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In adults with Prader–Willi syndrome, elevated ghrelin levels are more consistent with hyperphagia than high PYY and GLP-1 levels

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ABSTRACT

Objective: Prader–Willi syndrome (PWS) is a leading genetic cause of obesity, characterized by hyperphagia, endocrine and developmental disorders. It is suggested that the intense hyperphagia could stem, in part, from impaired gut hormone signaling. Previous studies produced conflicting results, being confounded by differences in body composition between PWS and control subjects.
Design: Fasting and postprandial gut hormone responses were investigated in a cross-sectional cohort study including 10 adult PWS, 12 obese subjects matched for percentage body fat and central abdominal fat, and 10 healthy normal weight subjects.
Methods: PYY[total], PYY[3–36], GLP-1[active] and ghrelin[total] were measured by ELISA or radioimmunoassay. Body composition was assessed by dual energy X-ray absorptiometry. Visual analog scales were used to assess hunger and satiety.
Results: In contrast to lean subjects ($p < 0.05$), PWS and obese subjects were similarly insulin resistant and had similar insulin levels. Ghrelin[total] levels were significantly higher in PWS compared to obese subjects before and during the meal ($p < 0.05$). PYY[3–36] meal responses were higher in PWS than in lean subjects ($p = 0.01$), but not significantly different to obese ($p = 0.08$), with an additional non-significant trend in PYY[total] levels. There were no significant differences in self-reported satiety between groups, however PWS subjects reported more hunger throughout ($p = 0.003$), and exhibited a markedly reduced meal-induced suppression of hunger ($p = 0.01$) compared to lean or obese subjects.
Conclusions: Compared to adiposity-matched control subjects, hyperphagia in PWS is not related to a lower postprandial GLP-1 or PYY response. Elevated ghrelin levels in PWS are consistent with increased hunger and are unrelated to insulin levels.

1. Introduction

Prader–Willi syndrome (PWS), with an incidence of approximately 1 in 15,000, is a leading genetic cause of obesity, caused by a loss of expression of paternally imprinted genes on chromosome 15q11–q13 (Goldstone, 2004). After initial severe hypotonia and failure to thrive in infancy, children with PWS develop hyperphagia with weight gain, with onset at 2–6 years (Butler, 1990). The syndrome is also characterized by developmental defects and obesity-related complications (Cassidy, 1997). However, it is the hyperphagia that causes one of the main impediments to independent living in adults with PWS. From childhood, constant vigilance, behavioral restraints and environmental modifications are necessary to prevent morbid obesity. The underlying mechanisms behind defective appetite regulation in PWS are poorly understood, and as yet there is no specific treatment for hyperphagia in PWS.

Many gut-derived peptides have been shown to play a physiological role in the control of satiety and feeding (Karra and Batterham, 2010). Several recent studies have investigated the modulation of individual hormones in PWS, but disagreement

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exists as to which, if any, secretory hormone dysregulation contributes significantly to the hyperphagia of PWS.

Peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are both anorexigenic hormones released from the L-cells of the intestine in response to nutrient intake. PYY is a 36 amino acid peptide with high structural homology to neuropeptide Y and pancreatic polypeptide. Its major circulating form, PYY[3–36], exerts its anorexigenic actions through the centrally located Y2 receptor (Vincent and le Roux, 2008). Circulating PYY levels are suppressed in fasting and rise for several hours after a meal (Adrian et al., 1985; Yang, 2002). Infusion of PYY has been shown to decrease appetite and food intake in humans, leading to its being proposed as a potential obesity treatment (Degen et al., 2005; Chelikani et al., 2007).

GLP-1 has several physiological functions: GLP-1 increases insulin secretion and suppresses glucagon levels in a glucose-dependent manner, decreasing food intake, slowing gastric emptying and increasing satiety (Baggio and Drucker, 2004). Its active form (GLP-1[7–36]) is rapidly degraded by the enzyme dipeptidyl peptidase-IV (DPP-IV) within approximately 2 min after secretion (Drucker, 2002).

Both PYY and GLP-1, acting peripherally as well as in the hypothalamus, play a critical role in satiety and energy homeostasis. However, their role in PWS, if any, is yet to be established (Karra and Batterham, 2010). One study of adult males and females with PWS showed no difference in fasting GLP-1 levels compared to obese controls (Goldstone et al., 2004). However, as GLP-1 is secreted in response to nutrient intake, differences may exist postprandially. A small number of studies have investigated postprandial PYY in PWS with conflicting results and there is no consensus as to whether postprandial PYY levels differ to those of obese controls (Butler et al., 2004; Goldstone et al., 2005; Gimenez-Palop et al., 2007; Paik et al., 2007a; Bizzarri et al., 2010). However, results were limited due to small subject numbers and inadequate subject matching.

Some previous research in PWS has focused on ghrelin, an orexigenic peptide produced in the stomach and pancreas. Ghrelin levels are regulated by nutritional state; high in fasting, it is suppressed for several hours postprandially. It has been shown to acutely stimulate food intake and growth hormone release (Cummings, 2006) and its chronic administration causes obesity in rodents (Wren et al., 2001). Although ghrelin levels are usually negatively associated with BMI, in PWS subjects ghrelin is unrelated to BMI (Haqq et al., 2003). This and several other studies have consistently demonstrated markedly elevated fasting ghrelin in PWS children and adults, and it has been proposed that ghrelin hypersecretion may be implicated in hyperphagia and delayed meal termination in PWS (DelParigi et al., 2002; Goldstone et al., 2004; Tauber et al., 2004).

Adipose tissue distribution is a major predictor of metabolic state. A high ratio of visceral to subcutaneous fat predicts complications such as impaired glucose metabolism and hypertension (Matsuzawa, 2008). Despite the morbid obesity often associated with PWS, subjects have been reported to have a lower visceral adiposity compared to weight-matched controls, which is usually associated with greater insulin sensitivity (Goldstone et al., 2001, 2004). Differences in fat distribution can influence hormone levels, so inadequate adiposity matching in past studies might have hampered understanding of the role of appetite regulating hormones in PWS.

A clear understanding of the mechanisms behind abnormal appetite regulation in PWS is necessary for the development and implementation of therapeutic strategies. While no clear understanding exists and reports are contradictory, this study is the first to assess the postprandial PYY, GLP-1 and ghrelin profiles in PWS independently of adiposity and fat distribution, and assess their contribution to expressed hunger and satiety during a solid standard meal.

2. Subjects and methods

2.1. Subjects

This study was approved by the St. Vincent's and Royal Prince Alfred Hospital's Human Research Ethics Committee. Written informed consent was obtained from participants' parents or guardians. PWS subjects were recruited from the specialized New South Wales Prader–Willi Syndrome Clinic at the Royal Prince Alfred Hospital, Camperdown NSW, Australia. Lean and obese control subjects were recruited by public advertisement. The PWS group consisted of 10 subjects with a typical PWS phenotype (6 males and 4 females, aged 27.9 (2.7) years, BMI 37.0 (2.9) kg/m^2 (mean (SEM)). Each PWS subject had diagnosis confirmed by cytogenetic testing (5 deletions, 5 uniparental disomy). Hyperphagia was assessed by the 13-point hyperphagia questionnaire by Dykens et al. (2007), and mean behavior, drive and severity scores were as reported in the corresponding age range in PWS. 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/m^2) matched for age, gender and BMI with the PWS group. The lean control group consisted of 10 gender- and age-matched subjects (5 males and 5 females, aged 28.8 (1.1) years, BMI 21.4 (0.4) kg/m^2). Because of the heterogeneity of the PWS cohort, matching was conducted by recruiting control groups with similar gender, age and BMI, rather than by individual case matching. Three PWS subjects had type 2 diabetes (T2D) (treated with metformin alone, metformin and gliclazide, and metformin and Mixtard 30/70, mean HbA1c 7.3%). Similarly, two obese control subjects had T2D (treated with metformin and gliclazide, and metformin, sitagliptin and rosiglitazone, mean HbA1c 7.6%). Three PWS subjects and 1 obese control subject had diagnosed obstructive sleep apnoea syndrome. Three PWS and 2 obese control subjects were treated with statins, and 1 PWS and 1 obese control subject were treated with aspirin 300 mg/day. Four PWS subjects took psychotropic medication, including anticonvulsants and antidepressants, whereas 2 obese control subjects took antidepressants. Five of 6 male PWS subjects were treated with low dose testosterone, whereas 1 of 4 PWS females took hormone replacement therapy. No PWS subjects had received growth hormone. All lean control subjects were healthy non-smokers taking no medication. Subjects were excluded from this study if they had acute infection, were taking appetite reducing agents or had recently undergone rapid weight loss.

2.2. Study design

All subjects fasted for 10 h before presenting at the Garvan Institute Clinical Research Facility at 0800 h. All PWS subjects were supervised by parents or carers to ensure that the fasting instructions were followed. Weight and height after voiding were measured using a wall-mounted stadiometer and electronic scale (TANITA, Wedderburn) in a hospital gown. Body mass index (BMI) was calculated by dividing weight (in kg) by height (in meters) squared (kg/m^2). Waist circumference was measured as the narrowest distance between the lower end of the ribs and the anterior superior iliac spines, and hip circumference as the widest circumference between the anterior superior iliac spines and the greater trochanters. Brachial blood pressure was measured by an Automatic Oscillometric Digital Blood Pressure Monitor (OMRON HEM-705CP, OMRON Corp., Tokyo, Japan). Two measurements were taken under standardized conditions, the subject sitting at approximately 45° in bed. 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. The study meal was specifically designed to be similar to a usual breakfast for PWS subjects, all subjects finishing eating in <20 min. Additional blood samples were collected 15, 30, 45, 60, 90, 120, 180 and 240 min after meal commencement for measurement of glucose and hormones as described below. Serum and plasma samples were stored at −80 °C until assayed.

2.3. Body composition

Whole body dual energy X-ray absorptiometry (DXA) (Lunar DPX GE-Lunar, Lunar Corp., Madison, WI) was used to analyze body composition. As previously described (Carey et al., 1996), a central abdominal window was outlined manually extending from the upper border of L2 to the lower border of L4 and laterally to the outer margin of the rib cage. The fat in this window was measured and expressed as mass (central abdominal fat in kilograms) and as percentage of the total soft tissue content in this area. Although this window contains both intra-abdominal fat and subcutaneous abdominal fat, it excludes 30% of the latter, has a relatively high intra-abdominal and low subcutaneous abdominal fat content, and has been shown to be strongly inversely related to insulin sensitivity assessed by the euglycemic-hyperinsulinemic clamp. Our group has previously demonstrated a CV of less than 6% for central abdominal fat, based on data from 10 female subjects scanned on four separate occasions (Carey et al., 1996). Central abdominal fat mass measured by DXA correlates highly with visceral fat mass measured by other methods such as MRI and CT, and has been validated previously (Park et al., 2002; Stewart et al., 2003).

2.4. Assessment of hunger and satiety

To achieve most reliable ratings of hunger and satiety before and after the test meal, we adapted visual analog scales (VAS) specifically for the capabilities of PWS patients. It is difficult to know if PWS adults really are able to distinguish between hunger and satiety, therefore, we chose two questions from the commonly used questionnaire by Hill and Blundell (1982), "how much could you eat now?" to assess hunger and "how full do you feel" to assess satiety. Pre-tests at the PWS clinics showed that PWS subjects (n = 10) had difficulties rating their feelings on a merely numeric scale, and their answers were often influenced by the ample explanations by investigators and carers. This was avoided by enhancing the appetite VAS with simple pictures of increasing amounts of the meal above the numeric scale. Satiety was assessed according to Shapira et al. (2004), where the VAS was enhanced with cartoons of increasingly protruding bellies above the numeric scale (Supplementary Fig. 1).

2.5. Biochemical measures

Glucose was measured immediately in whole blood by the glucose oxidase method YSI glucose analyzer (model 2300 STAT PLUS 230V, YSI, Inc., Yellow Springs, OH, USA). Serum insulin and plasma PYY[total], PYY[3–36] and ghrelin(total) were measured by radioimmunoassay with commercially available kits (LINCO Research, Inc., St. Charles, USA). The sensitivity of the insulin assay was 2 μU/mL. Inter- and intra-assay coefficients of variation (CVs) were <5%. The sensitivity of the PYY[total] assay was 10 pg/mL, specificity was 100% for human PYY[1–36], PYY[3–36], [Pro34]PYY and [Leu31, Pro34]PYY and <0.1% for human pancreatic polypeptide and neuropeptide Y. Inter- and intra-assay CVs were <3.5%. The sensitivity of the PYY[3–36] assay was 20 pg/mL, specificity was 100% for PYY[3–36] and not detectable up to 1000 pg/mL for PYY[1–36]. Inter- and intra-assay CVs were <8.5%. The sensitivity of the ghrelin[total] assay was 93 pg/mL, specificity was 100% for human ghrelin, ghrelin 14–28 and des-octanoylghrelin. Inter- and intra-assay CVs were <4%.

GLP-1[active] was measured in plasma by enzyme-linked immunosorbent assay (LINCO Research, Inc., St. Charles, USA). For PYY and GLP-1[active] measurements, 500 KIU/mL Aprotinin (Sigma, Sydney, Australia) and 10 μL/mL DPP-IV inhibitor (LINCO Research, Inc., St. Charles, USA) were added to EDTA blood collection tubes before blood samples were taken to prevent proteolysis. The sensitivity of the GLP-1 assay was 2 pM, specificity was 100% for GLP-1[7–36 amide] and GLP-1[7–37] and not detectable for GLP-1[9–36 amide], GLP-2 and glucagon. Inter- and intra-assay coefficients of variation (CVs) were <8%.

HOMA-IR and HOMA-β were used to estimate insulin resistance and beta-cell function, respectively, as described previously (Matthews et al., 1985).

2.6. Statistical analysis

Results are expressed as mean (SEM). Meal responses were calculated as average postprandial values obtained from areas under the curves (AUC, 0–240 min) using the trapezoidal rule, divided by time. Analyses were performed using JMP 8.0 (SAS Institute Inc.). All hormone data were Ln-transformed for analysis. Differences in subject characteristics between the PWS group and obese and lean controls were assessed using Dunnett's test. Two-way repeated measures ANOVA was used to assess differences in baseline and meal responses between groups, with repeats in time (baseline, average postprandial) and planned contrasts between PWS and obese controls and PWS and lean controls; residuals from all models were normally distributed ($p > 0.05$, Shapiro–Wilk W test). $P < 0.05$ was considered significant.

3. Results

3.1. Clinical parameters

Characteristics of all subjects are summarized in Table 1. Groups were matched for gender and age. PWS and obese subjects were matched for BMI with similar total body and abdominal fat. HOMA-IR and HOMA-B, estimates of insulin resistance and beta-cell function respectively, were similarly elevated in PWS and obese subjects compared to lean controls. Height and lean body mass, intrinsic features of the syndrome, were significantly lower in PWS

Table 1
Anthropometric measures of PWS, obese and lean subjects.

Variables	PWS	Obese	Lean
n (m/f)	10 (6/4)	12 (7/5)	10 (5/5)
Age (years)	27.9 (2.7)	31.9 (2.5)	28.8 (1.1)
Height (cm)	154.9 (4.0)	167.8 (2.1)*	168.7 (2.9)*
Weight (kg)	88.0 (7.1)	95.9 (2.2)	60.9 (2.2)*
BMI (kg/m^2)	37.0 (2.9)	34.3 (1.2)	21.4 (0.4)*
Waist (cm)	112.1 (5.8)	106.3 (2.4)	72.7 (1.3)*
WHR	0.93 (0.02)	0.90 (0.02)	0.79 (0.02)*
Whole body fat mass (kg)	42.6 (5.3)	40.3 (3.4)	14.6 (1.7)*
Whole body fat mass (%)	49.0 (2.5)	41.8 (2.9)	24.3 (2.9)*
Whole body lean mass (kg)	47.3 (2.7)	52.7 (2.6)*	43.7 (2.7)
Whole body lean mass (%)	49.9 (2.7)	55.2 (2.9)	71.5 (2.9)*
Abdominal fat mass (kg)	3.0 (0.4)	3.4 (0.3)	1.0 (0.1)*
Abdominal fat mass (%)	46.3 (2.4)	46.3 (2.2)	24.8 (2.2)*
HOMA-IR	3.4 (0.6)	3.4 (0.4)	1.7 (0.1)*
HOMA-B	60.8 (8.2)	60.2 (6.2)	35.5 (3.1)*

Data expressed as mean (SEM).
HOMA, homeostasis model assessment for insulin resistance (-IR) or beta-cell function (-B); WHR, waist-to-hip ratio.
* Significantly different to PWS group (p<0.05, Dunnett's test).

than obese subjects. PWS lean mass was similar to lean control subjects.

3.2. Hunger and satiety

Feelings of hunger and satiety were regularly assessed using a visual analog scale in the fasting state and during the meal. While suppression of hunger after the meal was similar in obese and lean subjects, it was absent in PWS (Table 2, Fig. 1A). However, there was no difference in reported satiety between groups (Fig. 1B).

3.3. Glucose and insulin levels

Fasting glucose levels were not different between groups (Table 2). However, the postprandial rise in plasma glucose (expressed as area under the curve (AUC) divided by time) was higher in PWS (6.2 (0.5) mmol/L) compared to obese (5.6 (0.3) mmol/L, $p = 0.04$) and lean controls (5.0 (0.1) mmol/L, $p = 0.04$) (Table 2, Fig. 2A).

Fasting insulin levels were similarly elevated in PWS (16.0 (2.7) μU/ml) and obese subjects (15.1 (1.5) μU/ml) compared to lean subjects (7.8 (0.6) μU/ml, $p = 0.005$) (Table 2).

The postprandial insulin response tended to be higher in PWS (51 (12) μU/ml) and obese subjects (43 (5) μU/ml) compared to lean controls (26 (3) μU/ml, p = n.s.), without statistical significance (Table 2, Fig. 2B).

3.4. Anorexigenic hormones PYY and GLP-1

PWS, obese and lean subjects all had similar fasting levels of PYY[total], PYY[3–36] and GLP-1[active] (Table 2). The postprandial response of PYY[total] tended to be higher in PWS than obese subjects (75 (7) vs. 71 (12) pg/ml, $p = 0.08$), without statistical significance (Fig. 2C). The postprandial PYY[3–36] response was significantly higher in PWS vs. lean subjects (59 (5) vs. 53 (4) pg/ml, $p = 0.02$), and tended to be higher than in obese controls (54 (8), $p = 0.08$) (Fig. 2D). The postprandial meal response of GLP-1[active] was not statistically different between groups (Fig. 2E).

3.5. Orexigenic hormone ghrelin

Subjects with PWS had significantly higher fasting and postprandial ghrelin levels than obese subjects (fasting levels 1071 (170) vs. 632 (48) pg/ml, $p = 0.04$) (Table 2, Fig. 2F). PWS subjects demonstrated similar postprandial ghrelin suppression to lean subjects (−15.1 (2.2)% vs. −13.2 (3.5)%, p = n.s.), but levels remained well above those of lean and obese subjects. Conversely, ghrelin suppression in obese subjects tended to be lower compared to PWS subjects (−6.4 (3.7)% vs. −15.1 (2.2)%, $p = 0.048$).

4. Discussion

Our results show that, independent of total and central adiposity, as well as gender, age and BMI, the postprandial response of PYY and GLP-1 in PWS subjects was not lower than controls. Importantly, the meal response of PYY[3–36] was higher in PWS compared to lean subjects. Despite high levels of satiety-inducing hormones and self-reported feelings of satiety, significantly greater hunger persisted in PWS subjects. This may relate to the significantly higher levels of ghrelin observed in PWS relative to obese controls, even when matched for adiposity, which is known to influence circulating ghrelin levels.

It has been reported that PWS, compared to BMI matched controls, had lower visceral adiposity, with a lower ratio of visceral to subcutaneous adipose tissue (Goldstone et al., 2001; Sode-Carlsen et al., 2010). The authors also noted that visceral fat predicted insulin, glucose, C-peptide, triglycerides and high-density and low-density lipoprotein cholesterol, while subcutaneous fat had no effect on these parameters (Goldstone et al., 2001). This highlights the importance of controlling for differential fat deposition as a possible cause of differences detected in hormone profiles. The present study was able to avoid potential confounding factors because of the similar levels of total adiposity, in addition to matching for age, gender and BMI. Interestingly, the two groups were also matched for central adiposity and insulin resistance without specific selection of control subjects. Although our PWS cohort might seem unusual, as a lower visceral fat mass and preserved insulin sensitivity has been reported previously (Brambilla et al., 1997; Goldstone et al., 2001, 2004, 2005; Talebizadeh and Butler, 2005; Kennedy et al., 2006; Theodoro et al., 2006; Haqq et al., 2010; Sode-Carlsen et al., 2010), other investigators recently reported similar observations to ours, i.e. obese children and adults with PWS having the same amount of visceral fat mass, the same degree of insulin resistance and a similar prevalence of metabolic syndrome compared to carefully weight-matched obese subjects

Table 2
Fasting and postprandial hormone levels and VAS scores.

		PWS	Obese	Lean	p (Repeated measures ANOVA)		
					Group	Meal	Group * Meal
Glucose (mmol/l)	Baseline	4.7 (0.2)	4.8 (0.3)	4.4 (0.1)	0.16	0.0001	0.04 [a,b]
	Meal	6.2 (0.5)	5.6 (0.3)	5.0 (0.1)			
Insulin (μU/ml)	Baseline	16.0 (2.7)	15.1 (1.5)	7.8 (0.6)	0.005 [b]	0.0001	0.6
	Meal	51 (12)	43 (5)	26 (3)			
PYY[total] (pg/ml)	Baseline	75 (7)	71 (12)	70 (5)	0.26	0.0001	0.08
	Meal	116 (8)	92 (13)	87 (4)			
PYY[3–36] (pg/ml)	Baseline	43 (5)	46 (7)	47 (5)	0.86	0.0001	0.02 [b]
	Meal	59 (5)	54 (8)	53 (4)			
GLP-1[active] (pmol/L)	Baseline	5.9 (0.6)	6.0 (0.4)	6.0 (1.0)	0.6	0.001	0.3
	Meal	9.1 (1.5)	6.9 (0.6)	8.5 (2.0)			
Ghrelin[total] (pg/ml)	Baseline	1071 (170)	632 (48)	831 (86)	0.04[a]	0.0001	0.2
	Meal	904 (142)	592 (50)	725 (83)			
Fullness (VAS score)	Baseline	1.2 (0.5)	0.8 (0.3)	0.3 (0.2)	0.75	0.0001	0.14
	Meal	2.9 (0.4)	3.3 (0.5)	3.1 (0.4)			
Hunger (VAS score)	Baseline	6.0 (0.6)	5.3 (0.6)	4.1 (0.6)	0.003 [a,b]	0.0001	0.01 [a,b]
	Meal	5.8 (0.6)	3.0 (0.4)	2.3 (0.4)			

Data expressed as mean (SEM).
Meal = average calculated as AUC/time.
[a] PWS vs. Obese $p < 0.05$, contrasts within ANOVA effects.
[b] PWS vs. Lean $p < 0.05$, contrasts within ANOVA effects.

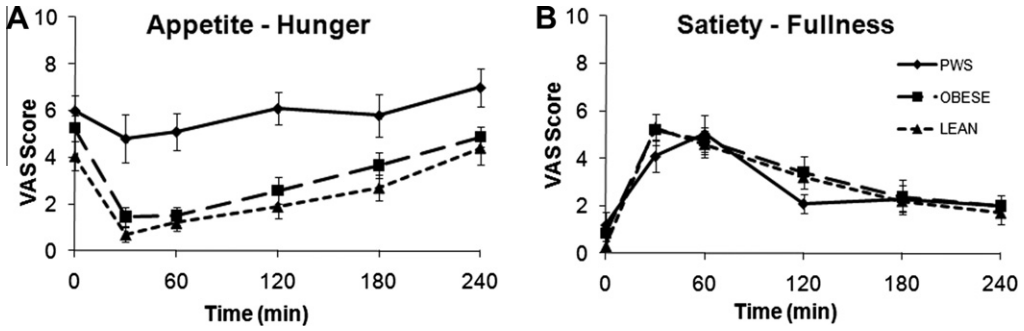

Fig. 1. Visual analog scores (VAS) during the test meal (600 kcal) assessing hunger (A, 'how much could you eat now?') and satiety (B, 'how full does your belly feel?') in PWS (solid line), obese (dashed line) and lean subjects (dotted line). Data are presented as mean (SEM).

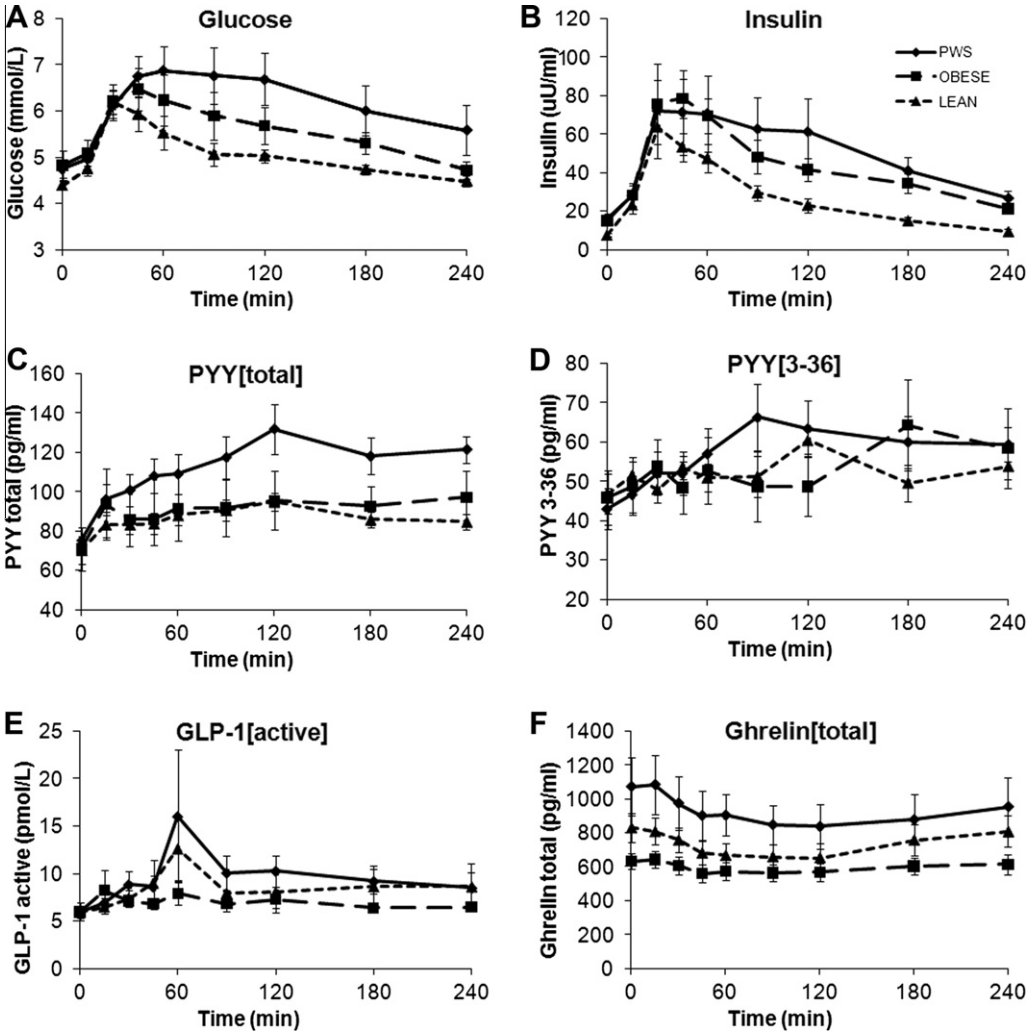

Fig. 2. Glucose (A), insulin (B), PYY[total] (C), PYY[3–36] (D), GLP-1[active] (E) and ghrelin[total] (F) levels during the meal test in PWS (solid line), obese (dashed line) and lean subjects (dotted line). Data are presented as mean (SEM).

(Talebizadeh and Butler, 2005; Brambilla et al., 2010). The present study was not designed to compare body composition and fat distribution between PWS and obese subjects, as a larger cohort would be needed to that end. However, the fact that PWS and obese controls had similar central and total adiposity provided an opportunity to examine gut hormone levels independent of a major confounding factor, helping to understand the underlying pathophysiology of appetite dysregulation in PWS.

Several reports have described the role of PYY in PWS, but with conflicting results. Some have found that adults and children with PWS have lower fasting and postprandial PYY levels than controls (Butler et al., 2004; Gimenez-Palop et al., 2007), while others reported no difference (Goldstone et al., 2005; Paik et al., 2007a) or increased PYY levels (Bizzarri et al., 2010).

A 2004 study (Goldstone et al., 2004) assessed fasting plasma GLP-1[total] in 12 female and 16 male adults with PWS, and found

no difference in levels compared to obese and lean controls. However, as the authors noted, there may be postprandial changes in secretion of GLP-1 in PWS not measured in their study. Furthermore, it may be that the level of the GLP-1[active] form, GLP-1[7–36], not previously measured in PWS, is physiologically more important than GLP-1[total]. In the present study, GLP-1[active] showed a similar pattern in PWS subjects as did PYY, being similarly high postprandially as in obese and lean control subjects.

We found no evidence of an impaired postprandial satiety response in the PWS subjects in our study. The secretion of anorexigenic hormones PYY and GLP-1 was not reduced compared to control subjects, nor were VAS satiety scores altered. Despite this, hunger scores remained significantly higher in the PWS group, with a rapid return to fasting levels after meal termination. Results from the current study suggest that satiety hormones such as PYY and GLP-1 are unlikely to cause the lack of satiety in subjects with PWS, and simple pharmacological strategies involving replacement therapy with PYY or GLP-1 might not be effective in treating PWS hyperphagia. Indeed, a study by Zipf et al. demonstrated that infusion of pancreatic polypeptide, which has similar function as a satiety factor as PYY, did not decrease reported hunger in children with PWS (Zipf et al., 1990). However, it remains possible that treatment with supraphysiological doses of a GLP-1 or PYY agonist, which may also affect other receptors, may still be beneficial.

The present study confirms previous reports (DelParigi et al., 2002; Haqq et al., 2003; Goldstone et al., 2004; Tauber et al., 2004) that fasting and postprandial ghrelin levels are significantly higher in PWS subjects compared to obese and lean subjects. However, in contrast to previous reports (Purnell et al., 2003; Goldstone et al., 2004, 2005; McLaughlin et al., 2004), our data suggest that the observed increase in ghrelin in PWS is not due to altered fat distribution or hypoinsulinemia because PWS and obese subjects were matched for total and central adiposity and showed similar insulin secretion and insulin resistance. Therefore, hyperghrelinemia could be a specific feature of PWS, possibly caused by underlying hypothalamic defects and an imbalance of vagal nerve activation.

Despite overall higher levels of ghrelin in PWS, these subjects still exhibited normal postprandial suppression of ghrelin in our study, consistent with results reported in 3 studies of children with PWS (Haqq et al., 2003; Paik et al., 2006, 2007b) and another of adults (Gimenez-Palop et al., 2007). Future trials are needed to elucidate the role of ghrelin in PWS using specific ghrelin antagonists, currently in development.

In healthy subjects, hunger and satiety operate in balance; when one is high the other is low. Interestingly, our findings in the present study confirm this pattern in lean and obese subjects but not in PWS subjects, who concurrently reported high satiety and high hunger scores. We observed an apparently normal satiety response in PWS adults, namely an increase in postprandial VAS fullness scores accompanied by a rise in PYY and GLP-1 concentrations and a drop in plasma ghrelin levels. However, despite this seemingly appropriate physiological response to a meal, PWS subjects maintained high VAS hunger scores, even immediately after consumption of a large meal. The simple fact that PWS ghrelin levels remain higher than those of controls may be sufficient to drive appetite to override the observed satiety response, leading to hyperphagia.

One limitation of this study is a certain degree of heterogeneity of the cohorts, a problem almost impossible to prevent when investigating a rare disease with multiple co-morbidities and medications. In this study, all recruited PWS subjects were included regardless of medical treatment. Obese control subjects were selected to be similar to the PWS subjects regarding medications and presence of T2D. However, certain medications, such as metformin, sitagliptin, insulin, and psychotropic medications can potentially have an impact on circulating gut hormones. To minimize these possible influences, all subjects had their meal study prior to taking their usual medication and insulin. In this study, subjects with T2D had similar gut hormone levels as non-diabetic subjects, and our results were unchanged when excluding all subjects with T2D.

In conclusion, this is the first study to investigate postprandial orexigenic and anorexigenic gut hormones in PWS and their relationships with hunger and satiety independent of visceral adiposity, BMI, age and gender. While it has been postulated that impaired postprandial secretion of such hormones could contribute to the hyperphagia of PWS, only a possible role for ghrelin promoting hunger was found, without evidence for involvement of GLP-1 or PYY in the hyperphagia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.npep.2011.06.001.

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