CLINICAL STUDY

Children with Prader–Willi syndrome exhibit more evident meal-induced responses in plasma ghrelin and peptide YY levels than obese and lean children

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Abstract

Background and aims: Ghrelin is an orexigenic 28-amino acid peptide produced by the stomach. Circulating ghrelin levels rise shortly before and fall shortly after every meal. Peptide YY (PYY), an anorexigenic 36-amino acid peptide, is secreted primarily from the intestinal mucosa of the ileum and large intestine. Plasma PYY levels begin to rise within 15 min after starting to eat and plateau within ~90 min, remaining elevated for up to 6 h. Recently, some studies have tried to evaluate the potential role of ghrelin and PYY in the hyperphagia of patients with Prader–Willi syndrome (PWS). While hyperghrelinemia is well characterized in PWS, conflicting results have been reported for PYY. The aim of the study was to investigate ghrelin and PYY responses to a standard liquid high-fat meal in children with PWS.

Patients and methods: Circulating levels of total ghrelin and PYY levels were assayed by RIA after overnight fasting and 45, 60, 90, and 180 min following a standard meal (Ensure 6 ml/kg) in 16 patients with PWS (11 boys and five girls, aged 4.6–10.7 years, including ten receiving 0.02 mg/kg per day rhGH for 2–18 months; body mass index (BMI) z-score: 0.6 ± 0.2 and 1.6 ± 0.5 for children treated or not treated with rhGH respectively), ten obese (eight boys and two girls, aged 9.2–15.6 years; BMI z-score: 2.4 ± 0.2 , i.e. BMI >97th centile for chronological age and sex) subjects, and 16 normal-weight controls (five boys and 11 girls, aged 5.8–17.3 years; BMI z-score: 0.6 ± 0.2).

Results: PWS children showed higher fasting levels of ghrelin than obese and lean controls. Postprandial ghrelin drop was more pronounced in PWS than in the other study groups. No significant difference on fasting levels of PYY was found among groups. PWS showed a higher postprandial PYY rise than obese and lean controls. PWS patients treated and not treated with GH showed similar fasting and postprandial levels of ghrelin and PYY. Fasting PYY levels correlated negatively ($P < 0.05$; $r = -0.68$) with those of ghrelin only in PWS.

Conclusions: The results of this study confirm fasting hyperghrelinemia in PWS. Since in PWS adults an impaired postprandial suppression of plasma ghrelin was previously reported to be associated with a blunted postprandial PYY response, the finding of a meal-induced decrease and increase in ghrelin and PYY levels respectively in PWS children would imply that the regulation of appetite/satiety of these peptides is operative during childhood, and it progressively deteriorates and vanishes in adulthood when hyperphagia and obesity worsen.

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Introduction

The epidemic of childhood obesity in Europe and elsewhere (1–4) has recently focused attention on understanding the endogenous signals that regulate appetite and energy balance. Ghrelin and peptide YY (PYY) are two such hormones secreted by the gut, which have important effects on appetite via their interactions with the hypothalamus (5, 6).

Ghrelin is a somatotrophic and orexigenic protein secreted primarily from specialized endocrine cells in the oxyntic glands of the stomach (7, 8). Serum concentrations of ghrelin rise during fasting and decline postprandially in adulthood (9, 10). Conversely, PYY is an anorexigenic hormone secreted mainly by L cells of the distal small bowel and colon (11). In adults, serum PYY levels increase after eating to a peak at 1–2 h (12).

Prader–Willi syndrome (PWS) is the most frequent cause of syndromic obesity occurring in 1 out of 8000–25 000 births. This syndrome is characterized by neonatal hypotonia and failure to thrive during the first months of life, followed by a rapid weight gain during the second year, which leads to a severe obesity with hyperphagia and a decreased satiety after the age of 3–4 years (13, 14). Early diagnosis, multidisciplinary care, and treatment with GH can improve the developmental outcome of these children and particularly reduce the incidence of obesity (15).

Recently, some studies have explored the potential role of ghrelin and PYY in the hyperphagia of patients with PWS (16–20).

High fasting ghrelin levels have been suggested to contribute to the absence of satiety in patients with PWS (21–25). Failure of circulating ghrelin titers to fall after a meal has previously been described in PWS adults (22). In contrast, in a study performed in a population of PWS children, a marked meal-induced decrease in ghrelin levels was found (16). This would imply that regulation of ghrelin function deteriorates progressively during the lifespan of PWS patients and is absent in adulthood when hyperphagia and obesity worsen.

This intriguing hypothesis might be valid for other orexigenic/anorexigenic peptides, including PYY, for which results in PWS are conflicting. In one study, lower fasting levels of PYY were reported in PWS children compared to a control population of newborns and infants (26). Two other studies in adult patients with PWS reported normal fasting and postprandial PYY levels after different calorie standard meals as well as a lack of any relationship between ghrelin and PYY levels (25, 27). Another report showed that a smaller postprandial suppression of plasma ghrelin in PWS adults was associated with a low fasting and a blunted postprandial PYY response (18).

The aim of this study was therefore to investigate whether in PWS children PYY production was stimulated by a standardized meal capable of reducing ghrelin levels. These results were compared to those of obese and lean children. In addition, in view of recent data which show that GH therapy in children with PWS decreases total but not acylated ghrelin concentrations and the total to acylated ghrelin ratio (28), a subgroup of PWS children treated with GH was also studied.

Subjects and methods

Subjects and clinical findings

The present study was undertaken at the Department of Pediatric Medicine, Unit of Endocrinology and Diabetology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, and at Pediatric Clinic, Insubria University,

Varese, Italy. Authorization of the clinical protocol was obtained from the local ethical committees. Informed written consent was obtained from the parents of all children, and assent for participation from the minor subjects.

Sixteen children with genetically confirmed diagnosis of PWS (11 boys and five girls, aged 4.6–10.7 years; body mass index (BMI): 20.5 ± 1.7 kg/m²; BMI z-score: 0.9 ± 1.0), ten children with morbid obesity (eight boys and two girls, aged 9.2–15.6 years; BMI: 32.0 ± 1.6 kg/m²; BMI z-score: 2.4 ± 0.2), and 16 control children (five boys and 11 girls, aged 5.8–17.3 years; BMI: 20.3 ± 0.9 kg/m²; BMI z-score: 0.6 ± 0.2) were enrolled in the present study. Twelve PWS children had a microdeletion, and four children had a uniparental maternal disomy.

The control group was represented by healthy non-obese children (BMI below the 97th percentile of Italian BMI curves (29)).

Ten children with PWS had been on treatment with rhGH (0.02 mg/kg per day rhGH) for a period ranging from 2 to 18 months. The remaining six children with PWS had not had rhGH treatment for 7 days.

All subjects were prepubertal or in early puberty by clinical assessment (Tanner stage 1–2 breast development in females, testicular size ≤ 5 ml in males). The children had no health problems (apart from PWS or morbid obesity) and were not taking any other medication than rhGH. Body weight and height were measured in all children at admission to the hospital. Height was measured with a Harpenden stadiometer. BMI and BMI z-score were then calculated (29).

Study design

Children were studied in the morning after an overnight fast. The subjects were asked to drink over 10 min a liquid mixed meal (6 ml/kg body weight, maximum 360 ml; Ensure, Abbott Srl). The meal provides 100 kcal in 100 ml (53% of the calories provided by carbohydrates, 17% by protein, and 30% by fat). Blood samples were drawn before the meal (mentioned as time 0 in the figures) and 30, 45, 60, 90, and 180 min after the end of the meal.

Biological measures

Blood glucose (mg/dl) was determined immediately after sampling by modified glucose oxidase method (Beckman Coulter, Brea, CA, USA). All other blood samples were drawn on ice, centrifuged immediately at 4 °C, and stored at –80 °C until assayed.

Total ghrelin (pg/ml) and total PYY (pg/ml) were measured by RIA (Linco Research, St Charles, MO, USA).

Total ghrelin RIA measures both octanoylated and des-octanoylated ghrelin, and has intra- and inter-assay coefficients of variation (CV) 3.3–10.0 and 14.7–17.8% respectively.

Total PYY RIA measures both PYY_{1–36} and PYY_{3–36}, but not NPY or pancreatic polypeptide, and has intra- and inter-assay CV 2.9–9.4 and 5.5–8.5% respectively.

Conversion from metric to SI units is as follows: ghrelin pg/ml $\times$ 0.3 = pmol/l and PYY pg/ml $\times$ 0.25 = pmol/l.

Insulin-like growth factor 1 (IGF1) concentration (ng/ml) was determined by enzyme immunoassay using a commercial kit (Mediagnost, Reutlingen, Germany). The sensitivity of the method is 0.09 ng/ml; intra-assay and inter-assay CV are 6.7 and 6.8% respectively.

Insulin concentration (mIU/ml) was determined by chemiluminescent immunometric assay using a commercial kit (Immulite 2000, DPC, Los Angeles, CA, USA). The sensitivity of the method is 0.002 mIU/ml; intra-assay and inter-assay CV are 4.1 and 5.2% respectively.

Triglycerides were quantified (mg/dl) using a colorimetric enzymatic method.

Levels of glucose, insulin, ghrelin, and PYY were measured in all samples, whereas those of IGF1 and triglycerides were measured only at baseline (at 0 min).

Statistical analysis

The GraphPad Prism 5.0 statistical software package (GraphPad Software, Inc., La Jolla, CA, USA) was used for data analysis.

Results were reported as mean $\pm$ s.e.m. A test of normality and an equal variance test were performed for all markers before the use of parametric tests. One-way ANOVA was employed to test differences between the children in preprandial hormonal and metabolic levels and demographic and clinical characteristics. Two-factor (group and time) repeated-measures ANOVA was used for ghrelin, PYY, glucose, and insulin to evaluate the significance of the main effects of interactions for group, time, and group $\times$ time (inter-group comparisons). One-factor (time) ANOVA was performed to evaluate the time-related variation of each (metabolic

or hormonal) parameter within each group (intra-group comparisons). When ANOVA was significant, a *post hoc* ANOVA test was performed for inter- or intra-group comparisons (Tukey's or Dunnett's test). Correlations between parameters were based on Pearson's correlation coefficient. $P < 0.05$ was considered to be significant.

As no differences were observed in fasting and postprandial ghrelin or PYY levels between males and females within each group, data were pooled.

Results

Demographic, clinical, and preprandial hormonal data

No significant difference for age emerged among the four groups except that between obese and PWS children (only the group treated with rhGH). BMI and BMI z-score were significantly higher in obese than lean children, but there was no difference between the two PWS subgroups treated or not treated with rhGH; BMI z-score was significantly lower in PWS children treated with rhGH than in the obese children. Preprandial ghrelin levels were higher in PWS children (irrespective of rhGH treatment) than in obese and lean children ($P < 0.05$), whereas fasting PYY levels were similar in all children. Fasting levels of insulin, glucose, and IGF1 did not differ significantly between the four groups. Baseline triglyceride levels were significantly higher in obese than lean children (Table 1).

Hormone and glucose responses to a meal

Plasma ghrelin and PYY The group $\times$ time repeated-measures ANOVA yielded a significant effect for group ($F(3,265) = 60.36$, $P < 0.05$) and time ($F(6,265) = 3.38$, $P < 0.05$), indicating that circulating ghrelin changed significantly across sampling times and that the groups displayed quantitative differences across the samplings.

Table 1 Demographic, clinical, metabolic, and hormonal data of the study children.

	Control	Obese	PWS	PWS + GH
Age (years)	9.8 $\pm$ 0.8	12.3 $\pm$ 0.7	8.8 $\pm$ 1.0	6.6 $\pm$ 0.6*
M/F	11/5	8/2	4/2	7/3
BMI (kg/m ²)	20.2 $\pm$ 0.9	32.0 $\pm$ 1.6 [†]	26.1 $\pm$ 3.6	17.2 $\pm$ 0.5
BMI z-score	0.6 $\pm$ 0.2	2.4 $\pm$ 0.2 [†]	1.6 $\pm$ 0.5	0.6 $\pm$ 0.2*
IGF1 (ng/ml)	326.4 $\pm$ 21.1	302.0 $\pm$ 34.5	280.8 $\pm$ 92.6	327.1 $\pm$ 27.4
Glucose (mg/dl)	77.3 $\pm$ 1.3	79.4 $\pm$ 2.6	75.8 $\pm$ 5.2	77.0 $\pm$ 3.2
Insulin (mIU/ml)	7.9 $\pm$ 1.0	13.1 $\pm$ 2.2	6.3 $\pm$ 2.7	10.0 $\pm$ 1.6
Triglycerides (mg/dl)	66.6 $\pm$ 6.2	97.5 $\pm$ 12.4 [†]	78.5 $\pm$ 11.0	61.9 $\pm$ 3.7
Ghrelin (pg/ml)	474.4 $\pm$ 53.9	414.7 $\pm$ 16.5	898.0 $\pm$ 238.2* [†]	1093.8 $\pm$ 120.4* [†]
PYY (pg/ml)	121.7 $\pm$ 8.9	123.3 $\pm$ 8.3	91.2 $\pm$ 16.2	149.6 $\pm$ 22.1

Data are expressed as mean $\pm$ s.e.m. Metabolic and hormonal parameters were measured in fasting conditions (at 0 min before administration of the meal). PWS, Prader–Willi Syndrome; PWS + GH, group of PWS children treated with rhGH. * $P < 0.05$ versus obese children; [†] $P < 0.05$ versus control (lean) children.

Indeed, the *post hoc* Tukey's test of group differences indicated that, as compared with lean and obese children, PWS children (irrespective of GH treatment) had significantly higher ghrelin levels at 45, 60, 90, 120, and 180 min after the meal (Fig. 1a).

PWS children displayed significant intra-group time-related variations for ghrelin concentrations, which decreased markedly after the meal (PWS children not treated with GH: at 45, 60, and 90 min; PWS children treated with GH: at 45, 60, 90, and 120 min; versus 0 min, $P < 0.05$). In the remaining groups (obese and lean children), administration of meal did not significantly change plasma ghrelin levels (Fig. 1a).

The group $\times$ time repeated-measures ANOVA showed a significant effect for group ($F(3,266) = 6.64$, $P < 0.05$) and time ($F(6,266) = 2.60$, $P < 0.05$) but no significant group $\times$ time interaction ($F(18,266) = 1.47$, $P = \text{NS}$), indicating that circulating PYY changed significantly across sampling times and that the groups displayed quantitative differences over the samplings (Fig. 1b).

Indeed, the *post hoc* Tukey's test of group differences indicated that, as compared with lean children, PWS children treated with GH had significantly higher PYY levels 60 min after the meal (Fig. 1b).

PWS children displayed significant intra-group time-related variations for PYY concentrations, which

progressively increased after the meal (PWS children not treated with GH: at 60 and 120 min; PWS children treated with GH: at 45 and 60 min; versus 0 min, $P < 0.05$). In the remaining groups (obese and lean children), administration of the meal did not significantly change plasma PYY levels (Fig. 1b).

For each subject, postprandial changes in nadir circulating ghrelin were calculated as percent variations of the preprandial ($t = 0$) values and analyzed by one-way ANOVA (control: -0.29 ± 0.16 ; obese: -0.32 ± 0.15 ; PWS: -0.41 ± 0.18 ; PWS+GH: -0.40 ± 0.15). This analysis showed no significant difference, although there was a trend towards decrease for PWS children.

Similarly, for each subject, postprandial changes in zenith circulating PYY were calculated as percent variations of the preprandial ($T = 0$) values and analyzed by one-way ANOVA (control: 0.41 ± 0.48 ; obese: 0.37 ± 0.29 ; PWS: 0.99 ± 0.92 ; PWS+GH: 0.79 ± 0.78). This analysis showed no significant difference, although there was a trend towards an increase for PWS children.

Plasma glucose and insulin Obese children had significantly higher glucose levels versus lean group 90 min after the meal. In addition, insulin levels were significantly higher in obese children than in the other groups (45, 60, and 120 min versus lean group, 60 min versus PWS groups not in therapy with GH, and 45 and 60 min versus PWS group treated with rhGH; after the meal; data not shown).

Correlations

Among correlations between baseline or food-induced changes in circulating ghrelin or PYY and the children's demographic, clinical, hormonal, and metabolic parameters (for all data and for each group), only the negative correlation between fasting PYY and ghrelin levels in the PWS groups was found to be statistically significant ($P < 0.05$; $r = -0.68$).

Discussion

In the present study, fasting levels of ghrelin, a stomach-derived orexigenic peptide with potent GH-releasing activity, were higher in PWS children (irrespective of rhGH treatment) compared with obese and lean children.

These results contrast with those of a previous study, where fasting plasma concentrations of ghrelin were similar in PWS and control children (16). These discrepant findings probably reflect the marked variability in fasting plasma ghrelin concentrations, which has also been reported in other studies, in which PWS children were compared to age-matched controls

Figure 1 Plasma ghrelin (panel a) and PYY (panel b) concentrations (pg/ml, mean $\pm$ S.E.M.) before and after a liquid high-fat meal administered at time 0 in control, obese, and PWS children, who were subdivided into two groups: treated or not treated with 0.02 mg/kg per day rhGH for 2–18 months. * $P < 0.05$ vs 0 min in the same group. See the text for other comparisons.

(23, 30, 31). Notably, in the work by Haqq *et al.* (20), only one-third of PWS children were hyperghrelinemic, a finding in clear contrast with the prevailing detection of high ghrelin levels in PWS adults (21, 22). Changes in ghrelin concentrations in PWS children during development could therefore be anticipated (19, 26). Longitudinal studies in a vast number of patients are essential to verify the occurrence of an age-related increase in ghrelin levels in PWS.

In the first years of life, hyperghrelinemia in PWS may be a response to failure to thrive or food restriction in infancy. Chronic or persistent hyperghrelinemia might then eventually promote hyperphagia, leading to progressive weight gain. In this connection, in the work by Feigerlová *et al.* (19), ghrelin dysregulation in PWS occurred very early and preceded the onset of obesity.

In our work, in contrast to ghrelin, fasting levels of PYY, an anorexigenic peptide derived primarily from the large intestine, were similar in PWS and obese and lean children. In fact, a negative correlation between fasting levels of ghrelin and PYY was found only in PWS children, suggesting the early occurrence of a dysregulation of other gastrointestinal appetite/satiety signals in PWS children (and not just of the ghrelin system), since the inhibitory effect of ghrelin on PYY is operative in healthy adults (12) but not in lean children (as shown in this study).

In the present work, the postprandial decrease in ghrelin concentrations was higher in PWS than the other two groups, and this did not respond to the inhibitory effect of the meal.

The mechanism(s) and the site(s) of ghrelin dysregulation present in PWS are currently unknown. The lack of ghrelin suppression after meals in PWS adults (22), but not in PWS children (this study), might indicate the existence of a multistep developmentally driven process in ghrelin dysregulation, as the result of the progressive deterioration through life of this genetic condition. Prospective studies enrolling a broader population of PWS patients are needed to test this hypothesis.

The most relevant finding of our study was the postprandial PYY increase in PWS children with no evident changes in plasma levels of the peptide in obese and lean children. Interestingly, this refractoriness of non-PWS children is in contrast with the postprandial PYY increase present in both lean and obese adults (12).

Recently, a smaller postprandial suppression of plasma ghrelin in PWS adults has been associated with low fasting and a blunted postprandial PYY response (18). This would imply that regulation of other gastrointestinal appetite/satiety peptides, including PYY, progressively deteriorates during the lifespan of PWS patients and is absent in adulthood when hyperphagia and obesity worsen. In any case, possible differences in populations of PWS patients enrolled in these studies cannot be ruled out.

The lack of suppression of ghrelin levels following a meal in control children confirms our previous results (16) and those of other authors (32). One possible explanation of this finding is the existence of a 'physiological' refractoriness to the inhibitory effect of feeding on ghrelin secretion in lean prepubertal children, as an anabolic setting of ghrelin system is deemed necessary at this age (32).

In the present study, administration of rhGH did not change ghrelin and PYY postprandial responses in PWS children. Although an increased mean acylated ghrelin to total ghrelin ratio was found in GH-treated PWS children due to a decrease in total ghrelin concentrations (28), other authors showed that neither GH deficiency nor GH replacement therapy alters ghrelin concentrations in either GH-deficient or PWS subjects (24, 33–36). A possible explanation of this discrepancy may depend on the duration of rhGH treatment, which was different in our group of PWS children, or on the too short duration of rhGH withdrawal in the group defined as 'not treated'.

Limitations of this study deserve acknowledgment and consideration. The sample size was somewhat small, which may have contributed to the lack of difference in fasting and postprandial ghrelin and PYY levels observed, particularly in the PWS group not treated with rhGH. However, the findings of this study confirm those of a previous work of ours (16). The groups were not matched for gender and age, although in fact no differences were observed in fasting and postprandial ghrelin or PYY levels between males and females within each group. The postprandial followup of PYY was short, although some studies showed a postprandial increase of this peptide lasting 6 h (12).

In conclusion, lean healthy children are hardly sensitive to the inhibitory/stimulatory effect of feeding on ghrelin and PYY secretion respectively, perhaps indicative of the existence of an anabolic setting of these peptides at this age. Based on previously reported impaired postprandial suppression of plasma ghrelin associated with a blunted postprandial PYY response in PWS adults, the finding of a meal-induced decrease and increase in ghrelin and PYY levels respectively in PWS children would imply that the physiological regulation of appetite/satiety of these peptides is operative during childhood, and progressively deteriorates and vanishes in adulthood when hyperphagia and obesity increase. Because in this study, the population of patients enrolled was not age matched, further studies are essential to confirm these results.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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