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Original Research Communications

Resting metabolic rate, plasma leptin concentrations, leptin receptor expression, and adipose tissue measured by whole-body magnetic resonance imaging in women with Prader-Willi syndrome[1–3]

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ABSTRACT

Background: Obesity in Prader-Willi syndrome (PWS) may be related to abnormalities in the adipocyte-leptin-hypothalamic pathway and may be exacerbated by reductions in the resting metabolic rate (RMR).

Objective: We compared body composition, body-composition–adjusted RMR, and adiposity-adjusted plasma leptin between women with PWS and control women. We also examined leptin receptor expression in the PWS group.

Design: We studied body composition using whole-body magnetic resonance imaging and measured plasma leptin by radioimmunoassay in 45 control women aged 18–56 y and in 13 women with PWS aged 20–38 y. RMR was measured by indirect calorimetry in 41 control women and in 8 women with PWS. Age, body composition, and regional adipose tissue (AT) depots were corrected for by multiple regression analysis. Messenger RNA expression of the leptin receptor was examined by reverse transcriptase–polymerase chain reaction in lymphocytes.

Results: In the PWS group, fat mass was greater after correction for fat-free mass, and RMR was normal after correction for both fat-free mass and fat mass. Leptin was influenced primarily by subcutaneous AT volume in both subject groups. Leptin concentrations were not significantly different between the 2 groups after adjustment for age and AT content or distribution. Full-length leptin receptor messenger RNA was expressed in the lymphocytes of the PWS group.

Conclusions: Differences in RMR in women with PWS are explained by abnormal body composition, suggesting that energy expenditure is normal at the tissue level in PWS. There is no evidence that defective leptin production causes obesity in PWS, and leptin receptor deficiency is not a primary consequence of the gene defects leading to leptin resistance. *Am J Clin Nutr* 2002;75:468–75.

KEY WORDS Magnetic resonance imaging, MRI, obesity, body fat, Prader-Willi syndrome, fat distribution, resting metabolic rate, growth hormone deficiency, hypogonadism, women

INTRODUCTION

Prader-Willi syndrome (PWS) is characterized by hyperphagia and life-threatening obesity from childhood, mental retardation, short stature due to growth hormone (GH) deficiency, and hypogonadism (1). The phenotype is thought to result from developmental abnormalities in the hypothalamus (2) that are due to imprinted gene defects on chromosome 15q11-13 (3). On the basis of total body water measurements by bioelectrical impedance analysis or isotopic dilution and, more recently, dual-energy X-ray absorptiometry (DXA), PWS patients have been shown to have a peculiar body composition, ie, a high percentage of body fat and a low percentage of lean tissue (4–7). PWS patients have a low resting metabolic rate (RMR), which appears to be explained by their abnormal body composition and contributes to a reduction in 24-h energy expenditure (4, 7–9).

Leptin, the adipocyte-derived plasma hormone, interacts with brain pathways, particularly in the hypothalamus, to reduce food intake and control energy expenditure and body weight (10). The hyperphagia, obesity, and hypogonadism observed in PWS patients are also observed in patients with leptin deficiency and leptin receptor defects (11–13), raising the possibility that defects in leptin pathways in the brain may explain these phenotypes in PWS patients. Other researchers found no evidence for leptin deficiency in PWS patients (14–22). However, their findings are complicated by the use of body mass index (BMI) as a measure of body composition, which underestimates obesity in PWS (6, 23), and by our recent finding of a high ratio of subcutaneous to visceral fat in women with PWS (24) because leptin is differentially expressed in various fat depots (25). No studies of the leptin receptor in PWS have been reported. The leptin receptor *OBR* occurs in several alternative splice variants, of

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TABLE 1
Characteristics of the lean and obese control women and of the female patients with Prader-Willi syndrome (PWS)[1]

	Control group		
	Lean[2] (n = 31)	Obese[3] (n = 14)	PWS group (n = 13)
Age (y)	31 ± 8 (18–45)	36 ± 10 (18–56)	27 ± 7[4] (20–38)
Weight (m)	66.2 ± 8.6 (49.4–81.1)	100.9 ± 14.9[5] (79.2–130.0)	82.3 ± 27.7[6,7] (54.0–144.0)
Height (m)	1.66 ± 0.06 (1.53–1.78)	1.66 ± 0.05 (1.54–1.77)	1.49 ± 0.08[6,8] (1.38–1.67)
BMI (kg/m^2)	23.9 ± 2.4 (19.6–28.3)	36.9 ± 5.7[5] (30.2–51.9)	36.6 ± 9.9[5] (23.6–51.6)
Total AT volume by MRI (L)	27.3 ± 6.7 (13.9–41.7)	59.3 ± 14.0[5] (45.2–90.0)	55.7 ± 25.8[5] (27.5–109.0)
Percentage body fat by MRI (%)	29.4 ± 4.5 (18.5–37.1)	41.9 ± 4.1[5] (36.8–50.5)	46.9 ± 6.9[5] (35.3–54.5)
SCAT volume by MRI (L)	23.3 ± 5.7 (12.1–35.2)	49.8 ± 12.1[5] (37.8–77.0)	48.6 ± 23.0[5] (23.1–94.3)
VAT:SCAT	0.068 ± 0.021 (0.039–0.118)	0.108 ± 0.021[5] (0.078–0.162)	0.067 ± 0.017[8] (0.048–0.099)
Plasma leptin (μg/L)	13.6 ± 8.2 (3.2–35.8)	42.1 ± 22.5[5] (19.8–90.4)	48.2 ± 38.5[5] (7.7–119.2)

[1] $\bar{x}$ ± SD; range in parentheses. AT, adipose tissue; MRI, magnetic resonance imaging; SCAT, subcutaneous AT; VAT, visceral AT.
[2] BMI ≤ 30.
[3] BMI > 30.
[4,7,8] Significantly different from obese (ANOVA with post hoc Tukey's test): [4] $P < 0.05$, [7] $P < 0.005$, [8] $P < 0.001$.
[5,6] Significantly different from lean (ANOVA with post hoc Tukey's test): [5] $P < 0.001$, [6] $P < 0.02$.

which only the long isoform (*OBRb*) is fully functional (26). A lack of *OBRb* expression might explain the obese and hypogonadal phenotypes of PWS patients.

Whole-body magnetic resonance imaging (MRI) is a reliable, safe, and accurate method for measuring body fat content and distribution (27, 28). MRI avoids the ionizing radiation of computed tomography scanning, and whole-body MRI avoids the sample bias seen with single- or selected-slice computed tomography or MRI (29). Therefore, in the present study we used whole-body MRI to examine body composition in healthy control subjects and women with PWS. We looked for any evidence of a lower RMR in the PWS patients than in the control subjects after correction for body composition. We also looked for differences in plasma leptin between the 2 subject groups after correction for both the content and distribution of adipose tissue (AT). To assess whether leptin receptor deficiency is a primary abnormality in PWS, we used reverse transcriptase–polymerase chain reaction (RT-PCR) to examine *OBR* and *OBRb* expression in the lymphocytes of the PWS patients.

SUBJECTS AND METHODS

Subjects

Subject characteristics are given in **Table 1**. Forty-five control women (n = 31 lean and 14 obese) and 13 women with PWS underwent whole-body MRI to assess body composition. Four women with PWS were taking oral contraceptives and one woman with PWS was receiving hormone replacement therapy. In addition, lymphocytes were obtained from 2 men with PWS for the study of leptin receptor expression.

Ethical approval for the study was obtained from the Research Ethics Committee of Hammersmith Hospital, London (registration numbers 92/3995 and 96/4807). Control subjects were recruited from hospital staff, dietetic clinics, and the general public via advertisements. Women with PWS were recruited through the Department of Psychiatry (University of Cambridge, United Kingdom) and the UK Prader-Willi Syndrome Association. Consent was obtained from both the PWS patients and their caregivers or next of kin. All subjects were aged >18 y and nondiabetic. Control subjects had no known endocrine disease and were premenopausal. All subjects reported stable body weight in the 2 mo prior to the study. However, some PWS patients had been involved in weight-reduction programs in the previous year. All PWS patients met the diagnostic criteria for PWS (1) and had childhood-onset obesity requiring vigorous behavioral modification because of their extreme hyperphagia and obsession with food. None of the PWS patients were receiving GH therapy and none had significant scoliosis or spinal rods. All PWS patients had experienced primary amenorrhoea or severe oligomenorrhea. All subjects had normal renal and hepatic function.

Body composition

BMI (in kg/m^2) was determined for each subject from height and weight measurements. Control subjects were divided into lean (BMI ≤ 30) and obese (BMI > 30) groups. Whole-body MRI was performed as previously described (29) with a 1.0T HPQ system (Marconi Medical, Cleveland).

with the use of a rapid T_1-weighted spin echo sequence with a repetition time of 36 ms, an echo time of 14 ms, a flip angle of 120°, a field of view of 60 cm, a 256 × 256 matrix, phase conjugate symmetry, and a slice thickness of 10 mm. Images in the arms and legs were collected with 2 averages and those in the trunk with 1 average. The subjects laid in the magnet in a prone position with their arms straight above the head. All images were acquired at single slices at the isocenter to avoid image distortion, and the subjects were moved through the magnet on a platter built for that purpose (28). Subjects were scanned from their fingertips to their toes with 10-mm thick transverse images every 10–30 mm. The total scanning time was only 20–30 min for each subject. Interactive computer analysis was used to quantify total and regional body AT volumes. Total AT was divided into total subcutaneous (SCAT), visceral (VAT), and extraabdominal internal (INAT) AT. VAT was calculated as the intraabdominal AT volume between the level of the diaphragm and femoral heads. INAT was calculated as total AT − SCAT − VAT and consisted primarily of the AT around the muscle fibers (intramuscular). The total fat mass (FM) was calculated from these results assuming a fat density of 0.9 kg/L (30) and a fat content of AT of 80% (29). MRI-derived total fat (as a percentage of total body mass, equal to 100 × absolute fat mass/total body weight) and fat-free mass (FFM, equal to total body weight – absolute fat mass) were then calculated by using total body weight.

Resting metabolic rate

RMR was measured by indirect calorimetry (Deltatrac II; Datex Division Instrumentarium Corp, Helsinki) after the subjects had fasted overnight. Subjects were studied while lying flat for ≥20 min ($\bar{x}$: 35 min).

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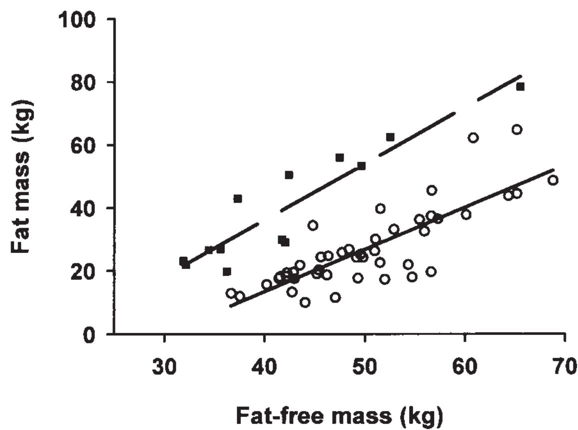

FIGURE 1. Relation between fat mass and fat-free mass measured by whole-body magnetic resonance imaging in healthy control women (○, solid regression line) and in female patients with Prader-Willi syndrome (■, dashed regression line). $r = 0.92$ ($P < 0.001$) in the PWS group and 0.81 ($P < 0.001$) in the control group.

Blood sampling

Subjects reported to the Endocrine Unit (Hammersmith Hospital) after fasting overnight for 12 h, and blood was drawn into tubes containing lithium heparin and aprotinin for plasma extraction. The tubes were immediately spun (1500 × *g*, 10 min, room temperature), and the plasma was separated and stored at −20°C for assay of leptin (Linco, St Louis).

Leptin receptor expression

Peripheral lymphocytes were isolated by differential sedimentation with the use of Lymphoprep (Nycomed Pharma, Oslo) and were transformed with the use of Epstein-Barr virus (EBV). Studies were performed on EBV-transformed lymphocytes from 2 men with PWS (age: 18–28 y; BMI: 21.3–22.4) and 3 women with PWS (age: 22–33 y; BMI: 41.8–51.6) and on untransformed lymphocytes from 1 woman with PWS (age: 22; BMI: 41.8). Untransformed and EBV-transformed lymphocytes from control subjects and postmortem samples of human hypothalamus were used as controls. Total RNA was isolated by extraction with guanidinium thiocyanate, phenol, and chloroform. First-strand complementary DNA (cDNA) was synthesized from 1 to 5 μg total RNA with the use of random hexamers. cDNA fragments covering the amino-terminal (nucleotides 164–630, common to all *OBR* isoforms) and carboxy-terminal (nucleotides 2831–3719, specific to the long isoform *OBRb*) coding sequence of the 1165–amino acid human long isoform *OBR* were amplified by nested PCR on 20% of the original RT reaction to generate 466– and 888–base pair fragments, respectively, as previously described (31). RT-PCR products were visualized on a 2%-agarose gel.

Statistical analysis

Pearson's product-moment correlation coefficients (r) were used to assess the relation between the variables in the control and PWS groups separately. If the slopes of the regression lines obtained between the control and PWS groups were not significantly different, the results for the 2 groups were combined in future multiple linear regression analyses, with PWS diagnosis included as an independent variable. Unadjusted and partially-adjusted regression coefficients (β) for the PWS group were then calculated to enable examination of the effect of PWS diagnosis on RMR and leptin, independent of age, body composition, and AT content or distribution. Adjustment of the measured RMR was made by using the multiple regression equations to correct for differences in age and body composition between the PWS, lean control, and obese control subjects. Log_{10} transformation was used to correct plasma leptin because it was not normally distributed. The correlation between plasma leptin concentrations and individual AT depots was examined with the use of multiple regression analysis in the control and PWS groups to assess the independent effects of regional adiposity. Between-group comparisons were made with the use of one-way analysis of variance (ANOVA) with a post hoc Tukey's test. Significance was set at $P < 0.05$. SIGMASTAT 2.0 (Jandel Corporation, Rafael, CA) and SYSTAT 8.0 (SPSS Inc, Chicago) were used for the statistical analyses.

RESULTS

Body composition in the PWS group

The PWS group had a significantly greater MRI-measured FM for a given FFM than the control group (PWS group: β = 24.8 kg, SE = 2.6 kg, $P < 0.001$; **Figure 1**). This finding was still significant after adjustment for differences in age and height (β = 19.1 kg, SE = 3.8, $P < 0.001$); however, only height (in m) was a significant independent variable (β = −40.5 kg, SE = 18.5, $P = 0.03$). This finding remained significant when the analysis was restricted to those PWS patients who were not taking oral contraceptives or receiving hormone replacement therapy: β = 18.1 kg, SE = 4.5, $P < 0.001$.

Resting metabolic rate

RMR data were available for 41 control subjects and 8 PWS patients (**Table 2**); the data for 4 control subjects and 5 PWS patients

TABLE 2
Characteristics and body composition of the lean and obese control women and of the female patients with Prader-Willi syndrome (PWS) who underwent measurements of resting metabolic rate (RMR)[1]

	Control group		
	Lean[2] (n = 28)	Obese[3] (n = 13)	PWS group (n = 8)
Age (y)	31 ± 1	36 ± 3	25 ± 2[4]
Weight (kg)	65.6 ± 1.7	101.6 ± 4.2[5]	95.7 ± 9.7[5]
Height (m)	1.66 ± 0.01	1.66 ± 0.02	1.50 ± 0.03[5,6]
BMI (kg/m²)	23.7 ± 0.5	37.1 ± 1.6[5]	42.1 ± 3.0[5]
Percentage fat by MRI (%)	29.1 ± 0.8	42.1 ± 1.2[5]	50.8 ± 2.0[5,6]
FFM by MRI (kg)	46.1 ± 0.9	58.5 ± 1.8[5]	46.1 ± 3.7[6]
FM by MRI (kg)	19.5 ± 0.9	43.2 ± 2.9[5]	49.6 ± 6.3[5]
FM:FFM	0.42 ± 0.02	0.74 ± 0.04[5]	1.06 ± 0.07[5,6]
RQ	0.84 ± 0.02	0.89 ± 0.03	0.85 ± 0.03
RMR (MJ/24 h)	5.460 ± 0.134	7.192 ± 0.293[5]	6.644 ± 0.464[7]
RMR (CV %)	11.4 ± 0.9	11.9 ± 1.2	10.1 ± 1.8

[1] $\bar{x}$ ± SEM. MRI, magnetic resonance imaging; FFM, fat-free mass; FM, fat mass; RQ, respiratory quotient.
[2] BMI ≤ 30.
[3] BMI > 30.
[4,6] Significantly different from obese (ANOVA with post hoc Tukey's test): [4] $P < 0.02$, [6] $P < 0.001$.
[5,7] Significantly different from lean (ANOVA with post hoc Tukey's test): [5] $P < 0.001$, [7] $P < 0.01$.

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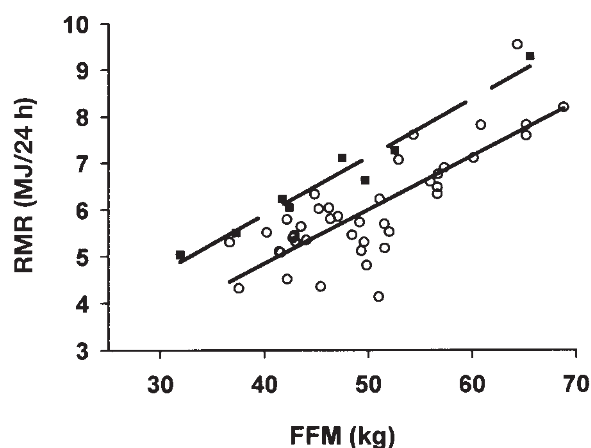

FIGURE 2. Relation between resting metabolic rate (RMR) and fat-free mass (FFM) measured by whole-body magnetic resonance imaging in healthy control women (○, solid regression line; $n = 41$) and in female patients with Prader-Willi syndrome (■, dashed regression line; $n = 8$). $r = 0.98$ ($P < 0.001$) in the PWS group and 0.80 ($P < 0.001$) in the control group.

were excluded because they had hyperventilated or did not remain still during the measurements. Although mean BMIs were not significantly different between the obese control and PWS groups, MRI-derived percentage fat and the ratio of FM to FFM were significantly higher in the PWS group. Mean respiratory quotients were not significantly different between the groups.

Positive correlations were found between RMR and FFM (**Figure 2**) and between RMR and FM ($r = 0.71$ and 0.89 in the control and PWS groups, respectively; both $P < 0.005$). No significant difference in the slopes of the regression lines were found between the control and PWS groups, allowing assessment of β values for the PWS group with the use of dummy variables in a combined data set with multiple linear regression analysis. RMR was significantly greater in the PWS group than in the control group after correction for FFM and age (PWS group: β = 1.046 MJ/24 h, SE = 0.268 MJ/24 h, $P < 0.001$), with RMR as the dependent variable and age, PWS diagnosis, and FFM as the independent variables. In contrast, RMR was significantly lower in the PWS group than in the control group after correction for FM and age (PWS group: β = −0.975 MJ/24h, SE = 0.372, $P < 0.02$), with RMR as the dependent variable and age, PWS diagnosis, and FM as the independent variable.

RMR was significantly lower in the PWS group than in the lean and obese control groups after adjustment for age, height, and weight and after adjustment for age and FM, but were significantly greater after adjustment for age and FFM (**Figure 3**). Only after adjustment for age, FFM, and FM was there no significant difference in RMR between the control and PWS groups (PWS group: β = 0.615 MJ/24 h, SE = 0.481, $P = 0.2$) with RMR as the dependent variable and age, PWS diagnosis, FFM, and FM as the independent variables.

Plasma leptin and adiposity

In both the control and PWS groups, plasma leptin was positively correlated with total and individual AT (SCAT, VAT, and INAT) volumes measured by whole-body MRI (**Table 3** and **Figure 4**). However, only the relation with SCAT remained significant after adjustment for other AT depots (Table 3 and **Table 4**). There was also a tendency for a negative effect of age on plasma leptin in the control group, independent of total AT ($P = 0.06$) or SCAT ($P = 0.07$). With the use of these models, the final regression equations were as follows: $r^2 = 0.64$, $\log_{10}$ leptin = 0.833 − [0.0078 × age (y)] + [0.017 × total AT (L)]; and $r^2 = 0.64$, $\log_{10}$ leptin = 0.822 − [0.0075 × age (y)] + [0.020 × SCAT (L)]. Therefore, the difference in age was adjusted for in the comparison between groups.

Plasma leptin in the PWS group

No significant differences in the slopes of the regression lines were found between the control and PWS groups, which enabled the assessment of β values for the PWS group with the use of dummy variables in combined data sets. Leptin concentrations were not significantly different between the control and PWS groups after adjustment for age and SCAT, VAT, and INAT separately; only age and SCAT had a significant effect on plasma leptin (Table 4). Plasma leptin was not significantly different between the groups after adjustment for total AT or SCAT (Figure 4) and age (**Table 5**). Similar results were found when the analysis was restricted to those PWS patients who were not taking oral contraceptives or receiving hormone replacement therapy (Table 5).

Leptin receptor expression

cDNA fragments common to all *OBR* isoforms and specific to the *OBRb* isoform (after the alternative splice site for the leptin receptor mRNA long isoform) were amplified from EBV-transformed lymphocytes from PWS patients (**Figure 5**) and from untransformed lymphocytes. In addition, the intermediate coding sequence of the long isoform leptin receptor (nucleotides 573–3079) was amplified by RT-PCR with the use of overlapping primers (31; AP Goldstone, unpublished observations, 1997).

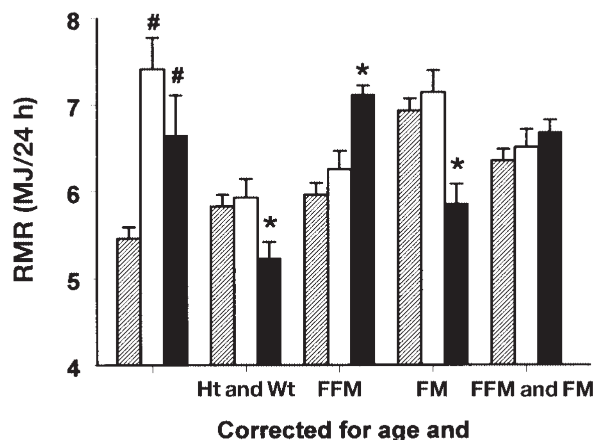

FIGURE 3. Mean (±SEM) resting metabolic rate (RMR) adjusted for differences in age, height (Ht), weight (Wt), fat-free mass (FFM), and fat mass (FM) in healthy, lean control women (hatched bar, $n = 28$); healthy, obese control women (open bar, $n = 13$); and female patients with Prader-Willi syndrome (PWS; solid bar; $n = 8$). Measured RMR was adjusted with the use of multiple linear regression equations, with measured RMR as the dependent variable and combinations of age, PWS diagnosis, Ht, Wt, FFM, and FM as independent variables. One-way ANOVA and a post hoc Tukey's test: #Significantly different from the lean control group, $P < 0.05$; *Significantly different from both the lean and obese control groups, $P < 0.05$.

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TABLE 3
Effect of adipose tissue (AT) depots on plasma leptin in the control women and in the female patients with Prader-Willi syndrome (PWS)[1]

Group and independent AT variable (L)	Univariate correlation		AT depot β coefficient		
	r^2	β Coefficient[2]	Adjusted for age	Adjusted for age and other AT depots	Adjusted for age and SCAT
Control group (n = 45)					
Total AT	0.61	0.016[3]	0.017[3] (0.64)	—	—
SCAT	0.61	0.019[3]	0.020[3] (0.64)	0.018[4] (0.64)	—
VAT	0.54	0.140[3]	0.155[3] (0.59)	0.028	0.025
INAT	0.46	0.221[3]	0.227[3] (0.47)	−0.005	−0.006
PWS group (n = 13)					
Total AT	0.71	0.013[3]	0.012[3] (0.74)	—	—
SCAT	0.73	0.015[3]	0.014[3] (0.76)	0.017[4] (0.77)	—
VAT	0.54	0.180[5]	0.157[6] (0.67)	0.006	0.007
INAT	0.37	0.160[4]	0.122 (0.46)	−0.099	0.086

[1] Partial regression coefficients (β) for the adipose tissue (AT) depots are from multiple regression analysis with $\log_{10}$ plasma leptin as the dependent variable, before and after adjustment for age and other AT depots (assessed by whole-body magnetic resonance imaging) as independent variables. Note that for both subject groups, leptin concentrations were affected primarily by the subcutaneous AT content. VAT, visceral AT; INAT, extraabdominal internal AT.

[2] Represents the value by which $\log_{10}$ leptin changes for a change in the specified AT depot, adjusted for other independent variables in the equation. r^2 values for the overall model are in parentheses.

[3] $P < 0.001$.

[4] $P < 0.05$.

[5] $P < 0.005$.

[6] $P < 0.02$.

DISCUSSION

Body composition in the PWS group

Our finding that the PWS group had a significantly greater MRI-measured FM for a given FFM than did the control group agrees with the findings of a previous study that used DXA to measure FM in children and young adults with PWS (6). A potential confounding factor in the measurement of FM with MRI is the assumption that the fat content of AT and the fat density are identical between groups with and without PWS. Nevertheless, there is no published evidence of any reduction in the uptake of fat into AT, in the fatty acid composition of AT, or in the fat cell size in patients with PWS that would otherwise contradict our findings (32, 33). Assumptions about lean tissue hydration, the ratio of extracellular to intracellular water, body shape, and fat distribution may similarly affect measures of lean body mass (LBM) with methods of body-composition analysis other than MRI in subjects who are extremely obese or who have GH deficiency (5, 34, 35). Furthermore, all 2-component models of body composition have limitations when FFM is calculated indirectly from FM (whole-body MRI) or when FM is calculated from LBM (bioelectrical impedance analysis or isotopic dilution) (36). The abnormal body composition observed in patients with PWS can be explained by their high energy intakes, GH deficiency, hypogonadism, and low physical activity (4, 9, 34, 37–39). It is unclear whether the childhood onset of PWS or the magnitude of the high energy intakes, GH deficiency, hypogonadism, and low physical activity was sufficient to produce such a gross abnormality. Other factors possibly responsible for the abnormal body composition are *1*) defects in hypothalamic functions that regulate body composition, separate from their hormonal effects, for example, via the autonomic

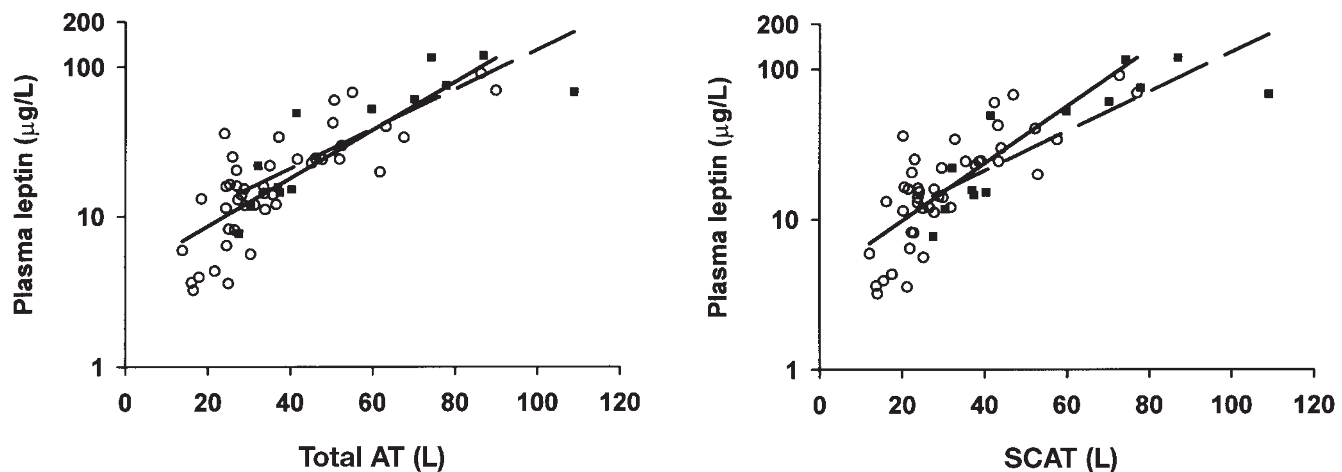

FIGURE 4. Relation between plasma leptin concentrations and total adipose tissue (AT) or subcutaneous AT (SCAT) volume as measured by whole-body magnetic resonance imaging in healthy control women (○, solid regression line) and in female patients with Prader-Willi syndrome (■, dashed regression line). $r = 0.85$ ($P < 0.001$) in the PWS group and $r = 0.78$ ($P < 0.001$) in the control group in both panels.

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TABLE 4
Multiple regression analysis of the effect of age, Prader-Willi syndrome (PWS) diagnosis, and adipose tissue (AT) depots on plasma leptin concentrations in the control women and in the female patients with PWS[1]

Independent variable	β Coefficient[2]	SE	*P*
Age (y)	−0.009	0.004	0.037
SCAT (L)	0.015	0.005	0.003
VAT (L)	0.059	0.041	0.159
INAT (L)	−0.043	0.048	0.378
PWS diagnosis[3]	0.031[4]	0.087	0.721

[1] Log_{10} leptin was used as the dependent variable, and the r^2 value for the overall model was 0.71. Note that age and subcutaneous AT (SCAT) are the only factors that affected plasma leptin significantly. VAT, visceral AT; INAT, extraabdominal internal AT.

[2] Represents the degree to which log_{10} leptin changes for a change in the specified variable, adjusted for other independent variables in the equation.

[3] The grouping variable PWS diagnosis was expressed as control = 0, PWS = 1. The interaction variables PWS × SCAT, PWS × VAT, and PWS × INAT were not significant.

[4] This value is equivalent to plasma leptin in a PWS patient being 107.5% (SE range: 87.9–131.5) that of the control subjects after adjustment for age, SCAT, VAT, and INAT.

nervous system, and *2*) the lack of expression of PWS genes outside the brain, such as the DNA binding protein necdin, which is normally expressed in muscle (40).

Resting metabolic rate

Previous studies showed low 24-h energy expenditures, physical activities, and RMRs in PWS patients (4, 7–9). We found a lower RMR in the PWS group than in the control group after adjustment for age, height, and weight, as found previously by others (4). However, we found that RMR was normal in the PWS group after adjustment for their lower FFM and higher FM as measured by whole-body MRI. This finding confirms the conclusion of previous studies that adjusted for LBM measured by isotopic-dilution methods (7, 9), ie, that low RMRs in children with PWS are explained by their abnormal body composition. These results suggest that there is no change in energy expenditure at the tissue level in PWS patients. Furthermore, the estimation of dietary energy requirements from RMRs calculated with the use of standard predictive formulas involving age, height, and weight in adults with PWS will be in error because the formulas do not accurately assess body composition. The finding of a normal RMR in subjects with PWS after adjustment for body composition does not detract from the importance of an increase in physical activity in PWS patients to improve their body composition (4, 9, 37) or of the potential benefits of novel drugs that increase peripheral thermogenesis.

Plasma leptin and adiposity in the control group

Plasma leptin concentrations increase with total adiposity (41) and, although there was a positive relation between plasma leptin and individual AT depots in the control group in the present study, only the relation with SCAT remained independent of other fat depots. This finding contrasts with previous findings for markers of insulin sensitivity, such as fasting insulin and triacylglycerol concentrations, which are primarily influenced by VAT (24). This finding with the use of whole-body MRI agrees with that seen with the use of BMI or DXA to assess total fat and selected-slice MRI or computed tomography to measure VAT in both male and

TABLE 5
Plasma leptin concentrations adjusted for age and adiposity in the control women and in the female patients with Prader-Willi syndrome (PWS)[1]

	Plasma leptin in the PWS group	
PWS group	Adjusted for age and total AT	Adjusted for age and SCAT
	% of control value	
All (*n* = 13)	99.5 (82.9–119.4)	96.1 (79.9–115.6)
Those not taking OCPs or receiving HRT (*n* = 8)	111.3 (89.8–138.1)	106.1 (85.3–132.0)

[1] $\bar{x}$; SE range in parentheses. Values were calculated from the partial regression β coefficients for the PWS group ($\bar{x} \pm$ SE) with the use of multiple regression analysis of the combined data from the control group (*n* = 45) and the PWS group [either all subjects, or those not taking oral contraceptive pills (OCPs) or receiving hormone replacement therapy (HRT)]. In the regression analysis, log_{10} leptin was the dependent variable and PWS diagnosis (control = 0, PWS = 1), total adipose tissue (AT) or subcutaneous AT (SCAT) volume, measured by whole-body magnetic resonance imaging, were independent variables. β represents the value by which log_{10} leptin changes in PWS compared with control subjects after adjustment for age and total AT or SCAT. This value was then converted into a value for the PWS group that was a percentage of the control value, equal to $100/10^{-\beta}$. Note that there was no significant difference in plasma leptin between the control and PWS groups after adjustment for age and total AT or SCAT.

female control subjects (14, 42–44). The importance of SCAT is further supported by the findings of higher leptin mRNA expression and secretion rates in SCAT than in VAT (25, 45).

Plasma leptin in the PWS group

Plasma leptin was higher in PWS females with a greater AT content, in agreement with previous studies that used BMI or DXA to measure body composition (14–17). However, there was no significant difference in plasma leptin between the PWS and

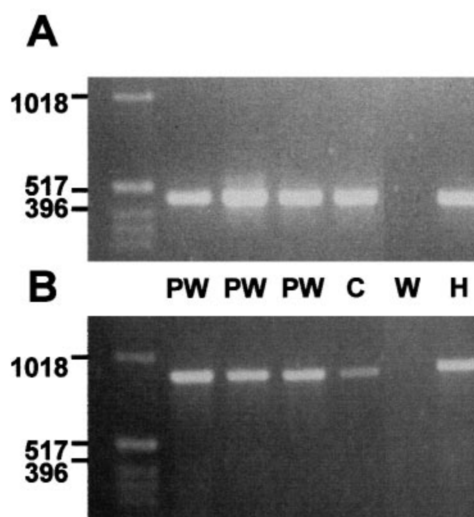

FIGURE 5. Reverse transcriptase–polymerase chain reaction of leptin receptor messenger RNA in lymphocytes from healthy control women (C), from female patients with Prader-Willi syndrome (PWS), in a postmortem sample of human hypothalamus (H), and in a negative (ie, does not contain leptin receptor mRNA) water control (W). A: extracellular common *OBR* isoform (nucleotides 164–630, 466 base pairs); B: intracellular long *OBR* isoform (nucleotides 2831–3719, 888 base pairs) (30).

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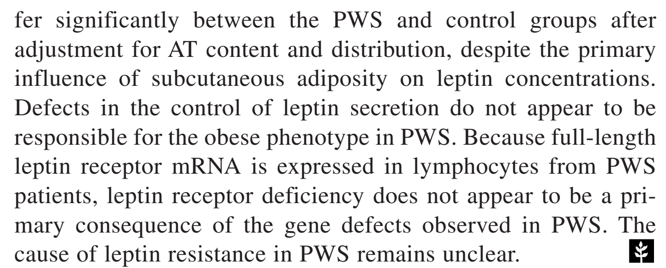
the control groups after adjustment for total AT or for SCAT, VAT, and INAT. Correction for SCAT, as opposed to just total AT, was necessary because of both the primary effect of this fat depot on plasma leptin concentrations and the secondary effect of the higher ratio of SCAT to VAT in PWS patients (24). A high ratio of SCAT to VAT is expected to be associated with high plasma leptin concentrations (42–45). Indeed, one study found higher leptin concentrations in the PWS group than in the control group, relative to the total fat content measured by DXA (16), that could be explained by abnormal body fat distribution in the PWS group. Other possible explanations for the contrast with our findings include differences between MRI- and DXA-measured body composition and the inclusion of both children and adults and males and females in that study (16) because hypogonadism in males has an effect on leptin concentrations opposite to that in females (46). Leptin concentrations are also affected by GH (47, 48) and sex hormones (46). The finding that plasma leptin concentrations were similar between our control subjects and our oligoamenorrheic, presumably GH-deficient, PWS females—even in those who were not receiving hormone replacement therapy—suggests that the effects of estrogen and GH on plasma leptin may be primarily mediated by overall changes in body fat content or distribution (25, 38, 47, 49). There is no evidence from our study to suggest that defects in leptin production are responsible for the hyperphagic, obese phenotype in the PWS group. In fact, the hyperphagia observed in the PWS patients was similar to that seen in humans with complete leptin deficiency or leptin receptor defects (11–13). There appears to be a complete dissociation between eating behavior and plasma leptin concentrations in PWS patients. A similar dissociation was seen in humans with other causes of hypothalamic disease, such as damage from childhood craniopharyngiomas, leading to apparent leptin resistance (50). It seems doubtful, therefore, that administration of leptin to adults with PWS would be beneficial in the treatment of hyperphagia.

Leptin receptor in the PWS group

Abnormal hypothalamic *OBRb* receptor expression or functioning may explain the hyperphagia, obesity, and hypogonadism associated with PWS (12). However, we showed that both common and long isoform *OBR* mRNA is expressed in the lymphocytes of PWS patients. This finding suggests that imprinted genes in the PWS region, such as *SNRPN*, thought to be involved in RNA splicing (51), are not essential for *OBR* alternative splicing or expression, at least in lymphocytes. These findings do not, however, exclude the possibility of absent *OBRb* expression in the hypothalamus of PWS patients, due directly to a hypothalamus-specific defect or indirectly to developmental hypothalamic abnormalities. Alternatively, the obese phenotype may result from defects in neuronal pathways downstream from the leptin receptor itself or in leptin-independent circuits. Future studies need to examine *OBRb* expression and neuroanatomic targets for leptin in postmortem samples of the hypothalamus from humans with PWS and from mouse models of PWS.

Conclusion

Using whole-body MRI, we showed that FM (relative to FFM) is greater in women with PWS than in healthy control subjects. In addition, RMR is normal in women with PWS after adjustment for their abnormal body composition. These findings suggest that a low RMR is not a primary abnormality of PWS contributing to obesity. Plasma leptin concentrations did not differ significantly between the PWS and control groups after adjustment for AT content and distribution, despite the primary influence of subcutaneous adiposity on leptin concentrations. Defects in the control of leptin secretion do not appear to be responsible for the obese phenotype in PWS. Because full-length leptin receptor mRNA is expressed in lymphocytes from PWS patients, leptin receptor deficiency does not appear to be a primary consequence of the gene defects observed in PWS. The cause of leptin resistance in PWS remains unclear.



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