Circulating Peptide YY Concentrations Are Higher in Preterm than Full-Term Infants and Correlate Negatively with Body Weight and Positively with Serum Ghrelin Concentrations

Tania Siahanidou, Helen Mandyla, Maria Vounatsou, Dimitris Anagnostakis, Ioannis Papassotiriou, and George P. Chrousos

Background: Peptide YY (PYY) and ghrelin are gastrointestinal tract–derived hormones involved in the short- and long-term regulation of food intake and energy balance (1, 2). PYY is synthesized predominantly by the endocrine cells of the terminal ileum and colon (2); it is released into the circulation in response to a meal and participates in signaling at the hypothalamus at the end of the meal (2). A Y2 receptor–mediated mechanism has been postulated for the satiety action of PYY (3). Chronic administration of PYY inhibits food intake and body weight gain in experimental animals (3).

Ghrelin is secreted predominantly from X/A-like endocrine cells of the oxyntic glands of the stomach. Circulating ghrelin concentrations are highest in the fasting state, decreasing within 1 h after a meal (2). The role of ghrelin in food intake and energy balance is opposite to that of PYY, and it exerts orexigenic effects through activation of the hypothalamic neuropeptide Y-Y1 pathway (4). Chronic ghrelin administration leads to a significant increase in cumulative food intake and body weight gain (1). Ghrelin also affects adipogenesis (4, 5) by stimulating increased food intake and, possibly, by decreasing fat oxidation (6) and energy expenditure (1). In addition to its important roles in the control of energy homeostasis, ghrelin stimulates growth hormone secretion in pituitary cells in culture and in vivo (7).

Methods: To measure serum concentrations of PYY in preterm and full-term infants and to investigate their correlations with anthropometric characteristics, food intake, and serum ghrelin concentrations, we measured serum PYY and ghrelin concentrations by RIA in 62 healthy preterm infants [mean (SD) gestational age, 32.0 (2.1) weeks; postnatal age, 40.9 (14.8) days] and 15 healthy full-term infants of comparable postnatal age. All the infants were formula-fed every 3 h.

Results: PYY concentrations were significantly higher in preterm [1126.2 (215.4) ng/L] than full-term infants [825.3 (234.4) ng/L; P < 0.001]. In the entire study population, serum PYY concentrations correlated negatively with gestational age and anthropometric measurements (birth weight, body weight, body length, body mass index, and head circumference) and positively with serum ghrelin concentrations, whereas there was no significant correlation between PYY concentration and caloric intake or weight gain. Multiple regression analysis, after correction for prematurity, revealed that serum PYY concentrations correlated independently with serum ghrelin concentrations and infant body weight or body mass index.

Conclusions: Circulating concentrations of PYY may increase in preterm infants to compensate for the negative body-weight balance. The physiologic mechanisms behind the correlation between PYY and ghrelin remain to be elucidated.

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been determined in healthy and diseased individuals in many studies (8–13), but little attention has been paid to preterm infants. In premature neonates hospitalized immediately after birth, energy balance is often negative, leading to lower anthropometric measurements at discharge than those expected on the basis of “normal” intrauterine growth rates (14). Because poor postnatal growth in preterm infants may have long-term consequences (15), mechanisms regulating food intake and growth in these infants are of great interest. We therefore compared circulating PYY and ghrelin concentrations in preterm infants with those in full-term infants of comparable postnatal age; we also investigated whether PYY and ghrelin concentrations were correlated with the infants’ anthropometric measurements, food intake, and growth rates.

Materials and Methods

Study Population and Protocol

The study population consisted of 62 preterm (gestational age, 28–36 weeks; birth weight, 940-2100 g) and 15 full-term (gestational age, 37–41 weeks; birth weight, 2520–3950 g) infants. Gestational age was estimated from the last menstrual period and supported by fetal ultrasound measurements and clinical examination of the neonate according to the Ballard score. Preterm infants admitted to our unit were included in the study provided that (a) they had no congenital malformations or major morbidities [necrotizing enterocolitis, bronchopulmonary dysplasia, or intraventricular hemorrhage ≥grade 1 (16)], (b) their mothers had elected formula feeding, and (c) they did not contract an infection before their last 10 days of hospitalization. The same commercial formula (S-26; Wyeth Nutritionals) was used for feeding all infants in the study.

Infants younger than 32 weeks of gestational age and/or those with immature sucking were initially given 2-h nasogastric tube bolus feeds. After sucking feeds had been established, the infants were offered formula on demand every 3 h. Finally, all studied infants were bottle-fed every 3 h during the last week of hospitalization or earlier. Body weight was obtained daily with a standard electronic scale. Recumbent length and head circumference were measured weekly by a single investigator. For length measurements, an infantometer containing a stationary headboard, a movable footboard, and a built-in centimeter scale was used. Head circumference was measured with a nonstretchable plastic insertion tape. Infants were discharged when they had reached a weight of at least 2100 g, provided that they were in good health and enteral feeding had been fully established. On the morning of the day of discharge and before feeding, venipuncture was performed for sample collection for routine blood tests as well as for serum PYY and ghrelin measurements.

A venous blood sample for PYY and ghrelin measurements was also obtained from 15 healthy full-term infants who were used as the reference group. Sex and postnatal age distributions were similar in the full-term and preterm infant groups. The full-term infants were admitted to our unit after birth and subsequently followed up as outpatients. They were exclusively formula-fed every 3–4 h and were offered the same kind of formula as the preterm infants. Their weight, length, and head circumference were measured weekly by the same method applied to preterm infants.

In the entire study population, the amount of formula consumed at each meal over the 3 days before blood sampling was recorded, and caloric intake (Kcal·kg⁻¹·day⁻¹) was calculated. Weight gain in g·kg⁻¹·day⁻¹ was estimated for the period since birth and the week before blood sampling. The weight increase during the study period was calculated as the difference between body weight z-score at the time of blood sampling and birth weight z-score (ΔWz-score). Similarly, the differences in body length z-score (ΔLz-score) and head circumference z-score (ΔHCz-score) were also calculated. Weight, length, and head circumference z-scores were evaluated with Eurogrowth, Ver. 2.0 2000, software (University of Vienna and the University of Nijmegen).

Informed parental consent was obtained for all infants studied.

Hormone Assays

Serum PYY concentrations were assayed with a human PYY RIA (Linco Research), which recognizes both the 1–36 and 3–36 forms of human PYY, with an interassay CV of 6.5% and intraassay CV of 4.5%. Serum ghrelin concentrations were measured with a commercial RIA (Phoenix Pharmaceuticals) with ¹²⁵I-labeled bioactive ghrelin as a tracer molecule and a rabbit polyclonal antibody against both octanoylated and des-octanoylated human ghrelin. This assay measures the total circulating ghrelin concentrations. The intra- and interassay CVs were 5.5% and 2.1%, respectively.

Statistical Analysis

Data are presented as the mean (SD). Values of PYY and ghrelin followed a gaussian distribution, both overall and for preterm and full-term infants separately, so that no transformation was necessary. Differences between preterm and full-term infants in quantitative variables were evaluated by t-test or Mann–Whitney U-test, as appropriate. Pearson correlations were used to analyze relationships among the variables of interest. Multiple regression analysis was also performed to evaluate whether several variables were correlated independently with PYY and ghrelin concentrations or whether they were confounding factors.

A P value <0.05 was considered significant. All statistical analyses were performed with the SPSS statistical package.
Results

The characteristics of preterm and full-term infants are shown in Table 1. As expected, all anthropometric measurements [body weight, length, body mass index (BMI), and head circumference] were significantly lower in preterm than in full-term infants. No difference was recorded in caloric intake (Kcal·kg⁻¹·day⁻¹) between groups. In preterm infants, weight gain (g·kg⁻¹·day⁻¹) over the study period, as well as over the last week before blood sampling, was significantly higher than in full-term infants (P = 0.03 and 0.01, respectively) but was insufficient to cover the deficit in weight z-score; thus, in preterm infants the ∆Wz-score was more negative than in full-term infants (P < 0.001 between groups). The ∆Lz-score and the ∆HCz-score, however, did not differ significantly between groups.

Serum PYY concentrations were significantly higher in preterm than in full-term infants [1126.2 (215.4) ng/L vs 825.3 (234.4) ng/L; P < 0.001; Fig. 1A], as were serum ghrelin concentrations [721.7 (98.0 ng/L) vs 602.5 (93.7) ng/L; P < 0.001; Fig. 1B]. In the entire study population, there was no significant difference between females and males in serum PYY or ghrelin concentrations [PYY, 1076.0 (256.9) ng/L in females vs 1058.0 (243.9) ng/L in males (P = 0.75); ghrelin, 708.7 (96.1) ng/L in females vs 689.1 (117.6) ng/L in males (P = 0.41)]; there also was no difference between females and males in either the preterm or full-term infant groups.

In the entire study population, serum PYY and ghrelin concentrations were negatively correlated with gestational age [r = −0.45 (P < 0.001) and r = −0.36 (P = 0.001), respectively], birth weight [r = −0.43 (P < 0.001) and r = −0.33 (P = 0.003), respectively], body weight [r = −0.58 (P < 0.001) and r = −0.45 (P < 0.001), respectively; Fig. 2], body length [r = −0.54 (P < 0.001) and r = −0.54 (P < 0.001) between groups]. In preterm compared with full-term infants, BMI [r = −0.60 (P < 0.001) and r = −0.38 (P = 0.004), respectively; Fig. 3], and head circumference [r = −0.47 (P < 0.001) and r = −0.39 (P = 0.001), respectively]. There was no significant correlation between PYY or ghrelin concentrations and caloric intake or weight gain during the entire study or the week before blood sampling. Serum PYY concentrations correlated negatively with the ∆Wz-score (r = −0.33; P = 0.004), whereas serum ghrelin concentrations correlated negatively with the ∆Lz-score (r = −0.35; P = 0.03). In the group of preterm infants, only body weight and BMI correlated with serum PYY concentrations [r = −0.44 (P < 0.001) and r = −0.46 (P = 0.002), respectively], whereas none of the variables studied correlated significantly with serum ghrelin concentrations. In full-term infants, serum

<table>
<thead>
<tr>
<th>Table 1. Characteristics of preterm and full-term infants.</th>
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<tbody>
<tr>
<td><strong>Variables</strong></td>
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<tr>
<td>F/M, n</td>
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<tr>
<td>Gestational age, weeks</td>
</tr>
<tr>
<td>Birth weight, g</td>
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<tr>
<td>Postnatal age, days</td>
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<tr>
<td>Body weight, g</td>
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<tr>
<td>Body length, cm</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Head circumference, cm</td>
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<tr>
<td>Caloric intake, cal·kg⁻¹·day⁻¹</td>
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<tr>
<td>Weight gain, g·kg⁻¹·day⁻¹</td>
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<tr>
<td>Entire study period</td>
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<tr>
<td>∆Wz-score</td>
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<tr>
<td>∆Lz-score</td>
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<td>∆HCz-score</td>
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*All data except for sex are the mean (SD). |

*Comparing with full-term infants: *P* < 0.01; *P* < 0.05.
PYY concentrations correlated negatively with body weight ($r = -0.59; P = 0.01$), body length ($r = -0.62; P = 0.02$), and head circumference ($r = -0.59; P = 0.04$), whereas serum ghrelin concentrations correlated negatively with body length ($r = -0.71; P = 0.009$) and head circumference ($r = -0.75; P = 0.01$). Serum PYY and ghrelin concentrations were significantly positively correlated in the total study population ($r = 0.50; P < 0.001$) as well as in the separate groups of preterm ($r = 0.33; P = 0.009$) and full-term infants ($r = 0.53; P = 0.03$; Fig. 4).

In multiple regression analysis, the only variables that correlated independently with serum PYY concentration, after correction for prematurity, were ghrelin concentrations and body weight or BMI (Table 2). Body length tended to independently correlate with both serum PYY and ghrelin concentrations (Table 3).

**Discussion**

Preterm infants at discharge from the neonatal unit had higher serum PYY and ghrelin concentrations than full-term infants of comparable postnatal age. To our knowledge, this is the first study in which circulating concentrations of both PYY and ghrelin were measured in preterm infants and the correlations between these hormones and anthropometric measures, food intake, and growth rates were examined. Circulating PYY concentrations have been measured in a limited number of studies involving preterm infants (17–19), and the concentrations reported were as high as in our study. Berseth et al. (19) compared PYY concentrations in preterm and full-term infants and reported higher concentrations in preterm infants, although the difference did not reach statistical significance. We are not aware of any reports of circulating ghrelin concentrations in preterm infants of similar age to those in our study, but ghrelin measurements have been reported for cord blood after preterm birth (8, 20, 21).

In our study, the increased PYY and ghrelin concentrations in preterm infants could be attributed to increased synthesis/secretion and/or to decreased clearance of these peptides. Very little is known about the clearance of PYY (22); ghrelin, however, has been more extensively studied. There is evidence that the kidney is the primary site of ghrelin clearance (10, 23), but the liver also plays an important role (23). Decreased clearance of both peptides as a consequence of immaturity of the relevant organs (kidney or liver) related to prematurity could be the cause of the increased circulating concentrations; however, the circulating concentrations of PYY were higher at day 12 of life than those observed at day 6 in healthy preterm infants (17). Similarly, circulating ghrelin concentrations have been reported to peak after the first postnatal month.
**Table 2. Multiple regression analysis model for serum PYY concentrations in the entire study population**

<table>
<thead>
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<th>Independent variables</th>
<th>β</th>
<th>P</th>
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<tbody>
<tr>
<td>Gestational age</td>
<td>-0.06</td>
<td>0.89</td>
</tr>
<tr>
<td>Body weight</td>
<td>0.57</td>
<td>0.38</td>
</tr>
<tr>
<td>Body length</td>
<td>-0.96</td>
<td>0.09</td>
</tr>
<tr>
<td>Head circumference</td>
<td>0.06</td>
<td>0.84</td>
</tr>
<tr>
<td>ΔLZ-score</td>
<td>-0.18</td>
<td>0.25</td>
</tr>
<tr>
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<td>Serum PYY concentrations</td>
<td>0.38</td>
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* Serum PYY concentration is the dependent variable.

**Table 3. Multiple regression analysis model for serum ghrelin concentrations in the entire study population**

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</table>

* Serum ghrelin concentration is the dependent variable.

**Fig. 4.** Correlations between serum PYY and ghrelin concentrations. ●, full-term infants; ○, preterm infants. The lines represent the regression slopes separately for the groups of full-term (---; P = 0.03) and preterm infants (----; P = 0.0092) and for the entire study population (-----; P < 0.001).

and up to the 24th month in healthy full-term infants (8). The increase with postnatal age of circulating PYY and ghrelin concentrations in healthy preterm and full-term infants makes the possibility of decreased clearance in our preterm infants unlikely.

Several factors, such as nutrient (protein, fat, carbohydrates) (24, 25) and caloric ingestion (26), cholinergic system stimulation (26, 27), hormones (e.g., somatostatin, growth hormone, gastrin, cholecystokinin, insulin, leptin, and glucocorticoids) (26, 28–34), and growth factors (e.g., insulin growth factor-1, transforming growth factor-α, and epidermal growth factor) (26, 35) have been implicated in the synthesis/secretion of PYY and/or ghrelin. In our study, the positive correlation between circulating PYY and ghrelin concentrations, reported for the first time, might indicate that there is a common modulator for the synthesis/secretion of both peptides or that each peptide regulates the synthesis/secretion of the other. Because nutrient and caloric intake were similar between groups, it is unlikely that they were the cause of the higher PYY and ghrelin concentrations in preterm than in full-term infants. However, because we did not study the other factors mentioned above, we cannot comment on their role as common modulators for the synthesis/secretion of ghrelin and/or PYY. In addition, a direct influence of PYY on ghrelin synthesis/secretion (or vice versa) has not been shown. It has been reported that PYY administration caused a decrease in circulating ghrelin concentrations (36), but this was disputed in a later study (37). Moreover, the possibility that serum ghrelin may exhibit a diurnal rhythm in infants as in adults (38), along with the fact that we measured both peptides at only a single time point on the morning of discharge, makes it harder to evaluate the significance of the observed correlation between PYY and ghrelin. Further studies are needed to elucidate the mechanism underlying this relationship.

Whatever the specific cause of the higher PYY and ghrelin concentrations in preterm than in full-term infants observed in this study, increased circulating ghrelin concentrations are a feature of states associated with negative energy/body weight balance, such as anorexia nervosa (9) and cardiac (11) and cancer cachexia (12). Ghrelin increases in these situations have been postulated to provide compensation through orexigenic and adipogenic
effects (4, 5). Circulating PYY concentrations in states of negative energy balance were studied by Stock et al. (9) in adolescents with anorexia nervosa. No difference was reported in PYY concentrations between patients and controls; however, the evidence on this topic is limited. In the present study, preterm infants had a negative balance in their body weight, which could be associated with the higher serum concentrations of ghrelin and, possibly, PYY. Although their weight gain was greater than that of full-term infants, the ΔWz-score was negative, and the deficit was significantly larger than that of full-term infants. Moreover, PYY and ghrelin correlated negatively with anthropometric measures in the entire study population, and interestingly, body weight (or BMI) was an independent predictor of PYY concentrations. The fact that body length was the anthropometric measure that tended (P = 0.09) to be an independent predictor of ghrelin concentrations could be related to the known growth hormone–releasing effect of ghrelin (7).

The negative correlation between PYY concentrations and body weight, along with (a) the negative correlations between ghrelin and anthropometric measurements and (b) the positive correlation between PYY and ghrelin suggest a more complex role of these peptides than initially recognized. Because PYY and ghrelin have opposite effects, the correlation of each peptide with the anthropometric data would have been expected to be the inverse of that of the other peptide. Thus, the pattern of correlations between PYY, ghrelin, and the anthropometric data of the infants observed in this study suggests that PYY and ghrelin may have had a similar effect on growth regulation in the infants. The assay we used for PYY determination did not differentiate between the 2 major molecular forms of PYY: PYY (1–36) and PYY (3–36) (26). A recent experimental study (39) showed that PYY (1–36), although less potent than PYY (3–36) in decreasing food intake, also exerted an antiorexigenic action. It has been postulated that PYY (1–36) inhibits food intake by acting similarly to PYY (3–36) at Y2 receptors, but interestingly, it can also potently stimulate food intake through Y1 receptors (39). Thus, predominance of Y1 over Y2 receptors could cause an orexigenic instead of the expected anorexigenic action of PYY (1–36). In preterm infants, the majority of circulating PYY-like immunoreactivity has been reported to be of a magnitude similar to that of the PYY (1–36) concentration (17), but the distributions of Y1 and Y2 receptors are not known.

We did not observe any correlation between PYY or ghrelin concentration and caloric intake or weight gain in our study population. The absence of such a correlation could be attributable to the complexity of the regulation of food intake and weight balance (2), which involves counteracting hormonal signals that might have masked the effects of the peptides studied. Whether ghrelin or PYY played a role in the significantly higher weight gain in preterm than in full-term infants is not known. In addition to the roles of PYY and ghrelin in the regulation of energy balance, their increased concentrations in preterm infants might also be related to the previously reported physiologic effects of these peptides on gut motility, secretion of gastrointestinal enzymes, and intestinal cell proliferation (12, 26, 40). Further studies are needed to elucidate the precise clinical significance of the increased PYY and ghrelin concentrations in preterm infants and to determine the usefulness of measuring these peptides in clinical practice.

References

18. Baker J, Berseth CL. Postnatal change in inhibitory regulation of


