n = 12), and PWS male (n = 6) adults. In controls and PWS, ghrelin was negatively correlated with visceral adiposity, fasting insulin, and homeostasis model insulin resistance index. There was no significant correlation with serum IGF-I in PWS. In stepwise linear regression, visceral adiposity ($P < 0.02$) had a stronger inverse correlation with ghrelin than sc fat depots in controls and PWS, possibly through hyperinsulinemia, as the correlations with insulin resistance were even stronger ($P < 0.01$). PWS females had significantly ($P < 0.001$) elevated ghrelin (mean ± SD, 661 ± 360 pg/ml), compared with both nonobese (363 ± 163) and obese (191 ± 66) controls. Ghrelin was increased 3.4- to 3.6-fold in PWS females adjusting for total adiposity, 3.2- to 3.4-fold adjusting for visceral adiposity, and 3.0-fold adjusting for insulin resistance. Fasting plasma glucagon-like peptide-1 was normal in PWS females. The hyperghrelinemia in PWS adults is therefore not solely explained by their reduced visceral adiposity and relative hypoinsulinemia. Its cause and consequences await further elucidation. (*J Clin Endocrinol Metab* 89: 1718–1726, 2004)

PRADER-WILLI SYNDROME (PWS) is a genetic obesity syndrome characterized by severe hyperphagia from childhood, mental retardation, and short stature, with GH deficiency and hypogonadism (1). The phenotype is thought to result from developmental abnormalities in the hypothalamus (2) due to chromosome 15q11-q13-imprinted gene defects (3).

Our previous studies, using whole body magnetic resonance imaging (MRI), in PWS women have found an increased fat mass/fat-free mass ratio, normal secretion of the adipocyte-derived anorexigenic hormone leptin, a reduced resting metabolic rate explicable by the abnormal body composition (4), and a selective reduction in visceral adiposity in both PWS men and women that appears protective against the adverse metabolic consequences of obesity, such as insulin resistance (5, 6).

Abbreviations: AGRP, Agouti-related protein; ASCAT, abdominal sc adipose tissue; AT, adipose tissue; BMI, body mass index; FFM, fat-free mass; FM, fat mass; GHS-R, GH secretagogue receptor; GLP-1, glucagon-like peptide-1; HOMA-IR, homeostasis model insulin resistance index; HRT, hormone replacement therapy; INF, infundibular nucleus; MRI, magnetic resonance imaging; NPY, neuropeptide Y; OCP, oral contraceptive pill; POMC, proopiomelanocortin; PVN, paraventricular nucleus; PWS, Prader-Willi syndrome; SCAT, total sc adipose tissue; VAT, visceral adipose tissue.

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Ghrelin is a hormonal ligand at the GH secretagogue receptor (GHS-R), located in the hypothalamus and pituitary (7), which stimulates pituitary GH secretion (8). Circulating ghrelin is secreted from the stomach, particularly when fasting, with its release inhibited by food (9). Ghrelin is also found in hypothalamic neurons and the pituitary (10–12). Ghrelin acutely stimulates food intake and GH secretion in rodents and humans, and chronic administration to rodents causes obesity (8, 13–15).

Recent studies have found grossly elevated plasma ghrelin levels in PWS adults (16, 17) and children (18), which might contribute directly to their hyperphagia and/or, perhaps though reduced GHS-R levels or receptor desensitization, GH deficiency. This is not seen in other causes of obesity, including genetic leptin deficiency, leptin resistance, or melanocortin-4 receptor mutations, where levels are reduced (16, 19), and it is not explicable by GH deficiency or incomplete puberty (20–22). The cause of elevated circulating ghrelin in PWS is therefore unclear.

Several studies have suggested that the negative correlation of fasting ghrelin levels with obesity in non-PWS subjects may be a result of insulin resistance and hyperinsulinemia suppressing ghrelin secretion (19, 21, 23–28). The relationship between body fat distribution and plasma ghrelin has not been examined in detail in this situation (26). We therefore hypothesized that the visceral fat depot may have a primary influence on ghrelin levels, and that the elevated plasma ghrelin in PWS may be related to their

reduced visceral adiposity, increased insulin sensitivity, and lower insulin levels.

In this study we have therefore looked at the relationship between fasting plasma ghrelin and different fat depots, as determined by whole body MRI, and examined differences in plasma ghrelin between control and PWS adults, adjusting for both adipose tissue (AT) content and distribution, and markers of insulin resistance. We also measured another gut hormone secreted by intestinal L cells, glucagon-like peptide-1 (GLP-1), involved in glucose and energy homeostasis (29).

Subjects and Methods

Recruitment

Ethical approval for the study was obtained from the research ethics committee of Hammersmith Hospital. Control subjects were recruited from hospital staff, dietetic clinics, and public advertisement. PWS adults were recruited through Department of Psychiatry, University of Cambridge, and the United Kingdom Prader-Willi Syndrome Association. Consent was obtained from both the PWS subjects and the caregiver or next-of-kin. All subjects were over 18 yr of age and nondiabetic. Control subjects had no known endocrine disease and were premenopausal. All subjects reported stable body weight in the previous 2 months and, other than PWS subjects, had not been involved in weight reduction programs in the last year. All PWS subjects met diagnostic criteria for PWS (1) and suffered from childhood-onset obesity, requiring vigorous behavioral modification, such as locking food and dietary supervision, for extreme hyperphagia and obsession with food. All PWS females had experienced primary amenorrhea or severe oligomenorrhea. All subjects had normal renal and hepatic function. Body composition and some metabolic data for the female patients in this study has been included in previous reports (4, 5).

Body composition

Subjects had height and weight measurements to determine body mass index (BMI). Controls were divided into nonobese (BMI, <30 kg/m^2) and obese (BMI, >30 kg/m^2) groups. Whole body MRI images were acquired on a Picker 1.0T HPQ system (Marconi Medical, Cleveland, OH) using a rapid T1-weighted SE sequence and a slice thickness of 10 mm, as previously described (4, 5). Interactive computer analysis was used to quantify and calculate total and regional body AT volumes, including total sc (SCAT), abdominal sc (ASCAT), and visceral (VAT) adipose tissue; total fat mass (FM), total fat (as a percentage of body mass), and fat-free mass (FFM), as previously described (4, 5, 30).

Blood sampling

Venous sampling was performed after a 12-h overnight fast, and blood was immediately spun, separated, and stored at −20 C for assay. Plasma glucose was measured using an automated analyzer (RA-1000, Technicon Instrument Co. Ltd., Basingstoke, UK). Plasma was assayed for ghrelin (RIA; Phoenix Pharmaceuticals, Belmont, CA), leptin (RIA; Linco Research, Inc., St. Charles, MO), and GLP-1 (RIA) (31), and serum was assayed for insulin (immunoradiometric assay; guinea pig antiinsulin antibody, Diagnostics Scotland, Edinburgh, UK). Serum IGF-I was also measured in PWS subjects (chemiluminescent assay; Nichols Institute, Inc., San Juan Capistrano, CA). The coefficients of variation for these assays were less than 10%. The homeostasis model insulin resistance index (HOMA-IR) was calculated using fasting insulin and glucose concentrations as previously described (32).

Statistical analysis

Pearson product-moment correlation coefficients (r) were used to assess the relationship between variables for control and PWS subjects separately. Plasma ghrelin levels were related to individual AT depots, and measures of insulin resistance, using multiple regression analysis in both control and PWS subjects, were used to assess the independent effects of regional adiposity. Unadjusted and partial adjusted PWS regression coefficients (β) were then calculated to enable examination of the effect of PWS on variables, independent of age, body composition, AT content or distribution, and insulin resistance. Log_{10} transformation was used to correct variables that were not normally distributed. Between-group comparisons were made using *t* test or one-way ANOVA with *post hoc* Tukey's test. Significance was taken as $P < 0.05$. Statistical analysis was performed using SigmaStat 2.0 (Jandel Corp., San Ramon, CA) and Systat 8.0 (SPSS, Inc., Chicago, IL).

Results

Subject characteristics

Thirty-nine control female adults (26 lean and 13 obese) and 12 PWS female adults were studied (Table 1) (4). Three PWS subjects were receiving the oral contraceptive pill [OCP; one subject each: ethinyl estradiol (20 μg)/norethisterone (1 mg), cyclical ethinyl estradiol (30 μg)/levonorgesterol (0.15 mg), and norethisterone (0.35 mg)], and one PWS female was receiving hormone replacement therapy (HRT) as cyclical estradiol valerate (2 mg)/levenorgestrel (75 μg). Six PWS male adults were studied, of whom two were receiving testosterone replacement (one orally and one im). No PWS subject was currently receiving GH therapy, but one female PWS subject taking the OCP, aged 22 yr, had received GH during childhood only. Four PWS females were receiving selective serotonin reuptake inhibitors and/or phenothiazines. Subject characteristics are given in Table 1.

Reduced visceral adiposity and insulin resistance in PWS

PWS females had reduced visceral adiposity (VAT volume and VAT/SCAT ratio) compared with obese non-PWS females (Table 1), as in the previous report that includes these subjects (5). This remained significant when adjusting for age and total AT volume by multiple regression analysis (PWS: $\beta = -1.3$; SE $= 0.3$; $P < 0.001$ and $\beta = -0.027$; SE $= 0.008$; $P = 0.002$, respectively). Fasting insulin and HOMA-IR were also lower in PWS females compared with obese control females (Table 1). This remained significant when correcting for age and total AT volume (PWS as a percentage of control: insulin: mean, 37.9; 95% confidence interval, 20.7–69.3; $P = 0.004$; HOMA-IR: mean, 38.2; 95% confidence interval, 20.8–70.1; $P = 0.005$) using $\log_{10}$-transformed data in multiple regression analysis.

Plasma ghrelin, regional adiposity, and insulin resistance

In control females, fasting plasma ghrelin was lower in obese than in nonobese subjects (Table 1; $P < 0.001$, by *t* test), and fasting ghrelin was negatively correlated to BMI, percent body fat, total body AT volume, and individual AT depots (Table 2 and Fig. 1). In forward stepwise multiple linear regression analysis, including regional AT depots, the strongest negative correlation was found with VAT, but there was no independent additional relationship with either total SCAT or ASCAT (Tables 2 and 3). Age had a small, but significant, positive relationship with plasma ghrelin independent of regional adiposity (Table 3).

In control females, plasma ghrelin was negatively correlated with fasting insulin, HOMA-IR, and leptin, but not with GLP-1 (Table 2). In forward stepwise multiple linear regression analysis, the relationships with markers of insulin re-

TABLE 1. Subject characteristics

	Controls		PWS	
	Nonobese female	Obese female	PWS female	PWS male
n	26	13	12	6
Age (yr)	32 ± 8 (18–45)	37 ± 9 (23–56)	27 ± 6 (20–38)[d]	25 ± 4 (19–29)
Weight (kg)	65.4 ± 8.3 (49.4–77.3)	100.4 ± 15.3 (79.2–130.0)[c]	84.7 ± 27.5 (55.0–144.0)[b,d]	76.3 ± 30.5 (49.9–126.9)
Height (m)	1.66 ± 0.06 (1.53–1.78)	1.65 ± 0.06 (1.54–1.77)	1.50 ± 0.08 (1.38–1.67)[c,e]	1.64 ± 0.07 (1.53–1.71)
BMI (kg/m^2)	23.8 ± 2.6 (19.6–28.3)	36.8 ± 5.9 (30.2–51.9)[c]	37.3 ± 10.0 (23.6–51.6)[c]	28.1 ± 9.6 (19.8–43.4)
Total AT volume (liters)	26.6 ± 6.5 (13.9–37.2)	59.1 ± 14.6 (45.2–90.0)[c]	57.8 ± 25.7 (27.5–109.0)[c]	40.8 ± 24.4 (18.7–79.9)
% body fat	28.9 ± 4.5 (18.5–35.6)	42.0 ± 4.3 (36.8–50.5)[c]	47.4 ± 7.0 (35.3–54.5)[c,d]	36.3 ± 8.3 (24.0–45.3)
VAT volume (liters)	1.5 ± 0.7 (0.5–3.3)	5.2 ± 1.4 (3.0–8.0)[c]	3.4 ± 1.6 (1.2–6.9)[c,e]	3.0 ± 1.8 (1.1–5.5)
VAT/SCAT	0.065 ± 0.022 (0.039–0.118)	0.104 ± 0.016 (0.078–0.121)[c]	0.068 ± 0.017 (0.048–0.099)[e]	0.090 ± 0.034 (0.061–0.152)
Plasma leptin (pg/ml)[f]	13.3 ± 8.6 (3.2–35.8)	42.1 ± 22.5 (19.8–90.4)[c]	51.2 ± 38.6 (7.7–119.2)[c]	18.7 ± 20.9 (3.8–59.6)
Serum insulin (mU/liter)[f]	4.1 ± 2.2 (1.0–7.9)	13.8 ± 9.1 (4.6–37.1)[c]	9.7 ± 8.3 (1.6–26.0)[a,d]	7.5 ± 5.7 (2.5–17.0)
HOMA-IR[f]	0.89 ± 0.50 (0.21–1.84)	3.55 ± 2.44 (1.10–8.59)[c]	2.09 ± 1.92 (0.30–5.55)[d]	1.74 ± 1.43 (0.58–4.23)
Plasma GLP-1 (pg/ml)[f]	98.0 ± 45.2 (42.2–258.7)	121.4 ± 72.6 (41.9–278.5)	125.4 ± 63.7 (67.3–231.0)	64.0 ± 8.9 (49.8–73.9)
Plasma ghrelin (pg/ml)	363 ± 163 (102–708)	191 ± 66 (93–344)	661 ± 360 (160–1369)[b,e]	564 ± 380 (141–1124)

Measurements were made in nonobese (BMI, <30 kg/m^2) and obese (BMI, >30 kg/m^2) control females, and PWS females and males. Figures are given as the mean ± SD (range). For conversion from metric units in the table to SI units (picomoles per liter), multiply the value by 7.5 for insulin, 0.30 for GLP-1, and 0.29 for ghrelin.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ *vs.* nonobese female subjects; [d] $P < 0.05$, [e] $P < 0.001$ *vs.* obese female subjects (ANOVA with *post hoc* Tukey's test for female subjects only).

[f] ANOVA was performed on $\log_{10}$-transformed data.

sistance (fasting insulin and HOMA-IR), but not with sc adiposity, remained significant when including age and VAT in the regression model (Table 3).

Plasma ghrelin in PWS

In PWS females, the negative correlations between plasma ghrelin and BMI, percent body fat, total AT, VAT, and leptin did not reach statistical significance, but the negative correlations with fasting insulin and HOMA-IR did reach significance (Table 2 and Fig. 1). When including PWS males, the negative correlation between plasma ghrelin and VAT, but not with other regional AT depots or measures of total adiposity, was significant (Table 2). In forward stepwise multiple linear regression analysis in all PWS subjects, the negative relationships between ghrelin and fasting insulin or HOMA-IR were stronger than that for VAT, as seen in controls (Table 3).

There was no significant correlation between plasma ghrelin and serum IGF-I in PWS females (r = −0.21; $P = 0.51$; IGF-I: mean ± SD, 183.0 ± 73.8; range, 48.4–296.8 ng/ml; multiply by 0.13 for conversion to nanomoles per liter), PWS males (r = −0.29; $P = 0.58$; IGF-I: mean ± SD, 150.7 ± 61.5; range, 82.3–247.6 ng/ml), or PWS females and males combined (Table 2). No significant relationship between serum IGF-I and plasma ghrelin was seen in PWS subjects when including age, total AT, VAT, fasting insulin, or HOMA-IR in multiple linear regression analysis (data not shown).

Fasting plasma ghrelin levels were higher in PWS females compared with both nonobese and obese control females (Table 1). Using multiple regression analysis, PWS females had significantly higher plasma ghrelin (between 3.0- and 3.6-fold elevation) than controls (all $P < 0.001$) when adjusting for age, percent body fat, total AT volume, visceral AT volume, fasting plasma leptin, insulin, or HOMA-IR (Table 4 and Fig. 1). Similar results were obtained if analysis was restricted to only those PWS females who either were (n = 8) or were not (n = 4) receiving the OCP or HRT, or those not receiving selective serotonin uptake inhibitor or phenothiazine treatment (n = 8; data not shown).

More detailed genetic testing to identify the molecular class was available in 10 of the PWS subjects. There was no significant difference in fasting plasma ghrelin (mean ± SD, 689 ± 490 *vs.* 616 ± 256 pg/ml; $P = 0.9$) between those with a chromosome 15q11-q13 deletion (four females and two males; percent body fat, 41.7 ± 11.5; total AT volume, 44.8 ± 24.8 liters) and those without a deletion (maternal uniparental disomy or imprinting center mutation; two females and one male; percent body fat, 45.9 ± 5.2; total AT volume, 55.5 ± 21.4 liters) or when adjusting for percent body fat, total AT volume, visceral AT volume, fasting insulin, or HOMA-IR ($P = 0.4–0.9$). In addition, one PWS male with an unbalanced chromosomal translocation (46,XYt15:Y), 35.8% body fat, and a total AT volume of 24.8 liters had a high fasting plasma ghrelin level of 1124 pg/ml.

There was no significant difference between plasma GLP-1 in PWS females and either nonobese or obese controls (Table 1) or between PWS and all control females when adjusting for

TABLE 2. Relationship between fasting plasma ghrelin and total or regional adiposity, circulating hormones, and measures of insulin resistance in control female and PWS adults

Independent variable	Control females		PWS females only		PWS females + males	
	r	*P*	r	*P*	r	*P*
n	39		12		18	
BMI	−0.36	0.02	−0.37	0.23	−0.36	0.14
% Body fat	−0.31	0.05	−0.15	0.65	−0.18	0.49
Total AT volume	−0.34	0.03	−0.43	0.16	−0.45	0.06
VAT volume	−0.38	0.02	−0.47	0.13	−0.60	0.008
Visceral fat (% mass)	−0.38	0.02	−0.27	0.39	−0.49	0.04
SCAT volume	−0.34	0.03	−0.42	0.17	−0.42	0.08
ASCAT volume	−0.36	0.03	−0.36	0.24	−0.39	0.11
Leptin[a]	−0.48	0.002	−0.43	0.16	−0.34	0.16
GLP-I[a]	−0.08	0.63	−0.21	0.51	−0.03	0.90
IGF-I	n/a	n/a	−0.21	0.51	−0.19	0.44
Fasting insulin[a]	−0.43	0.007	−0.78	0.003	−0.77	<0.001
HOMA-IR[a]	−0.40	0.01	−0.77	0.003	−0.76	<0.001

Pearson product moment correlation coefficients (r) using linear regression analysis for the relationship between fasting plasma ghrelin and measures of total and regional adiposity, circulating hormones, and insulin resistance. n/a, Not available.

[a] Independent variable was $\log_{10}$-transformed.

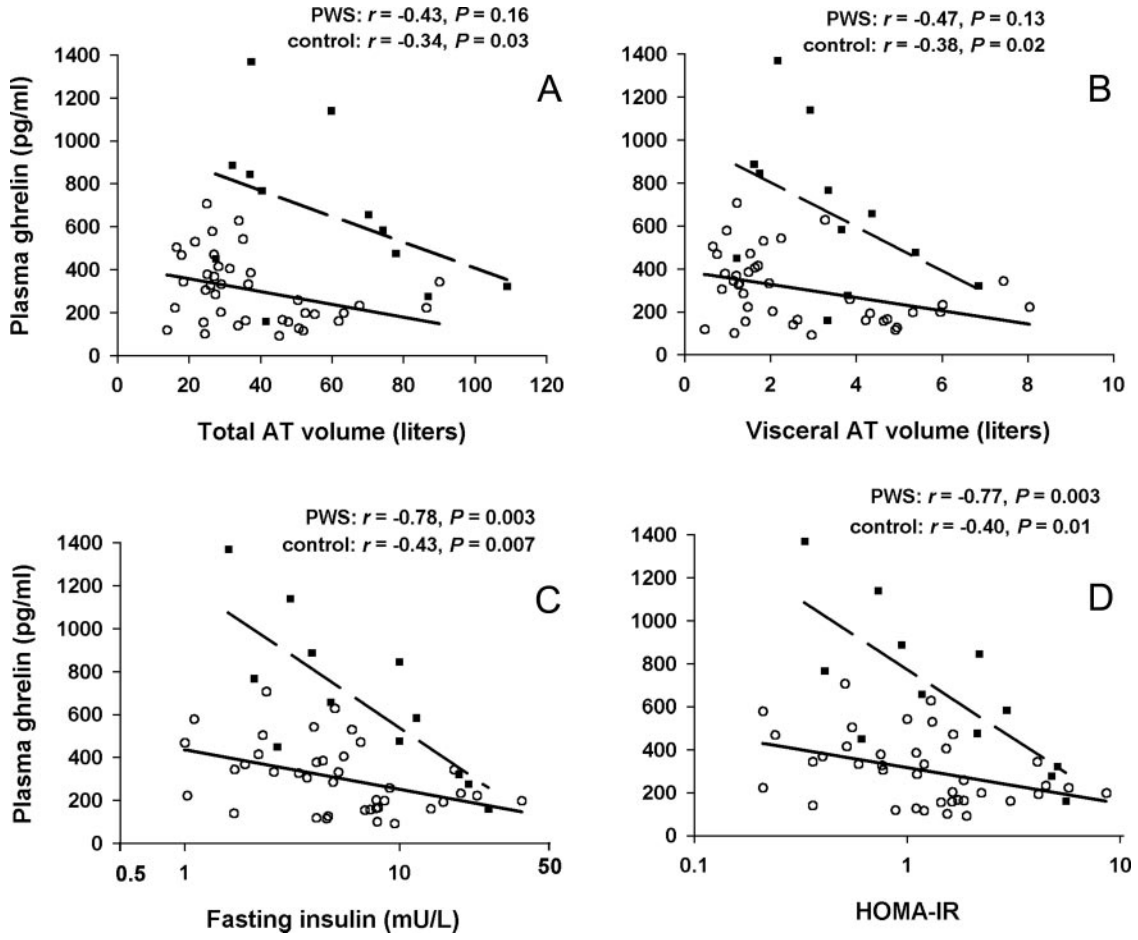

FIG. 1. Plasma ghrelin in control and PWS adult females. Relationship between fasting plasma ghrelin and total (A) or visceral (B) AT volume as determined by whole body MRI, fasting insulin (C), and HOMA-IR (D) in adult control females (○, *solid regression line*) and PWS females (■, *dashed regression line*). *r* indicates the Pearson correlation coefficient. Note that fasting ghrelin levels are higher in PWS females after adjusting for total or visceral adiposity or insulin resistance.

differences in age and total AT ($P = 0.5$), VAT ($P = 0.3$), fasting insulin, or HOMA-IR (both $P = 0.2$) using multiple linear regression analysis.

Discussion

Circulating ghrelin is an important metabolic hormone, stimulating GH secretion and appetite, as evident from both

TABLE 3. Effects of age, regional adiposity, and insulin resistance on fasting plasma ghrelin

Subjects	Model	Independent variable	Δ r^2 (%)	r^2 (%)	β	SE	P^β	P^E
Control females (n = 39)	A	Total AT	11.7	11.7	−2.984	1.346	0.03	0.03
		Age	6.4	18.1	Not included			0.36
	B	VAT	14.7	14.7	−39.54	12.43	0.003	0.02
		Age	9.0	23.7	5.778	2.808	0.047	0.05
		SCAT	0.01	0.25	Not included			0.39
	C	VAT	14.7	14.7	−39.54	12.43	0.003	0.02
		Age	9.0	23.7	5.778	2.808	0.047	0.05
		ASCAT	2.0	25.7	Not included			0.33
	D	Insulin[a]	18.1	18.1	−183.4	64.2	0.007	0.007
		VAT	5.0	23.1	Not included			0.45
		Age	0.9	24.0	Not included			0.53
	E	HOMA-IR[a]	15.9	15.9	−166.8	63.0	0.01	0.01
		Age	6.0	21.9	Not included			0.45
		VAT	1.8	23.7	Not included			0.38
PWS females and males (n = 18)	A	Total AT	19.9	19.9	−6.165	3.095	0.06	0.06
		Age	8.0	27.9	Not included			0.22
	B	VAT	36.2	36.2	−130.0	43.2	0.008	0.008
		Age	7.6	43.8	Not included			0.17
		SCAT	6.5	50.3	Not included			0.42
	C	VAT	36.2	36.2	−270.3	89.8	0.009	0.008
		ASCAT	10.8	47.0	35.0	20.0	0.099	0.10
		Age	7.3	54.3	Not included			0.17
	D	Insulin[a]	58.5	58.5	−727.9	153.4	<0.001	<0.001
		VAT	2.2	60.7	Not included			0.38
		Age	0.1	60.8	Not included			0.93
	E	HOMA-IR[a]	57.0	57.0	−694.2	150.6	<0.001	<0.001
		VAT	2.3	59.3	Not included			0.37
		Age	0.2	59.5	Not included			0.89

These results demonstrate the greater importance of VAT than SCAT or ASCAT depots in explaining the variance in fasting plasma ghrelin in nonobese and obese control females (n = 39) or in PWS females and males (n = 18). The relationship between hyperinsulinemia or insulin resistance and fasting ghrelin is stronger than the influence of VAT in both groups. Forward stepwise multiple linear regression was performed with plasma ghrelin (picograms per milliliter) as the dependent variable, and age (years), total or regional AT volumes (liters), fasting serum insulin (milliunits per liter), or HOMA-IR as independent variables. Independent variables are: model A, age and total AT; model B: age, VAT, and SCAT; model C: age, VAT, and ASCAT; model D: age, VAT, and fasting insulin; model E: age, VAT, and HOMA-IR Δ r^2 indicates the change in variability (percentage) in fasting ghrelin explained by each independent variable in the final model; r^2 indicates the cumulative variability (percentage) explained by the model after each step; β represents the regression coefficient for each independent variable included in the final equation ± SE; P^β represents the significance of β in the final model; P^E represents the significance of the F-to-enter for that independent variable in stepwise multiple linear regression, in addition to those variables already included in the equation (if $P^E > 0.10$ the independent variable was not included in the final model).

[a] Independent variable was $\log_{10}$-transformed.

rodent and human studies (8, 13–15). An understanding of the factors regulating ghrelin secretion by the stomach may reveal novel therapies for GH-deficient states, obesity, or cachexia. This current study demonstrates for the first time a relationship between visceral adiposity and low ghrelin levels that may be explicable by the associated insulin resistance and hyperinsulinemia. It confirms that PWS adults have high plasma ghrelin concentrations that may be partially contributed to, but are not solely explained, by their abnormal body fat distribution or reduced insulin resistance.

Using whole body MRI to determine body fat distribution, we have found that fasting plasma ghrelin levels are more strongly negatively correlated to the visceral AT volume than either total or abdominal sc adiposity. This is consistent with the previous report of a stronger negative association of fasting plasma ghrelin with waist circumference or plasminogen activator inhibitor type 1 concentrations (a marker of visceral adiposity) than with percent body fat (26). This negative association appears to be related to the associated insulin resistance and hyperinsulinemia seen with increasing visceral adiposity (5), because the correlation with visceral adiposity was less significant than that with fasting insulin or HOMA-IR. This is consistent with the previous reports of negative relationships between fasting ghrelin and measures of insulin resistance (19, 21, 24, 26–28) and an inhibitory effect of insulin on ghrelin secretion (23, 25). The acute lowering of plasma ghrelin after food intake does not, however, appear to be due to acute insulin release (9, 33). Nevertheless, it remains possible that other factors associated with visceral adiposity, such as hormones and cytokines secreted by visceral fat into the portal circulation or indirectly by the gut in response to such signals, inhibit gastric ghrelin secretion. Despite the negative correlation of plasma ghrelin with leptin in our study, leptin, which is predominantly secreted by sc fat (4), is unlikely to inhibit ghrelin secretion because ghrelin levels are low in obesity due to congenital leptin deficiency or leptin receptor mutations (16, 18).

PWS is associated with marked hyperphagia and obesity developing in childhood and GH deficiency (2). Using whole body MRI, we have previously demonstrated a selective relative reduction in visceral adiposity in PWS adults, which protects against the metabolic consequences of obesity, such

TABLE 4. Effect of PWS on fasting plasma ghrelin, correcting for age, body composition, fat distribution, and insulin resistance in female adults

PWS as % of control females, adjusting for age and	Mean	95% CI	*P*
Age alone	288	185–447	<0.001
BMI	341	208–558	<0.001
Total AT volume	340	219–547	<0.001
% Body fat	364	206–644	<0.001
VAT volume	323	209–498	<0.001
Visceral fat	337	217–521	<0.001
Leptin[a]	341	217–535	<0.001
Fasting insulin[a]	296	196–446	<0.001
HOMA-IR[a]	297	197–449	<0.001
VAT volume + fasting insulin[a]	301	195–464	<0.001
VAT volume + HOMA-IR[a]	304	197–470	<0.001

Fasting plasma ghrelin in PWS females compared to control females [mean and 95% confidence interval (CI)], adjusting for age, total or visceral adiposity, and markers of insulin resistance. Note that fasting plasma ghrelin is significantly greater (~2.9- to 3.6-fold) in PWS subjects when adjusting for total or visceral adiposity (either as AT volume or fat as percent body mass) and/or fasting insulin or HOMA-IR. PWS regression coefficients (β) were calculated using multiple regression analysis of combined nonobese and obese control females (n = 39) and PWS females (n = 12). Log_{10} plasma ghrelin was the dependent variable, and PWS diagnosis (control = 0; PWS = 1), age, BMI, total or visceral AT volume, total or visceral fat (percent body mass), fasting plasma leptin, insulin, and HOMA-IR were independent variables. β represents the value by which the metabolic parameter is altered in PWS compared to controls, correcting for the independent variables. This was converted into a figure for PWS as a percentage of the control, equal to $100/10^{-\beta}$. The interaction coefficients (PWS × independent variable) were all nonsignificant and were therefore excluded from analysis.

[a] Independent variable was log_{10}-transformed.

as insulin resistance and hypertriglyceridemia (5, 6). This unusual situation occurs despite the presence of many phenotypes that should increase visceral adiposity. Developmental, genetic, hypothalamic, or hormonal abnormalities might be responsible (5). The current study has confirmed the previous findings of markedly elevated fasting circulating ghrelin levels in PWS adults, as also seen in children (16–18). In addition, we have demonstrated that although there appears to be a small contribution to the hyperghrelinemia in PWS from their reduced visceral adiposity and hyperinsulinemia, this is not the sole explanation, because the finding persists, albeit to a slightly lesser extent, after correction for these covariates. There was a 3.4- to 3.6-fold increase in plasma ghrelin in PWS women after correcting for total body adiposity, using whole body MRI to measure body composition, of a similar magnitude to that previously reported in PWS using BMI or dual energy x-ray absorptiometry (16–18). Plasma ghrelin was increased 3.2- to 3.4-fold in PWS after correcting for visceral adiposity and 3.0-fold after correcting for measures of insulin resistance. The stronger inverse correlation between plasma ghrelin and visceral adiposity or insulin resistance than with total adiposity seen in PWS subjects as well as in controls suggests a normal response to the factors lowering ghrelin secretion in obesity, such as hyperinsulinemia, as previously hinted (16–18). Our finding that ghrelin levels are elevated in PWS females who are either not receiving or are receiving OCP/HRT suggests that it is not caused by their hypogonadism, supported by the lack of any association of plasma ghrelin with pubertal status in non-PWS subjects (21). Similarly, the elevated ghrelin in PWS was not related to concomitant use of antidepressant or antipsychotic medications. Hyperghrelinemia in PWS appears not to be due to GH deficiency itself, as confirmed by the lack of any significant inhibitory effect of GH replacement on fasting plasma ghrelin in both non-PWS and PWS subjects (20, 22, 34). Although correction was not made for the significant reduction in body fat with GH therapy in one study of PWS subjects, which might be expected in itself to increase fasting plasma ghrelin, there was no significant change in fasting insulin levels with GH therapy in that study (22). There was no significant effect of the PWS molecular class on the degree of hyperghrelinemia, as reported previously (18), although the numbers with such detailed genetic testing in our study were small.

The cause of the hyperghrelinemia in PWS, therefore, remains unclear. It is currently unknown whether this is due to increased secretion from only the stomach or perhaps other organs, including the small intestine and pituitary (35), or if the bioactive octanoylated ghrelin is increased proportionally (8). It is unknown whether there is any direct effect on ghrelin-secreting cells from the loss of PWS gene expression. As vagotomy increases plasma ghrelin in rodents (36), the elevated plasma ghrelin in PWS might result from reduced parasympathetic vagal efferent tone in PWS. Such an effect in PWS is suggested by reduced postprandial pancreatic polypeptide secretion (37) and disturbed parasympathetic cardiac autonomic function tests (38, 39). The reduced visceral adiposity in PWS (5, 6) might also be explicable by reduced parasympathetic tone, as an anabolic parasympathetic innervation of visceral fat has recently been demonstrated (40). Although reduced vagal tone might also be expected to reduce intestinal GLP-1 secretion (41), no reduction of fasting plasma GLP-1 in PWS was seen in our study, but it remains possible that postprandial GLP-1 secretion might be affected.

Disturbed parasympathetic vagal tone could result from hypothalamic abnormalities, as the dorsal motor nucleus of the vagus nerve in the brainstem, which innervates the stomach and visceral fat depots, receives descending efferent inputs from the hypothalamus, particularly the paraventricular nucleus (PVN) (40, 42–44), including oxytocin neurons (45–47). A reduction of hypothalamic oxytocin neurons has been seen in postmortem human PWS hypothalami (48) and *ndn* (an imprinted candidate PWS gene) knockout mice (49).

The consequences of hyperghrelinemia in PWS remain unclear. It has been hypothesized to potentially contribute to GH deficiency in PWS (16) through reduced GHS-R levels or receptor densensitization as a consequence of chronically elevated ghrelin, as has been found with chronic GHRH stimulation (50, 51). Indeed, pituitary cells show rapid desensitization to the effect of GHS *in vitro* (52), whereas *in vivo* intermittent infusion of ghrelin augments GH secretion, but continuous infusion of ghrelin suppresses GH secretion in moderately GH-deficient rats (53). An inverse correlation of plasma ghrelin with IGF-I in non-PWS subjects has been inconsistently found (21, 24). Although the relatively small number of subjects needs to be considered, the lack of any significant negative association of plasma ghrelin with serum IGF-I in PWS subjects in our study, even when adjusting for adiposity or hyperinsulinemia, calls into question any causative role for hyperghrelinemia in the GH deficiency of PWS. Similarly, although ghrelin is thought to stimulate GH secretion in part by stimulating hypothalamic GHRH neurons (54–56), no abnormalities of hypothalamic GHRH neurons have been seen in postmortem PWS hypothalami (57).

The degree of hyperghrelinemia seen in PWS would be sufficient to produce hyperphagia, as gauged by the acute stimulation of appetite and food intake with ghrelin infusions in non-PWS subjects (15). In rodents, ghrelin increases feeding via activation of hypothalamic neuropeptide Y (NPY) and agouti-related protein (AGRP) neurons (13, 58). It is therefore noteworthy that such neuronal activation is not seen in the infundibular nucleus (INF) of postmortem PWS hypothalami (59), using quantitative immunocytochemistry and *in situ* hybridization. Such postmortem studies do have their inherent difficulties, but because neuropeptides and their mRNA are stable in the postmortem human hypothalamus, technically reliable and reproducible quantitative studies of hypothalamic changes in pathophysiological states are possible if there is appropriate matching of controls, consideration of confounding variables, and detailed image analysis (60, 61). Nevertheless, the numbers of PWS hypothalami studied were small, assessment of neuronal peptide content and mRNA expression may not adequately reflect neuronal activity or neuropeptide release, and the confounding effects of premorbid illness in both PWS and non-PWS subjects may complicate the interpretation (57, 59). However, no evidence of increased activity of NPY/AGRP neurons (or deficiency of GHRH neurons) was even seen in those few PWS subjects who died suddenly (57, 59). Possible additional explanations for this discrepancy include resistance to the action of ghrelin in NPY/AGRP neurons, perhaps due to densensitization or reduced number of GHS-R, or orexigenic actions of ghrelin through other pathways, such as stimulation of vagal afferents (62). Recent work has also suggested that ghrelin may act on presynaptic NPY terminals to increase NPY release (12). It is therefore possible that the elevated ghrelin levels in PWS increases appetite by acting distal to NPY cell bodies to directly increase NPY inhibition of anorexigenic proopiomelanocortin (POMC) neurons in the INF or increase NPY release onto other downstream circuits in the PVN or lateral hypothalamic area. Given the importance of the NPY, AGRP, and POMC innervation of the PVN in the control of feeding (63), it remains to be seen whether the PVN, and other brain regions, can respond normally to changes in ghrelin in PWS, given the known hypothalamic abnormalities in PWS (48). A causative, rather than epiphenomenonal, role for the elevated plasma ghrelin in PWS hyperphagia and other phenotypes, such as sleep disturbance (64), has therefore yet to be proven. However, an understanding of the pathophysiology of increased ghrelin secretion in PWS may also indirectly benefit subjects with non-PWS obesity through the development of treatment strategies to reduce ghrelin secretion.

In conclusion, this study has demonstrated the principal negative relationship of visceral adiposity with ghrelin levels, which may be related to hyperinsulinemia reducing ghrelin secretion. The marked elevation of fasting plasma ghrelin in PWS adults is not solely explicable by their reduced visceral adiposity or improved insulin sensitivity, although these may provide small contributions. The development of ghrelin antagonists and the use of drugs, such as somatostatin analogs, to lower plasma ghrelin will help clarify the role of hyperghrelinemia in PWS phenotypes (65). Further study of PWS mouse models and postmortem PWS human hypothalami and other organs will help elucidate the cause of hyperghrelinemia in PWS, which could be an indirect consequence of hypothalamic abnormalities (2).

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