

# Metabolic Profile and Body Composition in Adults with Prader-Willi Syndrome and Severe Obesity

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Previous investigations of adults with the Prader-Willi syndrome (PWS) are few and have demonstrated severe obesity with increased morbidity and mortality in cardiovascular disease. It is, thus, important to identify risk factors and, if possible, start prevention. We studied the clinical, genetic, endocrinological, and metabolic findings in 19 adult PWS patients (10 men; mean age, 25 yr). The PWS karyotype was demonstrated in 13 patients. The mean body mass index was 35.6 kg/m<sup>2</sup>, and total body fat was increased. Two thirds were biochemically hypogonadal. Fifty percent had severe GH deficiency (GHD). Four were hypertensive. One patient had heart failure and diabetes. Impaired glucose tolerance was seen in 4 patients, elevated homeostasis model assessment index in 9 patients, and modest dyslipidemia in 7. IGF-binding protein-1

correlated negatively with insulin levels. Four patients had osteoporosis, and 11 had osteopenia. There was no significant difference between the group with the PWS karyotype and the group without the karyotype in age, body mass index, waist/hip ratio, percent body fat, insulin values, homeostasis model assessment index, or lipid profile, except for lipoprotein(a), which was significantly higher in the group with the negative karyotype. IGF-I and lumbar spine bone mineral density were significantly lower in patients with genetic alteration, indicating a more severe GHD. The risk factors found in this study predicting cardiovascular disease are interpreted as secondary to GHD. These findings point to the importance of evaluating treatment of GHD in adults with PWS. (*J Clin Endocrinol Metab* 87: 3590–3597, 2002)

THE PRADER-WILLI syndrome (PWS) was first described by Prader, Willi, and Labhart in 1956 (1) and is characterized by short stature, muscular hypotonia, hypogonadism, mild mental retardation, and hyperphagia, the latter leading to obesity (1–5). The findings vary with age, and muscular hypotonia is the most prominent symptom during infancy, whereas short stature, hyperphagia, and behavioral problems become more predominant with increasing age (2–4). The syndrome is seen in all ethnic groups and is equally common in males and females. The incidence is approximately 1 in 15,000 live births, *i.e.* 6–7 children with PWS are born in Sweden/yr. The criteria developed by Holm *et al.* (3) are helpful in establishing the diagnosis. PWS is often associated with an underlying imprinting defect in a segment (q11–13) of chromosome 15 (6–11). It is supposed that the genetic alterations lead to the dysfunction of several hypothalamic centers, but a complete pathological-anatomical explanation for the clinical picture has not yet been found (2, 12).

A well established endocrinological consequence of hypothalamic dysfunction is the frequent occurrence of secondary hypogonadism (1–3, 13, 14). As obesity hampers GH secretion, it has been difficult to evaluate the GH/IGF-I axis in PWS, but the short stature, low IGF-I, and the favorable response to GH therapy in children and adolescents indicate deficient GH secretion (15–22).

Most previous studies have described the disorder in children (2, 3, 15–27), whereas few studies have focused on the

situation in adults with PWS (28–32). It has been reported that diabetes, dyslipidemia, and cardiorespiratory dysfunction often lead to disability within the first 3 decades of life and early death in PWS (31). It is therefore, important to identify risk factors for future disability in these patients to institute preventive measures if possible. Hypogonadism is a known risk factor for osteoporosis, and GH deficiency (GHD) syndrome in the adult involves both reduced bone mineral density (BMD) and increased occurrence of fractures as well as dyslipidemia, truncal obesity, and increased cardiovascular morbidity and mortality (32). In the present study we describe the clinical, endocrinological, genetic, and metabolic findings as well as body composition data in a cohort of adults with PWS.

## Subjects and Methods

### Patients

Twenty consecutive adults with PWS, referred from other clinics or via the Swedish PWS patient organization were included. Informed consent was obtained from the patients or their guardians, and the study was approved by the committee for medical ethics at Karolinska Institute.

The patients had been diagnosed during childhood, except for 1 woman, who was diagnosed at the age of 30 yr. One patient suffered from chronic lymphatic leukemia and was excluded from further investigations. Thus, 19 patients [10 men and 9 women, 17–37 (mean, 25) yr of age] were studied. The patients were clinically reevaluated, and all fulfilled the requirements for the clinical diagnosis (Table 1). All patients also underwent molecular genetic testing by proving the absence of unmethylated PW71 DNA fragment (11). The PWS karyotype was indicated as methylation positive. All patients had been urged for many years to follow a strict, 1000 kcal/d diet, and 1 woman had been subjected to an unsuccessful intestinal resection to reduce weight.

Four patients had hypertension. One was treated with  $\beta$ -receptor-blocking agents, and 3 with angiotensin-converting enzyme inhibitors. One woman, aged 37 yr, suffered from cardiac insufficiency that was

Abbreviations: BMD, Bone mineral density; BMI, body mass index; GHD, GH deficiency; HDL, high density lipoprotein; HOMA, homeostasis model assessment; IGFBP, IGF-binding protein; LDL, low density lipoprotein; Lp(a), lipoprotein(a); OGTT, oral glucose tolerance test; PWS, Prader-Willi syndrome.

**TABLE 1.** Characteristics of 19 adults with clinical PWS

| Gender<br>(females/males) | Age<br>(yr) | Height<br>(cm) | BMI<br>(kg/m <sup>2</sup> ) | Holm's<br>criteria<br>(points) <sup>a</sup> | Methylation<br>test <sup>b</sup> | IGF-I SD<br>score | IGFBP-1<br>(μg/liter) | Serum leptin<br>(μg/liter) | OGTT     |
|---------------------------|-------------|----------------|-----------------------------|---------------------------------------------|----------------------------------|-------------------|-----------------------|----------------------------|----------|
| F                         | 19          | 157            | 38.1                        | 12                                          | Positive                         | −1.8              | 2.7                   | 79.5                       | Normal   |
| F                         | 19          | 155.5          | 34.4                        | 11.5                                        | Positive                         | −1.7              | 10                    | 68.5                       | Normal   |
| F                         | 22          | 158            | 33.2                        | 11                                          | Positive                         | −0.3              | 5.6                   | 64.9                       | Normal   |
| F                         | 23          | 165            | 36.5                        | 10.5                                        | Negative                         | −1.7              | 8.8                   | 75.5                       | Normal   |
| F                         | 25          | 157.8          | 31.3                        | 8                                           | Negative                         | 0.8               | 5.4                   | 52.8                       | Normal   |
| F                         | 29          | 152.6          | 36.5                        | 11                                          | Negative                         | −1.9              | 25                    | 46.1                       | Impaired |
| F                         | 31          | 151.2          | 31.4                        | 11                                          | Positive                         | −1.9              | 11                    | 89.3                       | Normal   |
| F                         | 32          | 148            | 50.4                        | 11                                          | Positive                         | −3.5              | 25                    | 56.4                       | Normal   |
| F                         | 37          | 149            | 49.1                        | 11.5                                        | Positive                         | −4.4              | 88                    | 50.9                       | Diabetes |
| M                         | 17          | 154.5          | 36.9                        | 10                                          | Positive                         | −3.8              | 10                    | 61.7                       | Impaired |
| M                         | 19          | 163.8          | 20.4                        | 8                                           | Negative                         | 0.0               | 11                    | 2.1                        | Normal   |
| M                         | 20          | 150.9          | 21.2                        | 12.5                                        | Positive                         | −2.0              | 8                     | 17                         | Normal   |
| M                         | 22          | 156.3          | 44.4                        | 12                                          | Positive                         | −3.2              | 11                    | 86.3                       | Impaired |
| M                         | 25          | 170            | 24.2                        | 11                                          | Positive                         | −3.7              | 30                    | 9.7                        | Normal   |
| M                         | 25          | 165            | 39.3                        | 8                                           | Negative                         | 0.3               | 3                     | 20                         | Normal   |
| M                         | 27          | 159.5          | 27.5                        | 12.5                                        | Positive                         | −2.5              | 30                    | 8.3                        | Normal   |
| M                         | 30          | 169            | 57.8                        | 10                                          | Negative                         | −1.7              | 12                    | 77.4                       | Normal   |
| M                         | 32          | 152.2          | 31.9                        | 11.5                                        | Positive                         | −2.3              | 8.6                   | 28.6                       | Normal   |
| M                         | 36          | 169.7          | 32.7                        | 11.5                                        | Positive                         | −3.2              | 10                    | 13.7                       | Impaired |

<sup>a</sup> Eight points are necessary for the diagnosis from 3 yr of age to adulthood.

<sup>b</sup> Standard cytogenetic analysis.

<sup>c</sup> According to the HOMA index, insulin resistance =  $\text{insulin}/(22.5e^{-\ln \text{glucose}})$  using the estimation in a single fasting sample (38, 39).

compensated with loop diuretics. She also had type 2 diabetes and was treated with metformin and insulin. All were reported to have behavioral problems; 2 patients were treated with neuroleptics, and another 2 with selective 5-serotonin reuptake inhibitors. Four of the 19 patients were attending school, 1 received a disability pension, and 14 had sheltered employment. Eight were living in group homes, and 9 were residing in their parental home; only 2 were living alone. Three of the 19 patients were heavy cigarette smokers.

### Anthropometric methods

Physical examination included measurements of height, weight, waist, hip, and blood pressure. Body mass index (BMI) and waist/hip ratio were calculated. A BMI between 20–25 kg/m<sup>2</sup> was defined as normal, 25–30 kg/m<sup>2</sup> as overweight, and above 30 kg/m<sup>2</sup> as obesity. Waist and hip were measured in the standing position. Waist was measured as halfway between the costal edge and the crista. Hip was measured as the greatest circumference around the nates. The cut-off level for waist/hip ratio was defined as more than 1.0 for men and more than 0.8 in women, because higher ratios are associated with an increased risk for cardiovascular disease (33, 34).

### Body composition studies

Body fat was determined by dual energy x-ray absorptiometry (QDR 4500, Hologic, Inc., Waltham, MA) according to a standard procedure described previously (35). This method estimates total body fat, as well as regional fat distribution in trunk and extremities. The BMDs of total body, lumbar spine (L1–L4), femoral neck, and trochanter region were assessed by the same instrument. The BMD values in the patients were compared with data from reference material provided by the manufacturer. The BMD values were expressed as SD scores from the mean of age- and gender-matched reference material (z-scores) and SD scores from the mean of young adults (T-scores). The WHO definition of osteoporosis and osteopenia was applied; *i.e.* BMD between −1.0 and −2.5 SD scores from the mean of young adults at any measured site was defined as osteopenia. Values below −2.5 SD scores were defined as osteoporosis (36).

### Diagnostic criteria for PWS

The diagnostic criteria reported by Holm *et al.* (3) include both major and minor criteria and supportive findings. Major criteria weighted as one point each comprise eight items: neonatal and infantile hypotonia,

feeding problems in infancy with poor weight gain, excessive or rapid weight gain between 1–6 yr of age in the absence of intervention, characteristic facial features, hypogonadism, mild to modest mental retardation and learning problems, hyperphagia and food obsession, and cytogenetic findings with paternal deletion or other abnormalities of the PWS gene.

The minor criteria weighted as 0.5 point each comprise: decreased fetal movements or weak cry in infancy, behavioral problems, sleep disturbances and apnea, short stature for genetic background, small hands, narrow hands with straight ulnar borders, eye abnormalities (esotropia, myopathy), viscous saliva, speech articulation defects, and skin picking. In adulthood a total score of eight is necessary for the diagnosis, and major criteria must comprise at least five points of the total score.

### Endocrinological and metabolic investigations

Blood samples were collected in the morning in the fasting state for the measurement (in serum) of hormones [insulin, T<sub>4</sub>, T<sub>3</sub>, TSH, cortisol, FSH, LH, testosterone, estradiol, PRL, IGF-I, IGF-binding protein-1 (IGFBP-1), and leptin] and lipid profile. Evaluation of serum lipids included analysis of triglycerides, total cholesterol, high density lipoproteins (HDL) and low density lipoprotein (LDL) cholesterol, and lipoprotein(a) [Lp(a)]. A total cholesterol less than 5 mmol/liter, LDL less than 3 mmol/liter, HDL cholesterol less than 1.0 mmol/liter, triglycerides less than 2.0 mmol/liter, and Lp(a) less than 0.3 g/liter were regarded as associated with low risk for cardiovascular disease (37).

An oral glucose tolerance test (OGTT) was also carried out. The patients ingested 75 g glucose dissolved in water, and blood glucose was measured before ingestion and 120 min after ingestion. Impaired glucose tolerance was defined as a 120 min glucose level between 7.8–11.0 mmol/liter, and diabetes as a glucose level above 11.1 mmol/liter. The homeostasis model assessment (HOMA) index [ $\text{insulin}/(22.5e^{-\ln \text{glucose}})$ ] using a single fasting sample was calculated as an estimation of insulin resistance (38, 39). We used 2.77 as a threshold for insulin resistance, as suggested in the Bruneck study (40).

Spontaneous GH secretion was measured by continuous venous blood sampling at 20-min intervals using a withdrawal pump (model 3003, Swemed, Västra Frölunda, Sweden) from 2000–0800 h (*n* = 12). The maximal GH level was measured after stimulation tests with either insulin-induced hypoglycemia (*n* = 9) or arginine infusion (*n* = 18). The stimulation tests were performed according to standardized protocols (41). The insulin tolerance test required the presence of hypoglycemic symptoms and blood glucose values lower than 2.2 mmol/liters after insulin administration.

## Assays

All patients underwent genetic testing using Southern blot analysis after cleavage with methylation-sensitive restriction enzymes (*Cfo*I and *Bgl*II) (11). The PWS karyotype was defined as methylation positive.

IGF-I was determined in serum by RIA after separation of IGFs from IGFBPs by acid-ethanol extraction and cryoprecipitation and with des(1–3)IGF-I as the radioligand to minimize interference of remaining IGFBPs in the extract (42). The normal range of IGF-I was established in 448 healthy subjects, aged 20–96 yr (43). The IGF-I values were also expressed as SD scores, calculated from the regression line of values in the subjects.

IGFBP-1 was analyzed by the method described by Póvoa and co-workers (44). The sensitivity of the RIA was 3 µg/liter, and the intra- and interassay coefficients of variation were 3% and 10%, respectively. The reference range was 7–100 µg/liter. The IGFBP-1 values were also expressed as SD scores, calculated from the regression line of values in the subjects.

GH and insulin were measured by fluoroimmunoassays (Delfia hGH and Autodelfia insulin, respectively, Wallac, Inc., Turku, Finland). The reference value for fasting serum insulin was less than 144 pmol/liter. The insulin assay did not have cross-reactivity with proinsulin.

Leptin was measured with a human leptin RIA kit (Linco Research, Inc., St. Charles, MO). Mean normal leptin values were: BMI of 18–25 kg/m<sup>2</sup> for men, 3.8 ± 1.8 µg/liter; and for women, 7.4 ± 3.7 µg/liter.

Serum concentrations of T<sub>4</sub>, T<sub>3</sub>, TSH, cortisol, FSH, LH, testosterone, estradiol, and PRL were measured using routine assays.

Serum cholesterol and triglycerides were measured with colorimetric methods (Vitos 900, Johnson and Johnson, Orthoclinical Diagnostics, Rochester, NY), and HDL was determined with direct colorimetry (Hitachi 911, Hialeah, FL). The LDL concentration was calculated according to the formula suggested by Friedewald *et al.* (45). Lp(a) was analyzed with nephelometry (NIH Image, Immunochemistry System, Beckman Instrument Inc., Fullerton, CA).

## Statistics

Results are presented as the mean ± SEM if not otherwise stated. Comparisons between groups were carried out with unpaired *t* test when variables were normally distributed. Otherwise, the Mann-Whitney rank-sum test was used. Correlations were analyzed using least square linear regression analysis, and variables with nonnormal distribution were log-transformed before analysis to obtain a more closely approximated Gaussian distribution. Statistical significance was set at *P* < 0.05. Statistical analyses were performed using SigmaStat for Windows (Jandel Scientific, Erkrath, Germany).

## Results

### Anthropometry

Height ranged between 148–165 cm (mean, 155 cm) in the females, and between 151–170 cm (mean, 161 cm) in the males. The corresponding height SD scores were –3 to –0.5 (mean –1.5) and –4 to –1 (mean, –2.5). The mean BMI was 35.6 kg/m<sup>2</sup>. Only three males had normal BMI; all of the other patients had elevated BMI, and the highest value found was 57.8 kg/m<sup>2</sup> (Table 1). The waist/hip ratio varied between 0.82–0.96 (mean, 0.89) in the females and 0.81–1.01 (mean, 0.90) in the males, *i.e.* all women had a ratio greater than 0.8, whereas all but one man had a ratio less than 1.0.

### Body composition and BMD

The percent body fat was 44.8–58.5% (mean, 52.9%) in the females and 19.4–55.5% (mean, 44.3%) in the males. In the three males with normal BMI (20.4, 21.2, and 24.2 kg/m<sup>2</sup>), the percent body fat values were 19.4%, 36.1%, and 40.1%, respectively.

BMD was lower than in sex- and age-matched normal

subjects. Mean levels, expressed as the SD score of age-matched reference material (z-score), were –0.65 ± 0.18 (*P* = 0.01) in total body, –1.2 ± 0.25 (*P* < 0.001) in lumbar spine, –1.5 ± 0.21 (*P* < 0.001) in femoral neck, and –1.06 ± 0.28 (*P* < 0.001) in the trochanter region. According to WHO criteria (36), four patients had osteoporosis (T-score below –2.5 SD score), and another 11 had osteopenia (T-score between –1 and –2.5 SD score).

### Endocrinological and metabolic evaluations

On the average, serum IGF-I was low (Table 1). The values were below –2 SD score of the mean of healthy age-matched subjects in eight patients, values were between mean and –2 SD score in nine patients, and two had higher values.

The peak GH response to a provocative stimulus was below the accepted cut-off of 3 µg/liter (46) in 9 of 18 patients. In the remaining 9 patients, values ranged between 3.7–16.0 µg/liter (mean, 7.7 µg/liter). The peak spontaneous GH secretion between 2000–0800 h was less than 3 µg/liter in 10 of 12 patients and 7.38 and 7.69 µg/liter, respectively, in 2 patients.

Pubic hair development corresponded to Tanner stage 5, *i.e.* adult stage, in all patients. Hypogonadism was diagnosed in 12 patients by signs and low serum concentrations of gonadal steroids and low to normal LH and FSH concentrations. One man received oral androgen substitution. Three men had normal testosterone values, and three women had irregular menstrual periods. Serum T<sub>3</sub>, T<sub>4</sub>, TSH, PRL, and cortisol were within the respective normal range in all patients (data not shown).

The individual leptin concentrations (Table 1) were significantly related to the percent body fat (*r* = 0.785; *P* < 0.01; Fig. 1).

Insulin levels varied between 29–151 pmol/liter, with a mean of 80 ± 10 pmol/liter (Table 1).

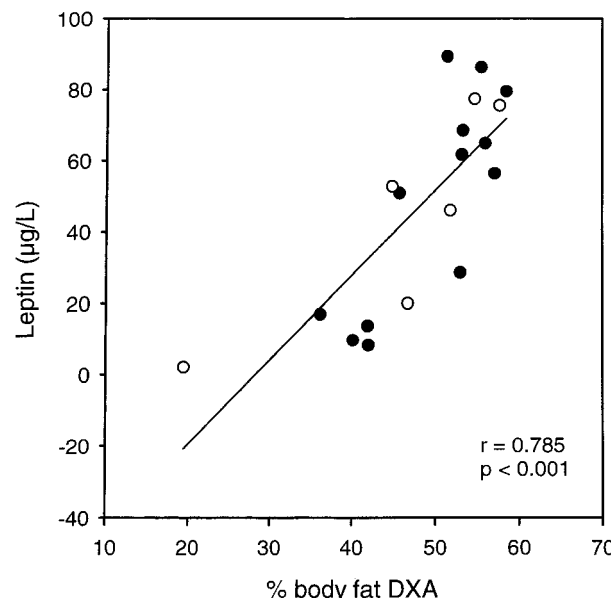


FIG. 1. Relationship between serum leptin concentrations and percent body fat estimated by dual energy x-ray absorptiometry in 19 adult patients with PWS. ●, Methylation-positive patients (PWS karyotype); ○, methylation-negative patients.

IGFBP-1 concentrations varied between 3–88  $\mu\text{g}/\text{liter}$  (mean, 16.6  $\mu\text{g}/\text{liter}$ ). Only one patient, who had diabetes, had an elevated IGFBP-I concentration. Two patients had subnormal values, the remainder had levels within the age-related normal range (Fig. 2). The correlation between IGFBP-1 and insulin was negative and significant ( $r = -0.49$ ;  $P = 0.034$ ; Fig. 3). When excluding the insulin-treated diabetic patient, who had the highest IGFBP-1, the correlation coefficient was  $r = -0.51$  ( $P = 0.032$ ).

OGTT confirmed diabetes in one patient, showed impaired glucose tolerance in four patients, and was normal in the remaining patients (Table 1). Insulin concentrations were similar in patients with normal and impaired OGTT. Using the HOMA index and the value of 2.77 as threshold for insulin resistance, five women and four men had values indicating increased insulin resistance. The patient with the highest index also had impaired OGTT. The other three patients with impaired OGTT had values less than 2.77.

Serum triglycerides were within the recommended limits ( $<2.0$  mmol/liter) in 18 of 19 patients, whereas one had a slightly elevated level (2.3 mmol/liter). Total cholesterol was less than 5 mmol/liter in 16 of 19 patients, and was slightly elevated in 3 patients (5.6, 5.7, and 6.2 mmol/liter, respectively). HDL cholesterol was less than 1.0 mmol/liter in 7 patients, and the lowest concentration found was 0.7 mmol/liter. Seven patients had LDL cholesterol above 3 mmol/liter, but the highest level found was 4.2 mmol/liter. Lp(a) concentrations were above 0.3 mmol/liter in 5 patients (2 men and 3 women). One patient with HDL of 0.9 mmol/liter, 1 patient with HDL of 0.9 mmol/liter and LDL of 3.4 mmol/liter, and 1 patient with total cholesterol of 5.7 mmol/liter as well as LDL of 3.9 mmol/liter had impaired glucose toler-

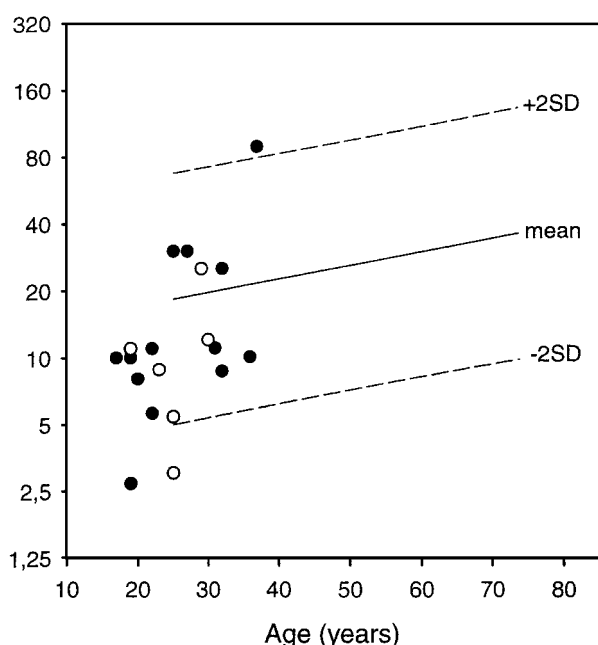


FIG. 2. Serum IGFBP-1 concentrations in 19 patients with clinical PWS in relationship to the age-related normal range. The normal range for age and the mean  $\pm$  2 SD score are indicated. ●, Methylation-positive patients (PWS karyotype); ○, methylation-negative patients.

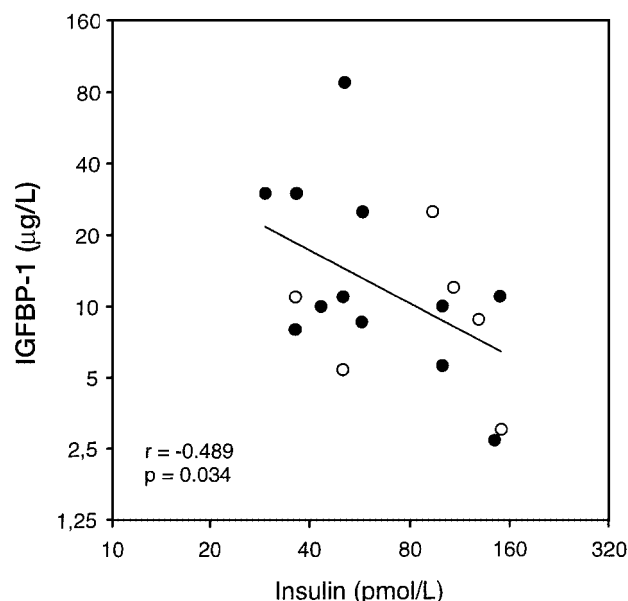


FIG. 3. Relationship between fasting insulin and IGFBP-1 concentrations in adult PWS patients. ●, Methylation-positive patients (PWS karyotype); ○, methylation-negative patients.

ance. Eight patients with a HOMA index above 2.77 had modest dyslipidemia in 1 or more of the measured lipids.

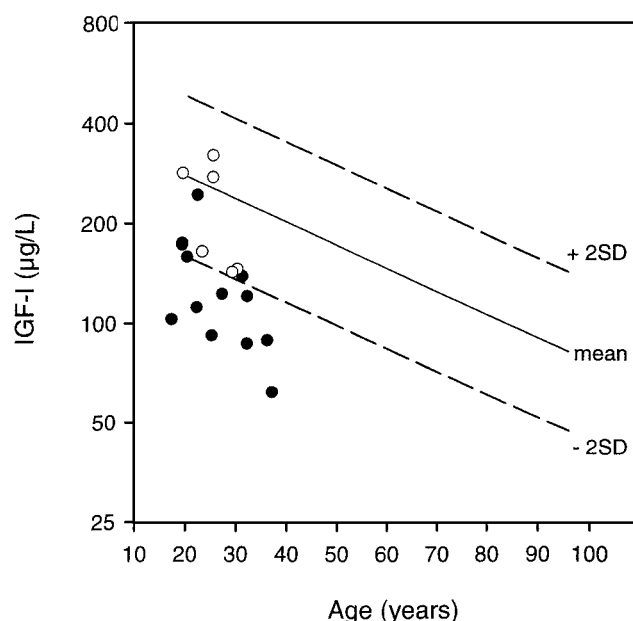
#### Comparison between PWS patients with and without chromosomal abnormalities

Although all patients fulfilled Holm's criteria for the PWS diagnosis, 13 patients had a pathological (positive) methylation test, whereas the remaining 6 patients were methylation negative. The mean score for the methylation-positive PWS patients was  $11.5 \pm 0.19$  points, and that for the methylation-negative patients was  $9.3 \pm 0.57$  points. This apparent difference is not statistically significant, taking into account that the genetic alteration adds 1 point to the total score. The scores were evenly distributed between major and minor points in both groups. We compared the patients with the PWS karyotype, *i.e.* positive methylation test (PWS), to those who had negative methylation test (PWS\*). The PWS group consisted of 13 patients (7 men and 6 women), and the PWS\* group consisted of 6 patients (3 men and 3 women; Table 2).

The groups were similar in age, BMI, waist/hip ratio, and percent body fat. Although the GH response to arginine was not significantly different between the groups, IGF-I serum concentrations were higher in the methylation-negative patients (Fig. 4), and they had a tendency to a higher height SD score. The lumbar spine BMD z values were significantly lower ( $P = 0.005$ ) in the PWS patients, but were similar at other measuring sites [total body ( $P = 0.134$ ), femoral neck ( $P = 0.102$ ), and trochanter region ( $P = 0.075$ )]. The groups had similar mean blood pressure, insulin, hemoglobin A<sub>1c</sub>, leptin, and lipid levels, except for Lp(a), which was significantly higher in the PWS\* group. The HOMA index was not significantly different between the PWS and PWS\* groups.

**TABLE 2.** Comparison between methylation-positive (PWS) and methylation-negative (PWS\*) patients (mean  $\pm$  SEM or number)

|                                       | PWS             | PWS*            | P value |
|---------------------------------------|-----------------|-----------------|---------|
| No.                                   | 13              | 6               |         |
| Age (yr)                              | 26 $\pm$ 2      | 25 $\pm$ 2      | NS      |
| Height (SD score)                     | −2.3 $\pm$ 0.2  | −1.5 $\pm$ 0.3  | 0.062   |
| Hypogonadism                          | 10              | 2               |         |
| BMI (kg/m <sup>2</sup> )              | 35 $\pm$ 2      | 37 $\pm$ 5      | NS      |
| Waist/hip ratio                       | 0.9 $\pm$ 0.02  | 0.9 $\pm$ 0.02  | NS      |
| Body fat (%)                          | 49.5 $\pm$ 2.0  | 45.9 $\pm$ 5.6  | NS      |
| IGF-I (SD score)                      | −2.6 $\pm$ 0.3  | 0.7 $\pm$ 0.5   | 0.003   |
| GH-peak ( $\mu$ g/liter) <sup>a</sup> | 3.99 $\pm$ 1    | 5.35 $\pm$ 2    | NS      |
| Insulin (pmol/liter)                  | 73 $\pm$ 11     | 95 $\pm$ 18     | NS      |
| HOMA index                            | 2.49 $\pm$ 0.46 | 3.08 $\pm$ 0.27 | NS      |
| Triglycerides (mmol/liter)            | 1.2 $\pm$ 0.4   | 1.2 $\pm$ 0.6   | NS      |
| Total cholesterol (mmol/liter)        | 4.6 $\pm$ 0.2   | 4.2 $\pm$ 0.3   | NS      |
| HDL (mmol/liter)                      | 1.1 $\pm$ 0.1   | 1.0 $\pm$ 0.2   | NS      |
| LDL (mmol/liter)                      | 2.9 $\pm$ 0.2   | 2.8 $\pm$ 0.2   | NS      |
| Lp(a) (mmol/liter)                    | 0.2 $\pm$ 0.1   | 0.6 $\pm$ 0.2   | <0.01   |

<sup>a</sup> After arginine stimulation.**FIG. 4.** Serum IGF-I levels in 19 adults with clinical PWS. Normal range for age and the mean  $\pm$  2 SD score are indicated. ●, Methylation-positive patients (PWS karyotype); ○, methylation-negative patients.

### Discussion

Our adult patients had clinically established PWS since childhood. They had short stature and were obese. Brambilla and co-workers (24) have previously shown that body composition in PWS is abnormal, with an increase in fat to lean tissue compared with both obese and normal weight individuals. In our patients the mean percent body fat of 53% and 44% in females and males, respectively, is similar to the findings in studies of children and adolescents (23, 24, 26, 27) as well as in one recent study of adult PWS women (47). In our study only three men and none of the women had a BMI less than 25 kg/m<sup>2</sup>. Two of the three men had percent body fat of 36% and 40%, *i.e.* clearly above the mean of 17.8  $\pm$  2.4 found in healthy young males with normal BMI assessed

with the same technique (48). The mean waist/hip ratio was the same in women and men, indicating a similar fat distribution in the two sexes. Unfortunately the amount of visceral fat tissue was not measured in our study. Goldstone and co-workers (47) found that visceral fat estimated with magnetic resonance imaging in adult PWS females was significantly reduced compared with that in other obese females, and this was not reflected by a difference in the waist/hip ratio. They also found that the proportion of visceral fat in PWS females was similar to that in nonobese controls. If this is true for the PWS men as well, the metabolic consequences of the increased fat mass might be less than previously thought. In fact, it was shown in a recent study of obese postmenopausal women by Brouchu *et al.* (49) that despite high levels of body fat, women with lower amounts of visceral adipose tissue had a favorable metabolic profile compared with women with higher amounts of visceral adipose tissue. This issue has also been extensively reviewed by Sims (50).

GHD may be one factor contributing to increased fat mass. The GH response to a provocative stimulus was heterogeneous. The majority of our patients had low spontaneous and low stimulated GH secretion; this is in accordance with previous reports in children and adults (15–22, 28–31). Forty percent of our patients had IGF-I levels below −2.0 SD score, whereas virtually all adults with childhood-onset severe GHD of other etiologies had IGF-I levels below the age-related normal range (44), indicating less severe GHD in our patients. However, IGF-I would be expected to be normal in obesity and low as a consequence of GH insufficiency. Our findings and the favorable response to GH therapy in children in terms of longitudinal growth and improvement of body composition (17–19, 25) emphasize the importance of evaluating GH treatment in adult PWS.

Approximately two thirds of our patients were biochemically hypogonadal. However, only one man received androgen replacement therapy, which had been commenced before he was admitted to our department. He had normal serum testosterone (26 nmol/liter) after this treatment. Sex hormone replacement in adults with PWS is not common in Sweden, because of the risk for increased aggressiveness in males and because it has been supposed that the peripheral conversion of adrenal androgens to estrogen might suffice for basic needs in women. Also, the increased risk of thromboembolic events when introducing estrogen therapy to obese women has been taken into account. It is recommended that stimulation tests for GHD, at least in peripubertal children, be performed in a sex steroid-replete milieu. As our patients were not receiving sex steroid replacement therapy, this could theoretically cause falsely low responses to the tests. It has previously been suggested that the effect of sex steroids on the GH/IGF-I axis is mediated by estrogen in both women and men (51). Our hypogonadal patients were obese and could therefore produce estrogen by peripheral conversion of androgens in adipose tissue.

The risk of diabetes is increased in PWS, and it has previously been reported that nearly 20% of adult patients with PWS have diabetes (31). It has been assumed that the reason is obesity causing insulin resistance. Studies of patients with PWS have, however, demonstrated normal or increased in-

sulin sensitivity (52, 53) and low insulin levels compared with other obese subjects (15). In our patients the HOMA index indicated increased insulin resistance in nine patients. Four patients had impaired glucose tolerance, but only one patient had overt diabetes. However, only one patient with a high HOMA index had impaired glucose tolerance. It is obvious that there is a risk for patients with PWS to develop diabetes, but the etiology is probably multifactorial, and we think that the risk is lower than previously suspected. In a study by Bonora *et al.* (40) it was found that the prevalence of insulin resistance in persons with BMI greater than 25 kg/m<sup>2</sup> was 43% in the absence of other metabolic disorders, increasing to 100% in the presence of four metabolic disorders. In our study seven of nine patients with a HOMA index indicating insulin resistance had modest dyslipidemia. These patients might eventually develop cardiovascular disease, but it can be argued that in the patients in whom we did not find any metabolic disorder, intense efforts to reduce weight would be unnecessary. The situation is complex in PWS, and because of the hyperphagia and the reduced metabolic rate (26), an already established lifestyle must be continued. Studies of larger cohorts than ours are needed to change diet recommendations.

We report for the first time serum levels of IGFBP-1 in adults with PWS. IGFBP-1 to -6 modify the actions of IGFs. Phosphorylation of IGFBP-1 increases the affinity for IGF-I and is in this form affecting the level of free IGF-I. Insulin is regarded as the principal regulator of hepatic production of IGFBP-1, and hyperinsulinemia leads to decreased levels of IGFBP-1 (54), as also documented in this study by the negative correlation. It may be hypothesized that low IGFBP-1 results in increased levels of the free fraction of IGF-I, which can suppress GH secretion. Therefore, a balance could exist between obesity and relative GHD in these patients.

Leptin levels in our study were similar to levels found in other studies of PWS patients (55, 56), and correlated to the percent body fat. This means that the relationship between leptin and adiposity was normal and does not offer an explanation to the hyperphagia.

Dyslipidemia is frequently found in GHD adults (57–59), and GHD is associated with increased plasma cholesterol levels due to an elevation of LDL cholesterol (57, 58). We found in the majority of our patients lipid profiles within recommended limits not associated with increased cardiovascular risk, and this may be due to the very strict diet our patients were prescribed. On the other hand, Lp(a) is an independent risk factor for cardiovascular disease that is not affected by diet or statins (60, 61). The level of Lp(a) is mainly influenced by hereditary factors (61), but estrogens can lower Lp(a) concentrations (60). Five of our PWS patients had increased Lp(a) levels, and three of them were women. They did not differ in estrogen levels from the rest of the women.

Four patients had hypertension, but were normotensive on adequate medication. Except for the two patients treated for edema and cardiac failure, respectively, none of the patients had cardiovascular disease.

To our knowledge, BMD data in adult PWS have not been reported previously. It has, however, been shown that BMD is reduced in adults with GHD (62). We found that BMD was low in adults with PWS compared with age- and sex-

matched controls, and the majority of the patients were classified as osteopenic or osteoporotic. This might be a result of the relative GHD as well as hypogonadism and represents a potential risk for fractures in the future. It should be remembered, however, that when assessing BMD using the dual energy x-ray absorptiometry technique, short stature may lead to an underestimation of BMD. On the other hand, obesity tends to cause an overestimate of BMD.

PWS is often associated with a loss of one or more normally active paternal genes in the proximal part of the long arm of chromosome 15 in the region q11-q13 (6–11). In this region the maternally derived genes are inactive. In approximately 70% of cases, PWS is caused by a deletion of paternal genes, in 25% by maternal disomy, and in 5% by translocation or other structural abnormalities (6–11). In recent years it has become widespread practice to request a molecular genetic confirmation of the clinical diagnosis (2). A fraction of adult PWS patients previously diagnosed by clinical signs and symptoms do not fulfil cytogenetic criteria for the diagnosis. In our study one third (6 of 19) of the patients were methylation negative, although they fulfilled the clinical criteria for the diagnosis. This is similar to the discrepancy between the established clinical diagnostic criteria and molecular genetics that has been seen in another study (28). Possibly, some of the patients may have translocation or other structural abnormalities that were not detected with the methylation test, or some patients with the PWS phenotype have associations to other hitherto unknown chromosomal abnormalities. It is noteworthy that when we carefully compared the phenotype and hormonal metabolic parameters in the methylation-positive group to those in the methylation-negative group, we did not find any significant differences between the groups, except that the methylation-negative patients had significantly higher IGF-I concentrations and tended to be taller. They also had significantly higher BMD in lumbar spine, all indicating less severe GHD. It should be remembered that the sample size and heterogeneity in the two groups limit generalizations.

In conclusion, we found that in this group of young adults with PWS, only three had a BMI less than 25 kg/m<sup>2</sup> despite a strict diet. The waist/hip ratio was increased in all women, and the mean percent body fat was high in both men and women. GH secretion was impaired, and approximately two thirds were biochemically hypogonadal. BMD was low compared with age- and gender-matched controls. Four patients were hypertensive; one patient had heart failure and diabetes. Four patients had an impaired glucose tolerance test, and the HOMA index was high in nine patients. IGFBP-1 was inversely correlated to insulin levels. A moderate dyslipidemia was seen in seven patients. Two thirds were methylation positive on genetic testing. The IGF-I sd score and lumbar spine BMD were significantly lower in the group with the mutation defect, indicating more severe GHD.

The PWS population has increased cardiovascular morbidity and mortality (31), and the only risk factors found predicting this were impaired OGTT and GHD. This emphasizes the importance of evaluating GH treatment in adults with PWS.

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