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High Circulating Ghrelin: A Potential Cause for Hyperphagia and Obesity in Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is a genetic disorder occurring in 1 of 10,000–16,000 live births and is characterized by excessive appetite with progressive massive obesity as well as short stature and mental retardation. Most patients have GH deficiency and hypogonadotropic hypogonadism. The causes of the hyperphagia and abnormal GH secretion are unknown. To determine whether ghrelin, a novel GH secretagogue with orexigenic properties, is elevated in PWS, we measured fasting plasma ghrelin concentration; body composition (dual-energy x-ray absorptiometry); and subjective ratings of hunger (visual analog scale) in seven subjects (6 males and 1 female; age, 26 ± 7 yr; body fat, 39 ± 11%, mean ± SD) with PWS (diagnosis confirmed by genetic test) and 30 healthy subjects (reference population, 15 males and 15 females; age, 32 ± 7 yr; body fat, 36 ± 11%) fasted overnight. All subjects were weight stable for at least 6 months before admission to the study. The mean plasma ghrelin concentration was higher in PWS than in the reference population (307 ± 164 *vs.* 109 ± 24 fmol/ml; $P < 0.001$), and this difference remained significant after adjustment for percentage body fat ($P < 0.001$). Plasma ghrelin was also higher ($P = 0.0004$) in PWS than in five healthy subjects fasted for 36 h. A positive correlation was found between plasma ghrelin and subjective ratings of hunger ($r = 0.71$; $P = 0.008$). Furthermore, in subjects with PWS, the concentration of the hormone was not different before and after ingestion of 2 ml and a satiating amount of the same liquid meal (ghrelin concentrations: 307 ± 164 *vs.* 306 ± 205 *vs.* 260 ± 134 fmol/ml, respectively; ANOVA for repeated measures, $P = 0.56$). This is the first evidence that ghrelin, a novel orexigenic hormone, is elevated in subjects with PWS. Our finding suggests that ghrelin may be responsible, at least in part, for the hyperphagia observed in PWS. (*J Clin Endocrinol Metab* 87: 5461–5464, 2002)

PRADER-WILLI SYNDROME (PWS) is a genetic disorder occurring in 1 of 10,000–16,000 live births. The vast majority of cases occur sporadically. Approximately 70–75% are due to a deletion of the proximal long arm of the paternally derived chromosome 15 (15q11,q13), 20–25% to maternal disomy of chromosome 15, 2–5% to imprinting mutations, and 1% to translocations (1). The condition, first described almost 50 yr ago (2), is characterized by excessive appetite with progressive massive obesity as well as short stature, low lean body mass, muscular hypotonia, mental retardation, behavioral abnormalities, and dysmorphic features (3). Most patients have GH deficiency and hypogonadotropic hypogonadism, suggesting hypothalamic-pituitary dysfunction (4). Obesity occurs in over 90% of affected individuals (5) and is the most prominent physical characteristic of untreated PWS as well as a major cause of morbidity and mortality (6). Infants with PWS usually experience feeding difficulties throughout the first year of life (due to a weak suckling response and poor head and neck control). Between 1 and 2 yr of age, the severe hypotonia of early infancy resolves, leading to the onset of hyperphagia and abnormal eating behaviors, including obsessive food seeking, hoarding, gorging, and consumption of nonfood items (7). The cause of this hyperphagia remains unknown, and there is no current effective pharmacological treatment.

Abbreviations: PWS, Prader-Willi syndrome.

We have recently shown that the novel hormone ghrelin, which is predominantly secreted by the stomach, induces adiposity by increasing food intake and decreasing fat utilization in rodents, an effect that is independent from the ability of the hormone to stimulate GH secretion (8, 9). Intracerebroventricular administration of ghrelin antiserum decreases food intake in rodents, indicating a central role of endogenous ghrelin in the control of energy balance (8). Plasma ghrelin concentration increases before every meal and decreases after nutrient intake (10, 11), suggesting a role in meal initiation. Plasma ghrelin levels are also lower in obese compared with lean individuals (12), suggesting that both acute and chronic changes in energy balance affect circulating ghrelin concentrations. Peripheral ghrelin administration at doses near the physiological range increases appetite and food intake in humans (13, 14), indicating that ghrelin is a peripheral signal to the brain to stimulate food intake.

We hypothesized that because ghrelin affects appetite and GH secretion, both of which are abnormal in PWS, disordered ghrelin secretion may be present in this condition. We report herein that fasting plasma concentrations of ghrelin were higher in PWS compared with healthy controls.

Subjects and Methods

All subjects were participants in studies of the pathogenesis of obesity at the Clinical Diabetes and Nutrition Section of the National Institutes of Health (Phoenix, AZ). Subjects with PWS were also participants in a

study of the neuroanatomical correlates of hunger, taste, and satiation (15). Some data from the reference population (15 males and 15 females; age, 32 ± 7 yr; body fat, 36 ± 11%) have been previously published (12, 16). A group of five control subjects (4 males and 1 female; age, 33 ± 7 yr; body fat, 28 ± 7%) who had undergone a prolonged fast (17) were also considered for some analyses. Apart from PWS, subjects were healthy and not taking any medication, as determined by medical history, physical examination, and laboratory screening tests. Among PWS patients (Table 1), subject 3 was taking testosterone cypionate (200 mg/month) until 14 d before admission, and subject 4 was taking recombinant GH (2.55 mg/d) until 19 months before admission. All subjects were weight stable for at least 6 months before admission.

The diagnosis of PWS was confirmed with a genetic test before admission. Diabetes was excluded by an oral glucose tolerance test (18). Women were studied in the follicular phase of their menstrual cycle. The protocol was approved by the Institutional Review Boards of the National Institute of Diabetes and Digestive and Kidney Diseases and the Indian Health Service, and all subjects gave written consent before participation. A team of independent consultants (*i.e.* psychiatrists and psychologists not directly associated with the study) evaluated each subject with PWS to assess the level of competence to understand all experimental procedures and to provide informed consent. Also, before consenting, individuals with PWS viewed a videotape detailing the main procedures of the study.

On admission, all subjects were placed on a weight-maintaining diet (50% carbohydrate, 30% fat, 20% protein), initially calculated on the basis of gender, weight, and height, and subsequently adjusted to maintain body weight within 1% of the weight. For individuals with PWS, the diet administered on the ward was designed to include food items that were familiar to the subject. Physical activity during admission was limited to sedentary activities, such as playing table games and watching TV, and an occasional supervised walk. Body composition was assessed by dual energy x-ray absorptiometry (DPX-l, Lunar Corp. Co., Madison, WI) as previously described (19). After at least 3 d on the weight maintenance diet and after either an overnight fast (PWS and reference population) or a 36-h fast (control subjects), blood samples were collected between 0600 and 0900 h. At the same time, PWS and control subjects were asked to rate their feelings of hunger on a 0–100 mm visual analog scale. Individuals with PWS ingested 2 ml and then a satiating amount (consumed over 25 min and delivering 50% of the subject's daily energy requirement) of a liquid formula meal (56% carbohydrate, 29% fat, 15% protein; Ensure Plus, 1.5 kcal/ml; Ross-Abbott Laboratories, Columbus, OH). A blood sample was collected after each of the two ingestions.

Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma ghrelin concentrations were determined using a commercially available RIA (Phoenix Pharmaceuticals, Inc., Mountain View, CA) as previously described (12). Because of the high ghrelin concentration in patients with PWS, ghrelin was measured at several dilutions between 1:1 and 1:20 to ensure validity and reproducibility of the measurements. Mean linearity of these samples was between 80% and 143% for dilutions of 1:5 and 1:10. Final measurements of all samples in one assay were performed on the basis of a 1:7 dilution, which was expected to generate concentrations close to the ED_{50} (57.6 fmol/ml) of the RIA.

Statistical analyses were conducted using the procedures of the SAS Institute (Cary, NY). Results are given as mean ± SD unless indicated otherwise. Differences between individuals with PWS and a reference population were tested using a general linear regression analysis with adjustment for percentage body fat. The relationship of plasma ghrelin concentration with subjective ratings of hunger was assessed by Spearman rank correlation analysis. Changes of plasma ghrelin concentrations in response to the meal were tested by ANOVA for repeated measures.

Results

Characteristics of the seven subjects with PWS are presented in Table 1. Fasting plasma ghrelin concentrations were negatively correlated with adiposity both in individuals with PWS ($r = -0.81$; $P = 0.027$) and in the reference population ($r = -0.49$; $P = 0.006$; Fig. 1, *top*). The mean fasting plasma ghrelin concentration was higher in individuals with PWS compared with the reference population (307 ± 164 *vs.* 109 ± 24 fmol/ml; $P < 0.001$), and this difference remained significant after adjustment for percentage body fat ($P < 0.001$). After excluding the PWS subject with the lowest percentage body fat and the highest ghrelin (Table 2, subject 1), the mean fasting plasma ghrelin concentration in the PWS group was 256 ± 99 fmol/ml and was still significantly higher than the reference population ($P < 0.001$). The mean fasting plasma ghrelin concentration was also higher in individuals with PWS compared with individuals fasted for 36 h (106 ± 62 fmol/ml plasma ghrelin; Fig. 1, *bottom*). We found suggestive evidence for a positive correlation between fasting plasma ghrelin concentration and subjective ratings of hunger (Fig. 2). In individuals with PWS, the mean plasma ghrelin concentration was not different before and after ingestion of 2 ml and a satiating amount of the same liquid meal (ghrelin concentrations: 307 ± 164 *vs.* 306 ± 205 *vs.* 260 ± 134 fmol/ml, respectively; $P = 0.56$, ANOVA for repeated measures). Glucose and insulin exhibited the expected postmeal increases (glucose, 4.8 ± 0.3 *vs.* 4.9 ± 0.4 *vs.* 6.6 ± 0.7 mmol/liter; insulin, 2.3 ± 2.1 *vs.* 2.4 ± 2.2 *vs.* 30.6 ± 29.7 mU/ml; both $P < 0.001$, ANOVA for repeated measures).

Discussion

PWS is the most common genetic form of obesity, but the cause of hyperphagia in this condition remains unknown. We report that fasting plasma concentrations of ghrelin, a novel orexigenic hormone, were 3-fold higher in seven adult individuals with PWS compared with healthy controls. We suggest that high ghrelin levels are responsible, at least in part, for the hyperphagia and obesity of PWS.

Mechanisms underlying elevated circulating ghrelin in PWS are unclear. The ghrelin gene is located on chromosome

TABLE 1. Characteristics of seven adult individuals with PWS

Subject	Gender	Age (yr)	Weight (kg)	Height (cm)	Body fat (%)	Ghrelin (fmol/ml)	Genetic test
1	M	27	49	152	19	616	Methylation assay
2	M	32	62	165	36	367	FISH
3	M	18	90	178	39	360	FISH
4	M	19	81	170	47	297	FISH
5	M	38	65	149	48	196	Methylation assay
6	M	27	70	156	33	181	Methylation assay
7	F	20	87	156	52	133	HRCA
Mean ± SD		25.9 ± 7.4	71.9 ± 14.7	161 ± 10	39 ± 11	307 ± 164	

M, Male; F, female; FISH, fluorescence *in situ* hybridization; HRCA, high resolution chromosomal analysis.

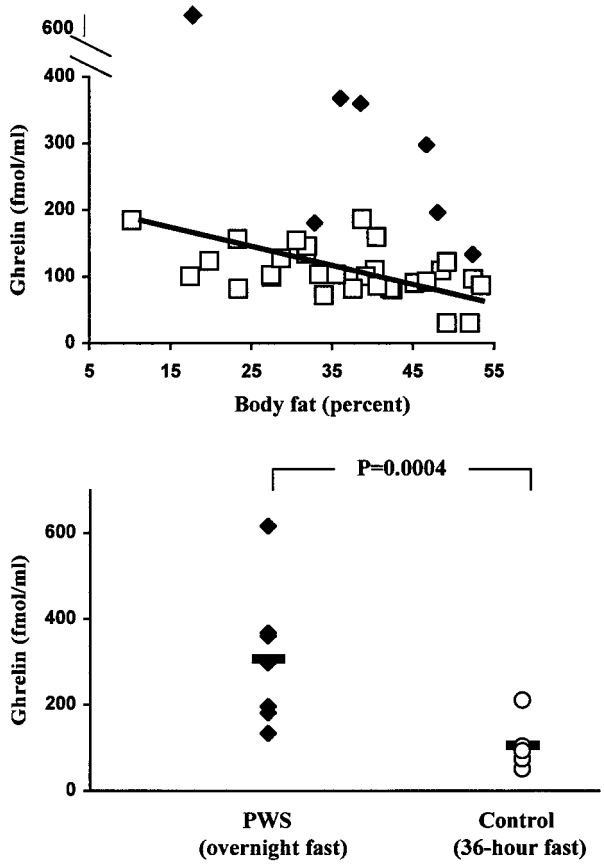

FIG. 1. Overnight fasting plasma ghrelin concentrations in subjects with PWS and a reference population, as well as 36-h fasting values in a control group. *Top*, Plasma ghrelin concentrations were negatively correlated with adiposity in subjects with PWS (♦, r = −0.81; $P = 0.027$) and in a reference population (□, r = −0.49; $P = 0.006$). The regression line applies only to the reference population. Using multiple linear regression analyses, the mean plasma ghrelin concentration was higher in PWS than the reference population after adjustment for age, sex, and percentage body fat ($P < 0.001$). *Bottom*, The mean plasma ghrelin concentration was higher in subjects with PWS (♦) than a group of control subjects (○) who had been fasted for 36 h.

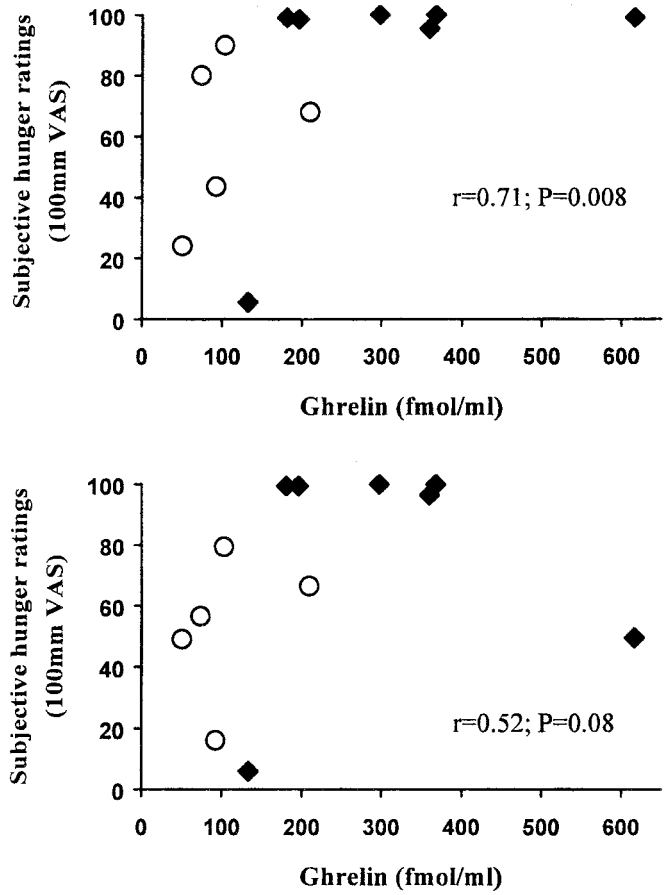

FIG. 2. Relationship between plasma ghrelin concentrations and subjective ratings of hunger. Individuals with PWS (♦, overnight fast) and controls (○, 36-h fast) were asked to rate their subjective sensation of hunger in response to the questions "How much food do you think you could eat right now?" (*top*) and "How hungry do you feel?" (*bottom*) on a 100-mm visual analog scale, ranging from 0 (nothing at all/not at all hungry) to 100 (a large amount/very hungry). The relationship of plasma ghrelin concentrations with subjective ratings of hunger was assessed by Spearman rank correlation analysis.

3 [http://www.ncbi.nlm.nih.gov/omim/, Online Mendelian Inheritance in Men (MIM no. 605353); Scott, A. F., personal communication in OMIM], whereas PWS arises from functional loss of several paternally expressed genes in an imprinted domain on chromosome 15. Because the majority of these genes appear to have regulatory functions (20), it is likely that the PWS genes regulate the expression of other genes at other loci, which represent the true genetic pathways that underlie the phenotypic features of PWS. Ghrelin signaling may represent one of these pathways.

Plasma ghrelin levels have not been reported to be elevated in individuals with GH deficiency, and GH replacement therapy for 1 yr did not modify circulating ghrelin levels (21), indicating that a GH-ghrelin feedback mechanism is unlikely to explain our findings. Among known physiological regulators of ghrelin secretion, macronutrient manipulations of the diet (22) and acute energy restriction (23) are also unlikely explanations of our findings, because the subjects with PWS in this study were on a weight-maintaining diet for at least 3 d before testing, yet had even higher ghrelin levels than individuals who were fasted for 36 h. Interestingly, the previously reported decrease in circulating ghrelin after a meal (10, 11) was not observed in the subjects with PWS in this study. Vagotomy has been shown to increase plasma ghrelin levels significantly (22). Secretion of pancreatic polypeptide, a recognized marker of parasympathetic nervous system drive to the splanchnic organs in response to a meal, has been reported to be deficient in PWS (24), which suggests that further exploration of the role of the autonomic nervous system in regulating ghrelinemia is warranted.

Plasma ghrelin concentrations in the same range as that of the PWS subjects in this study have previously been observed in cachectic states associated with anorexia (25, 26) and chronic heart failure (27). Although some of the PWS subjects in the present study were successfully maintaining a body weight below the clinical definition of obesity, the body composition data do not indicate cachexia in this group of people. Finally, the negative correlation between ghrelin and adiposity previously reported by us and others was also

observed among individuals with PWS. This suggests that although PWS produces a rightward shift in the relationship of ghrelin with adiposity, this genetic condition does not override the physiological mechanism that causes ghrelin to be lower in obese than in lean individuals.

We report a positive correlation between plasma ghrelin and subjective ratings of hunger, which is consistent with the orexigenic properties of the hormone. However, caution should be used in the interpretation of these results, because, to the best of our knowledge, visual analog scales for the assessment of hunger have not been validated in individuals with PWS.

In summary, we provide the first evidence [while preparing this manuscript, we became aware that similar findings were obtained by Dr. D. E. Cummings and colleagues in a separate group of 18 PWS subjects (28)] that ghrelin, a novel orexigenic hormone, is elevated in patients with PWS. Our data are consistent with the hypothesis that ghrelin is a physiological regulator of appetite in humans and suggest that high ghrelin levels may be responsible, at least in part, for the hyperphagia observed in PWS. Proof of this hypothesis will require intervention studies using agents that block ghrelin secretion and/or action and their effect on food intake.

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