Postprandial Metabolism in Adults with Prader–Willi Syndrome

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Abstract

Objective
Individuals with Prader–Willi syndrome (PWS) are commonly restricted to 60-75% of height-appropriate calorie intake because they rapidly become obese on a normal diet. This study measured changes in energy expenditure, glucose and lipid homeostasis, and metabolic flexibility in response to a meal in PWS adults.
Methods
11 adults with PWS were compared with 12 adiposity-matched and 10 lean subjects. Indirect calorimetry was conducted at baseline and 210 min after a standardized 600 kCal breakfast to assess energy expenditure and substrate utilization. Circulating glucose, insulin, C-peptide, glucagon, nonesterified fatty acids, and triglycerides were measured up to 240 min. Insulin sensitivity and insulin secretion rate were assessed by HOMA-IR and C-peptide deconvolution, respectively. Body composition was determined by dual-energy X-ray absorptiometry.
Results
The PWS group had lower lean mass than the obesity control group. Corrected for lean mass, there were no differences between the PWS and obesity groups in resting metabolic rate or metabolic flexibility. Total and abdominal fat mass, insulin sensitivity, and insulin secretion rate were also similar between these groups.
Conclusions
This study did not detect an intrinsic metabolic defect in individuals with PWS. Rather, lower lean mass, combined with lower physical activity, may contribute to weight gain on an apparent weight-maintenance diet.

Introduction

Prader–Willi syndrome (PWS), a genomic imprinting disorder, is one of the leading known genetic causes of obesity. It is caused by a loss of expression of a critical genomic region on the paternal allele of chromosome 15q11-q13 and is characterized by hypotonia and failure to thrive in infancy followed by hyperphagia in childhood and adulthood, with an onset at the age of approximately 2-6 years. Individuals with PWS rapidly become obese after early childhood, even while apparently consuming standard-sized portions of a "normal" diet. Consequently, the prescribed PWS-specific diet consists of only 60-75% of the caloric total of a normal diet for an individual of the same height and age [**1-3**]. This discrepancy in apparent energy utilization, whereby a person with PWS apparently gains weight on a diet on which a healthy person maintains body weight, appears central to understanding PWS obesity. This would appear to indicate that there is some difference in the way people with PWS metabolize or store energy. Adults and children with PWS have been found to have a lower resting metabolic rate (RMR) and sleeping metabolic rate than body mass index (BMI) -matched controls when assessed by indirect calorimetry [**4-6**]. These effects were not present, however, with appropriate correction for lean body mass, which is lower in individuals with PWS.

Even at rest, consumption and absorption of food lead to an upregulation of energy expenditure (EE). This process [known as the thermic effect of food (TEF)] can, depending on

macronutrient composition of the meal, account for up to 10% of EE [**7, 8**]. Factors influencing TEF include meal size and texture, meal frequency and regularity, and subject's insulin resistance [**9-12**]. Although TEF represents a relatively small component of EE, the cumulative effects of altered TEF over time could have a measurable impact on energy homeostasis and metabolic health. Increased TEF has been found to be associated with diseases such as anorexia nervosa and chronic obstructive pulmonary disease [**13, 14**]. The relationship between TEF and obesity is complex and still poorly understood, but chronically reduced TEF could potentially contribute to increased adiposity.

While a number of PWS studies have assessed RMR and other metabolic parameters in the fasted state, to our knowledge none has investigated the postprandial state. As well as being the physiological state in which humans spend most waking hours, the postprandial condition involves important adaptive changes in EE and substrate oxidation. This study aimed to determine whether there were any meal-related differences between PWS individuals and controls in these metabolic parameters, or in circulating lipids and glucose-regulating hormones, which could contribute to any degree towards the propensity towards weight gain in this syndrome.

Methods

Subjects

This study was approved by both St Vincent's and Royal Prince Alfred Hospital's Human Research Ethics Committees. Written informed consent was obtained from control participants and from the parents/guardians of participants with PWS. PWS subjects from the specialized New South Wales Prader–Willi Syndrome Clinic at the Royal Prince Alfred Hospital (Camperdown, NSW, Australia) were collectively invited to participate in the study. Those that volunteered were screened and recruited. Lean and obese control subjects were recruited by public advertisement.

The PWS group consisted of 11 subjects with a typical PWS phenotype (7 males and 4 females, aged 27.9 (±2.7) years, BMI 37.0 (±2.9) kg/m2 [mean (±SEM)]). Each PWS subject had diagnosis confirmed by cytogenetic testing; 6 had interstitial deletion of the PWS critical region on the paternally inherited allele of chromosome 15 and 5 had uniparental maternal disomy of chromosome 15. All participants with PWS had a specifically customized food plan according to their age, stature, and lifestyle, devised with the help of a nutritionist at the PWS clinic at Royal Prince Alfred Hospital.

The obese control group consisted of 12 subjects [7 males and 5 females, aged 31.9 (±2.5) years, BMI 34.3 (±1.2) kg/m2] matched for age, gender and BMI with the PWS group. The lean control group consisted of 10 gender- and age-matched individuals [5 males and 5 females, aged 28.8 (±1.1) years, BMI 21.4 (±0.4) kg/m2]. Control subjects were not undergoing dietary restriction at the time of the study and had maintained stable body weight for the previous 3 months.

Three participants with PWS had type 2 diabetes mellitus (T2DM) (treated with metformin alone, metformin, and gliclazide, and metformin and Mixtard 30/70, mean HbA1c 7.3%). Information about thyroid function was available for 10 out of 11 PWS subjects. Of these 10, all were euthyroid. Five of seven PWS males were treated with low dose testosterone and one of four females received sex hormone replacement therapy. None of the participants with PWS had been treated with growth hormone (GH). Two obese control subjects had T2D (treated with metformin and gliclazide, and metformin, sitagliptin, and rosiglitazone, mean HbA1c 7.6%).

Study design

Subjects attended the Garvan Institute Clinical Research Facility following an overnight fast. On the morning of the study, weight and height were measured after voiding, using a wall-mounted stadiometer and electronic scale (TANITA, Wedderburn) in a hospital gown. BMI was calculated by dividing weight (in kg) by height (in metres) squared (kg/m2). Waist circumference was measured at the level of the midpoint between the lower end of the ribs and the anterior superior iliac spines, and hip circumference was measured as the widest circumference between the anterior superior iliac spines and the greater trochanters.

An IV cannula was inserted into the antecubital vein and fasting blood samples were obtained before subjects consumed a standardized breakfast of mixed high carbohydrate and high fat content (600 kCal, 50% carbohydrate, 35% fat, 15% protein) within 20 min.

Indirect calorimetry
Indirect calorimetry (Deltatrac Metabolic Monitor, Datex Instrumentarium, Helsinki, Finland) was performed for two 30-min periods: at baseline (−60 to −30 min) and postprandially (210-240 min). During measurement, the subject's head was covered with a transparent airtight hood with a constant airflow from the metabolic monitor. O2 and CO2 exchange rates were recorded at 1-min intervals. The first 10 min of measurement were considered an equilibrium period; the subsequent 20 min of recording were averaged. Energy expenditure (kCal/h), respiratory quotient (RQ; ratio of CO2 production to O2 consumption) and carbohydrate and fat oxidation (mg/min) were calculated.

Biochemical analysis
Blood was collected at baseline and at 30, 60, 90, 120, 180, and 240 min postprandially. Serum and plasma samples were stored at −80°C until assayed. Glucose was measured immediately in whole blood by the glucose oxidase method [YSI glucose analyser (model 2300 STAT PLUS 230V, YSI, Yellow Springs, OH]. Insulin and C-peptide were measured in serum and glucagon in plasma by RIA (Linco Research, St. Charles, MO). Triglycerides and nonesterified fatty acids (NEFAs) were measured in serum by ELISA (Roche Diagnostics, Indianapolis, IN, and Wako Chemical Industries, Osaka, Japan, respectively). Inter/intraassay coefficients of variation were between 3.5 and 8%.

HOMA-IR and HOMA-β were used to estimate insulin resistance and beta-cell function, respectively, as described previously [**15**]. Insulin secretion rate was also calculated by C-peptide deconvolution according to the method described in Ref. [**16**]

Body composition
Whole body fat mass (in kg and as a percentage of body weight), abdominal fat mass (kg and as a percentage of abdominal soft tissue) and whole body lean mass (kg and % body weight) were determined by DXA as previously described [**17**].

Statistical analysis
Results are expressed as mean ± standard error (SE), unless otherwise stated. Analyses were performed using SPSS version 18 (SPSS, Chicago, IL), and graphs were generated using GraphPad Prism 5.0 (San Diego, CA). $P < 0.05$ was considered statistically significant.
Differences in subject characteristics between the PWS group and obese and lean controls were assessed using Dunnett's test.

EE was expressed both in absolute terms (kCal/h) and as adjusted means at a common total lean mass and total fat mass (46.80 and 33.52 kg, respectively), generated by ANCOVA with Bonferroni post hoc tests for comparison between the three groups.
Insulin secretion was calculated by C-peptide deconvolution according to the method described in Ref. [**16**].

Two-way repeated measures ANOVA was used to assess differences in baseline and meal responses between groups in glucose oxidation, fat oxidation, and RQ, and in time course data of glucose, insulin, C-peptide, glucagon, NEFAs, and triglycerides, with repeats in time (baseline, average postprandial), using the Bonferroni post hoc test for comparisons between groups. Differences in area under the curve (AUC) for the meal responses of glucose, insulin, C-peptide, glucagon, NEFAs and triglycerides were analyzed by one-way ANOVA.

Results

Body composition
Anthropometric characteristics are summarized in Table 1. PWS and obese groups had similar total and central fat mass (kg), while these parameters were lower in the lean group. PWS and lean groups had similar lean mass (kg). Lean mass was higher in the obese group.

Blood glucose and glucose oxidation
There was no group effect in blood glucose at baseline, and levels increased postprandially in all groups, with peak levels at 45-60 min (Figure 1A). There were no differences in glucose AUC between groups.

There were no differences between groups in fasting carbohydrate oxidation as assessed by indirect calorimetry (Figure 1B). There was an increase in postprandial carbohydrate

oxidation in PWS and obese groups ($P = 0.001$ and $P = 0.05$, respectively) and a similar nonsignificant trend in the lean group ($P = 0.2$).

TABLE 1 Anthropometric characteristics

	PWS			Obese			Lean		
	Mean	95% CI	Range	Mean	95% CI	Range	Mean	95% CI	Range
n (M/F)	11 (7/4)			12 (7/5)			10 (5/5)		
Age (years)	27.50	21.43, 33.57	17-39	32.25	26.88, 37.62	21-3	28.80	26.24, 31.36	24-37
Weight (kg)[a,c]	88.86	74.33, 103.4	56.9-134	95.87	90.97, 100.80	91.2-108.8	60.87	55.83, 65.91	52.95-74
Height (cm)[a,b]	154.70	146.70, 162.80	136-172	167.80	163.20, 172,40	153-176	168.70	162.10, 175.30	152-184
BMI (kg/m^2)[a,c]	37.35	31.42, 43.29	23.3-51.2	34.21	31.58, 36.84	30.1- 44.9	21.40	20.40, 22.40	18.5-23.5
Waist/hip ratio[a,c]	0.93	0.88, 0.98	0.85-1.06	0.90	0.85, 0.95	0.81-1.03	0.79	0.74, 0.84	0.68-0.91
Whole body fat mass (kg)[a,c]	43.26	32.56, 53.96	21.80-72.00	40.33	32.88, 47.79	27.29-62.35	14.63	10.77, 18.5	7.08-23.57
Whole body fat mass (%)[a,c]	47.68	42.10, 53.26	31.83-56.39	41.77	35.32, 48.22	29.60-59.32	24.32	17.69, 30.95	10.86-38.45
Abdominal fat mass (kg)[a,c]	3.06	2.16, 3.96	0.43-5.12	3.37	2.79, 3.96	2.28-4.83	1.03	0.82, 1.24	0.62-1.63
Abdominal fat mass (%)[a,c]	46.53	41.59, 51.46	29.8-56.0	46.25	41.46, 51.04	36.0-57.0	24.87	19.73, 29.83	12.2-37.6
Whole body lean mass (kg)[b,c]	43.21	37.48, 48.95	28.87-59.47	52.67	46.97, 58.36	40.41-66.14	43.70	37.51, 49.89	34.83-59.02
Whole body lean mass (%)[a,c]	49.54	44.12, 54.96	40.74-65.09	55.22	48.90, 61.54	38.47-66.68	71.54	65.00, 78.07	57.89-85.36
HOMA-IR[a,c]	3.36	2.09, 4.64	1.6-8.3	3.36	2.50, 4.22	1.5-6.0	1.70	1.45, 1.95	1.1-2.2
HOMA-B[a,c]	61.05	44,43, 77,66	32.0-106.1	60.18	46.59, 73.76	35.5-96.9	35.48	28.49, 42.47	26.6-47.9

Data expressed as means with 95% confidence intervals (CI). HOMA, homeostasis model assessment for insulin resistance (−IR) and beta-cell function (−B).
[a]PWS vs. lean, $P < 0.05$.
[b]PWS vs. obese, $P < 0.05$.
[c]Obese vs. lean, $P < 0.05$ (Dunnett's test).

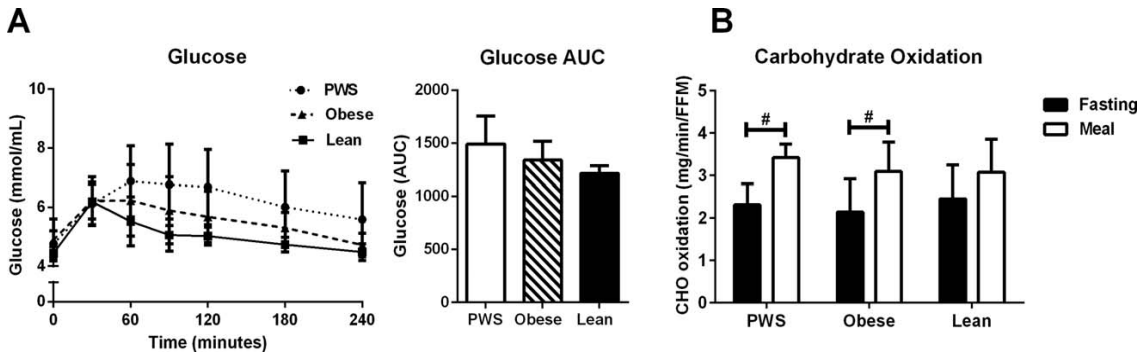

Figure 1 Fasting levels and meal responses of (**A**) blood glucose and (**B**) carbohydrate oxidation in PWS, obese, and lean. Data expressed as mean ± SEM. #Fasting vs. meal, $P < 0.05$.

Glucose-regulating hormones

Baseline insulin levels were not different between groups; they became elevated after the meal with a peak at 30-45 min (Figure 2A). There was no difference in AUC between groups. Similarly, C-peptide was not different between groups but levels rose postprandially, with peaks between 45 and 120 min (Figure 2B). Insulin secretion, as assessed by C-peptide/insulin deconvolution, increased postprandially (Figure 2C). The AUC for insulin secretion was significantly greater in PWS vs. obese ($P < 0.01$) and in obese vs. lean ($P < 0.05$). Glucagon levels were higher in PWS vs. lean throughout the study ($P = 0.03$), however there were no meal effects on glucagon (Figure 2D).

Lipid metabolism

There were no group effects at baseline in circulating NEFA levels, which exhibited a similar postprandial decrease in all three groups (Figure 3A).

PWS, obese, and lean groups had similar levels of fasting fat oxidation as assessed by indirect calorimetry. There was a nonsignificant trend in all three groups for fat oxidation to

decrease after the meal (Figure 3B).

There were no group differences in triglyceride levels, and levels of circulating triglycerides did not change after the meal (Figure 3C). The obese group had a higher triglyceride AUC compared to the lean group (0.04).

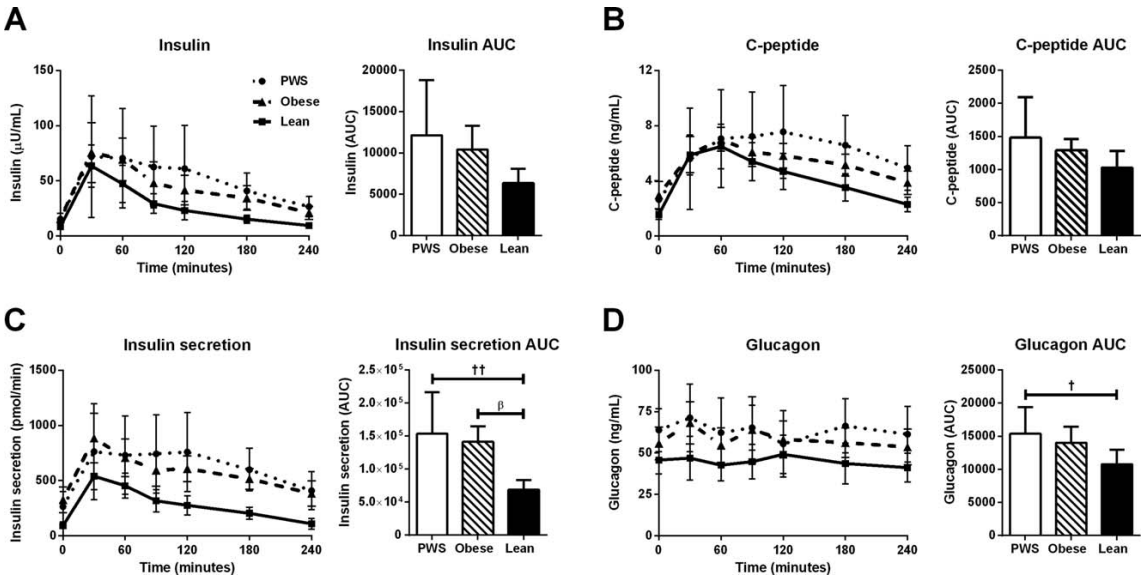

Figure 2 Postprandial circulating levels of (**A**) insulin, (**B**) C-peptide, (**C**) insulin secretion, and (**D**) glucagon in PWS, obese, and lean [left panel: time course response; right panel: area under the curve (AUC)]. Data expressed as mean ± SEM. †PWS vs. lean, $P < 0.05$; ††PWS vs. lean, $P < 0.01$; β obese vs. lean, $P < 0.05$.

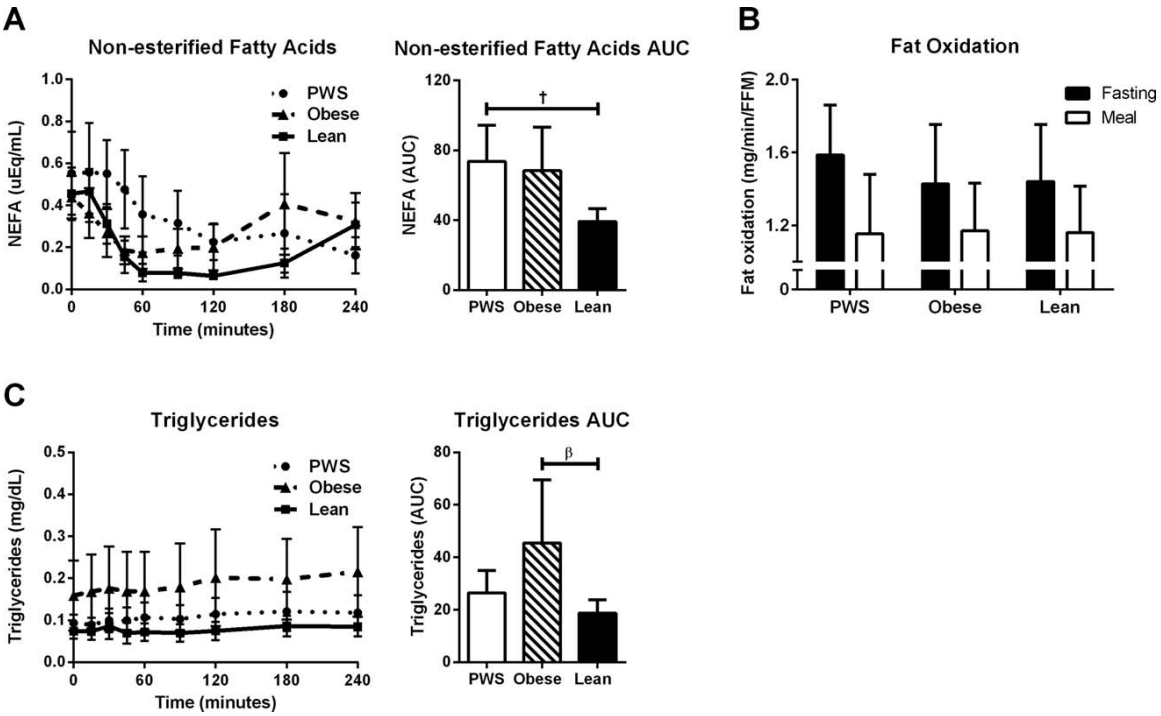

Figure 3 Fasting levels and meal responses of (**A**) nonesterified fatty acids, (**B**) fat oxidation, and (**C**) postprandial circulating levels of triglycerides in PWS, obese, and lean [left panel: time course response; right panel: area under the curve (AUC)]. Data expressed as mean ± SEM. †PWS vs. lean, $P < 0.05$; β obese vs. lean, $P < 0.05$.

Energy expenditure and substrate utilization

There were no group differences in fasting absolute EE (Figure 4A). Meal absolute EE was higher in the obese group compared to the lean group ($P = 0.04$). When adjusted for both total lean and total fat mass, there was no difference in fasting EE between PWS and obese groups or between PWS and lean groups; adjusted fasting EE was higher in the lean group than in the obese group (Figure 4B). There was no difference between groups in adjusted postprandial EE. Compared to baseline, neither absolute nor adjusted EE increased from fasting levels at the postprandial time point in any of the three groups to a statistically significant extent (Figure 4A,B).

RQ was not different between groups at baseline, with mean group values between 0.816 and 0.826. Postprandial RQ was elevated compared to baseline similarly in all groups (with mean group values between 0.858 and 0.870), indicating that all groups increased carbohydrate metabolism over fat as a metabolic substrate after the meal (Figure 4C).

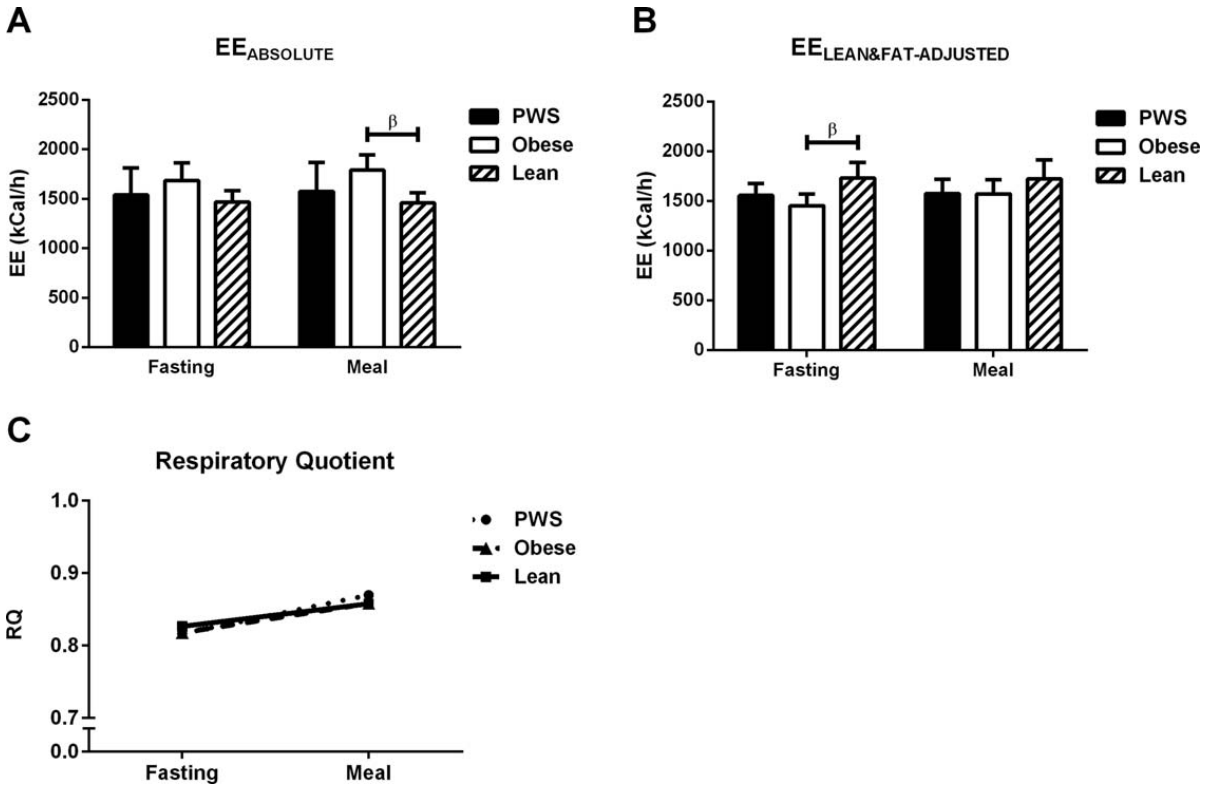

Figure 4 Fasting levels and meal responses of (**A**) absolute energy expenditure, (**B**) lean- and fat-adjusted energy expenditure, and (**C**) respiratory quotient in PWS, obese, and lean. Data expressed as mean ± SEM. β obese vs. lean, $P < 0.05$.

Discussion

The development of lifelong obesity in people with PWS is no longer a foregone conclusion. Indeed, some children and adults with PWS now manage to maintain a healthy range BMI through strictly-enforced dietary restraint and regular exercise. But in most individuals, apparent adherence to these measures is not sufficient to prevent obesity, raising the question of whether additional factors besides food intake and exercise could influence fat accumulation in PWS.

All the individuals with PWS who participated in the current study had closely supervised food plans, with some living at home and some in PWS-specific group housing. Despite this, however, they were, on the whole, obese. All except one had a BMI of over 28, with a group average BMI of 37.

We measured EE at baseline and postprandially. Baseline EE was used to represent RMR, and the difference between postprandial and fasting EE was attributed to TEF. Interestingly,

unlike previous studies, RMR was not reduced in PWS compared to obese in absolute values, highlighting the variability often observed between separate PWS cohorts. To adjust for variability between individuals due to body composition, RMR was analysed corrected for both total lean mass and total fat mass, whereby there were no intrinsic differences between PWS and obese or between PWS and lean. These findings do not support a role for reduced RMR in the development of obesity in adults with PWS.

The timing of postprandial changes in energy expenditure depends to a great extent on meal composition, particularly fat content [**18**]. We did not detect a statistically significant TEF in any of the three groups. This does not necessarily indicate, however, that no such changes occur. Previous studies in our lab have been able to detect a postprandial rise in EE in lean and obese subjects when measured between 210 and 240 min after meal ingestion [**19**]. The study design and meal composition were similar to the present study, and the same equipment and software were used. However the meal size in the present study was considerably smaller (600 kCal compared to 1000 kCal, specifically chosen to make the test meal representative for calorie-restricted PWS subjects). It is possible that the meal was insufficiently large for a measurable TEF response; alternatively, the peak in TEF may have occurred outside the window of indirect calorimetry measurement. Therefore, no final conclusion can be drawn on a possible difference in TEF between PWS, obese, and lean.

No subjects in our adult PWS group had been treated with GH replacement. Long-term GH treatment during childhood has been shown to have beneficial effects on many aspects of PWS, including lean body mass, physical activity and maintenance of a healthy body weight [**20-25**]. Similar effects have been observed in adults with PWS treated with GH, with studies showing decreased adiposity, increased lean mass and improved muscle strength [**26, 27**]. As low lean mass may contribute to reduced RMR in PWS, GH-driven increases in lean mass are likely to improve RMR, thus attenuating a positive energy balance.

The ratio of exhaled CO2 to inhaled O2 (RQ) gives information about substrate oxidation, with a physiological range of 0.7-1.0 (with 0.7 indicating 100% fat utilization and 1.0 indicating 100% carbohydrate utilization). RQ alters with the availability of dietary and stored macronutrients, rising with a high carbohydrate meal, and falling during fasting or after a high fat meal. The efficiency of the switch between low and high RQ and vice versa in response to nutritional state has been called 'metabolic flexibility' [**28**].

Interestingly, we found no differences in metabolic flexibility (represented by change in RQ) between groups, although there was large inter-individual variability. Neither the PWS group nor the obese group showed a defect in the capacity to increase RQ after a mixed high carbohydrate high fat meal that may have been predicted by their BMI. Our previous study, while finding no difference in RQ after a high carbohydrate meal between healthy subjects and those with a family history of T2DM (a group at high risk of developing obesity), identified a defect in metabolic flexibility in relatives of people with T2DM after a high fat meal [**19**]. Therefore, it could still be of interest to study the effect of a high fat meal on RQ in PWS.

It has been widely reported that people with PWS are more insulin sensitive than their degree of obesity would predict based on several reports of low fasting insulin and normal glucose levels in children with PWS [**23, 29-31**] and a study finding low insulin levels during an oral glucose tolerance test [**32**]. However, increased insulin sensitivity has yet to be convincingly demonstrated in adults with PWS, with one recent study showing similar frequency of metabolic syndrome between obese PWS and BMI-matched controls [**33**].

In this study, we found no differences between PWS and obese controls in insulin sensitivity, determined by HOMA-IR estimate. Further, there were no differences in circulating glucose, insulin and glucagon levels or in insulin secretion (assessed by C-peptide deconvolution). These results do not support the premise of increased insulin sensitivity in PWS. It remains, however, to assess insulin sensitivity by hyperinsulinaemic–euglycaemic clamp, the gold standard, but more invasive, method.

Reduced or normal triglyceride levels have been found in adults with PWS [**34-36**], but triglyceride meal responses have not been previously assessed. Interestingly, in this study we found that fasting plasma triglyceride levels showed a trend towards being lower in PWS than in obese individuals, which persisted postprandially. Indeed, PWS triglyceride levels were similar to the lean control group. This observation suggests that triglyceride levels are not a factor in the high cardiovascular morbidity observed in PWS.

In conclusion, this study found that most metabolic parameters measured, notably energy expenditure and metabolic flexibility, were not different between PWS and obese

controls. We found no support for the increased insulin sensitivity in PWS reported in some studies, possibly due to our using more stringent matching criteria for adiposity. Our data did not find evidence for a metabolic defect in PWS *per se*. Nevertheless, the reduced lean mass could be improved by currently recommended GH and sex steroid therapy combined with increased physical activity which could potentially lead to enhanced energy output.

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