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Hyperghrelinemia Does Not Accelerate Gastric Emptying in Prader-Willi Syndrome Patients

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Prader-Willi syndrome (PWS) is the most common form of syndromic obesity associated with hyperphagia. Because ghrelin stimulates gastric motility in rodents, and PWS patients have 3- to 4-fold higher fasting plasma ghrelin concentrations than normal subjects, we hypothesized that hyperphagia associated with PWS may be partly explained by rapid gastric emptying due to the increased gastric motility caused by ghrelin. We determined gastric emptying times (GETs) and measured ghrelin levels in 11 PWS children and 11 age-, sex-, and body mass index-matched controls using a standard meal containing [^{99m}Tc]diaminetriaminepentacetate. Median plasma ghrelin levels before (precibum) and after the GET study were higher in PWS patients than in controls ($P = 0.004$ and $P = 0.001$, respectively). Median percent gastric retentions at 90 min after the standard meal were 57.1% (range, 34.0–83.2%) in PWS patients and 40.2% (range, 27.2–60.2%) in controls ($P = 0.03$). In particular, precibum ghrelin concentrations were not significantly correlated with the rate of gastric emptying in PWS patients ($P = 0.153$; $r = 0.461$) or controls ($P = 0.911$; $r = 0.048$). Our results show that gastric emptying in PWS is reduced despite higher ghrelin levels, and that the voracious appetite associated with PWS is related to another mechanism. **(*J Clin Endocrinol Metab* 90: 3367–3370, 2005)**

PRADER-WILLI SYNDROME (PWS) is the most common form of human syndromic obesity. Infants with PWS present with diminished fetal activity and muscular hypotonia. However, excessive eating habits and food foraging in early childhood result in obesity in most cases. In terms of eating habits, subjects with PWS tend to eat steadily until food is unavailable and, on the average, consume 3 times more calories than normal (1). It is remarkable to observe that feelings of hunger start to reemerge shortly after food has been removed from PWS patients (1) regardless of the amount of food consumed. Moreover, it is well known that PWS patients hardly ever vomit, which is a supportive diagnostic finding in PWS (2). These characteristics of PWS led us to hypothesize that PWS patients may show accelerated gastric emptying.

Ghrelin is a centrally acting food intake stimulator (3, 4) and is secreted by X/A-like endocrine cells of the stomach oxyntic mucosa. It is a GH-releasing peptide and is considered a member of the motilin-related family of regulatory peptides (5).

Recently, the effect of ghrelin on gastroduodenal motility in a conscious rat model was described (6). Ghrelin administered into the lateral cerebral ventricle or a tail vein of fasted animals potentiated phase III-like contractions in the antrum and duodenum (6). Another study showed that ghrelin stimulates gastric emptying in mice, but that it has no discernable effect on acid secretion by gastric endocrine cells in rats (7).

PWS patients have 3- to 4-fold higher plasma fasting ghrelin concentrations than normal subjects, which tend to persist at this level (8, 9). Moreover, several studies have found that high levels of circulating ghrelin are associated with an increased appetite and obesity in PWS (10, 11). However, the mechanism by which elevated ghrelin affects the appetite has not been clearly elucidated in this syndrome. Therefore, we hypothesized that elevated ghrelin levels accelerate gastric emptying, thus inducing hunger, reducing the tendency to vomit, and resulting in the voracious eating characteristic of PWS.

To confirm this hypothesis we determined gastric emptying times (GETs) in PWS children and in age-, sex-, and body mass index (BMI)-matched controls and measured the corresponding plasma ghrelin levels.

Subjects and Methods

Subjects

Eleven PWS children (median age, 8.0 yr; range, 6–17 yr; male/female ratio, 6/5; median BMI, 22.2 kg/m^2; range, 16.7–47.1 kg/m^2) and 11 age-, sex-, and BMI-matched controls (median age, 9.0 yr; range, 6–16 yr; male/female ratio, 6/5; BMI, 23.3 kg/m^2; range, 16.7–46.0 kg/m^2) were enrolled in this study. Each of the PWS subject was individually matched with a control subject. PWS patients had typical symptoms and were genetically confirmed using the standard methylation test. Of the 11 PWS patients, nine harbored a deletion of the paternally transmitted chromosome 15q11, and two had uniparental disomy. The details of the PWS patients' characteristics are described in Table 1. Control subjects were volunteers recruited in either a hospital or a school. The criteria for control enrollment included the absence of a history of significant gastrointestinal disease, disturbed glucose metabolism, neuromuscular dis-

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Abbreviations: BMI, Body mass index; GET, gastric emptying time; PWS, Prader-Willi syndrome.

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TABLE 1. Age, sex, height, body weight, BMI, genotype, and tissue fat percentage of the PWS patients

Patient no.	Age (yr)	Sex	Height (cm)	Body weight (kg)	BMI	Genotype	Tissue fat (%)
1	6	F	124	31.5	20.5	Deletion	41.7
2	6	M	116	36.5	27.1	Deletion	53.5
3	6	F	105	18.5	16.8	Deletion	49.3
4	7	F	131.5	33	19.1	Deletion	39.1
5	8	M	133.3	39.5	22.2	Deletion	49.8
6	8	M	128.5	27.5	16.7	UPD	48.3
7	11	M	138	60	31.5	Deletion	50.5
8	12	F	139	37.5	19.4	UPD	30.4
9	12	F	143.5	61.3	29.8	Deletion	52.2
10	13	M	152.5	53	22.8	Deletion	47.7
11	17	M	142	95	47.1	Deletion	67.9

Controls were matched for age, sex (F, female; M, male), and BMI. UPD, Uniparental disomy.

ease, or the taking of any medication. Tissue fat percentage was measured by dual energy absorptiometry in PWS patients, but it was not measured in controls. The study design was reviewed and approved by the Samsung Medical Center institutional review board, and the parents of the participants provided informed consent.

GETs

After an overnight fast of 12 h, PWS patients and control subjects were imaged at the Department of Nuclear Medicine at our institute. On the morning of the test, subjects were fed a standard meal, which consisted of one egg cooked with 74 MBq [^{99m}Tc]diaminetriaminepentacetate, a bowl of rice, soup, and 50 ml water. They were instructed to consume the total test meal within 10 min, and we ensured that the egg had been consumed by all subjects. Scintigraphic imaging began within 1–2 min of the completion of the test meal. Each patient was imaged using a dual head γ-camera (Biad XLT, Trionix Research Laboratory, Twinsburg, OH) equipped with a low energy. all purpose collimator using a symmetric 20% energy window centered at the 140-KeV ^{99m}Tc peak. Subjects were imaged standing with cameras located anteriorly and posteriorly. The acquisition consisted of a series of 1-min static images acquired every 10 min for 120 min. Between images, subjects sat immediately outside the imaging room and were asked to refrain from any significant activity. For data processing, stomach counts on static images were measured from regions of interest that delineated the stomach boundary. The geometric mean of the total stomach counts of anterior and posterior images was used to calculate percent gastric retention at 90 min and the half-emptying time ($t_{1/2}$).

ELISA for ghrelin

Plasma ghrelin levels were measured before (precibum ghrelin) and after (postcibum ghrelin) the GET study in all subjects. Ghrelin levels in plasma were measured using a commercially available ghrelin ELISA kit (Phoenix Pharmaceuticals, Belmont, CA). The details of the procedure have been previously described (8). All samples were measured in triplicate.

Statistical analysis

Data normality was tested using the Shapiro-Wilkes test. If data deviated from a normal distribution, the Wilcoxon signed-rank test was used (*i.e.* for postcibum ghrelin and $t_{1/2}$). The paired *t* test was used to determine differences between percent gastric retentions at 90 min and precibum plasma ghrelin levels, which were normally distributed. Data are expressed as medians and ranges.

Results

Median percent gastric retentions at 90 min were 57.1% (range, 34.0–83.2%) in PWS patients and 40.2% (range, 27.2–60.2%) in controls ($P = 0.03$, by paired *t* test); their corresponding $t_{1/2}$ values were 102.2 min (range, 52.3–254.8 min) and 62.8 min (range, 37.1–98.2 min), respectively ($P = 0.049$, by Wilcoxon signed-rank test; Fig. 1).

Median plasma ghrelin levels before the GET study were 19,600 pg/ml (range, 6,490–52,060 pg/ml) in PWS patients and 6,298 pg/ml (range, 1,010–10,610 pg/ml) in controls ($P = 0.003$); after the GET study, they were 11,762 pg/ml (range, 3,770–38,160 pg/ml) in PWS patients and 3,060 pg/ml (range, 1,190–10,160 pg/ml) in controls ($P = 0.001$, by Wilcoxon signed-rank test). There was no significant correlation between BMI and the rate of gastric emptying in PWS patients (BMI *vs.* $t_{1/2}$: $P = 0.096$; $r = -0.527$; BMI *vs.* 90 min after the standard meal: $P = 0.259$; $r = -0.373$) or in control subjects (BMI *vs.* $t_{1/2}$: $P = 0.606$; $r = 0.217$; BMI *vs.* 90 min: $P = 0.967$; $r = -0.017$) or between the percentage of body fat and the rate of gastric emptying in PWS patients (fat percentage *vs.* $t_{1/2}$: $P = 0.272$; $r = -0.364$; fat percentage *vs.* 90 min after the standard meal: $P = 0.612$; $r = -0.173$).

Moreover, precibum ghrelin concentrations were not significantly related to the rate of gastric emptying in PWS patients (ghrelin *vs.* $t_{1/2}$: $P = 0.250$; $r = 0.379$; ghrelin *vs.* 90 min after the standard meal: $P = 0.153$; $r = 0.048$) or in control subjects (ghrelin *vs.* $t_{1/2}$: $P = 0.433$; $r = -0.324$; ghrelin *vs.* 90 min: $P = 0.911$; $r = 0.048$; Table 2). Postcibum ghrelin concentrations were not significantly related to the rate of gastric emptying in PWS patients (ghrelin *vs.* $t_{1/2}$: $P = 0.340$; $r = 0.318$; ghrelin *vs.* 90 min after the standard meal: $P = 0.179$; $r = 0.436$) or in controls (ghrelin *vs.* $t_{1/2}$: $P = 0.501$; $r = -0.227$; ghrelin *vs.* 90 min: $P = 0.417$; $r = -0.272$).

Discussion

To our knowledge this is the first investigation of gastric emptying in PWS. Before commencing the study, we believed that PWS patients would show rapid gastric emptying because of their characteristic voracious appetites and tendency not to vomit, and because they have 3- to 4-fold higher plasma ghrelin concentrations than normal (8), which are known to stimulate gastric motility in rodents (6, 7). However, our results contradicted this expectation. In the event, GET in PWS patients was found to be delayed, and precibum and postcibum ghrelin concentrations were not significantly related to gastric emptying rates in PWS patients or controls.

Two recent animal studies suggested that ghrelin promotes gastric emptying (6, 7). However, only one study determined a relationship between ghrelin and gastric motility in man (12). This study showed that the iv administration of ghrelin stimulated appetite and food intake in nine healthy volunteers. However, the same study found that ghrelin had

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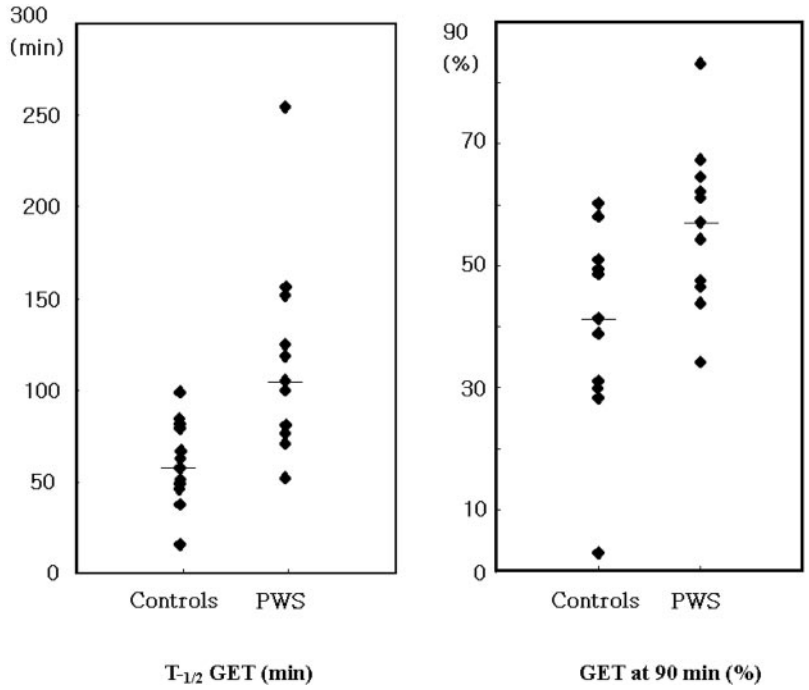

FIG. 1. Median percent gastric retentions at 90 min after the standard meal and GET $t_{1/2}$ values in PWS and control subjects. Median percent gastric retentions at 90 min after the standard meal and the corresponding $t_{1/2}$ values were increased in PWS patients *vs.* controls ($P = 0.03$ and $P = 0.049$, respectively).

no effect on gastric emptying, as assessed by the paracetamol absorption test (12). Despite the small number of subjects involved, these findings suggest that human and animal ghrelin function in different ways, at least in terms of gastric emptying. Our findings confirm that although there is a large difference in ghrelin levels in PWS and normal control subjects, these elevated ghrelin levels are associated with a reduction in the rate of gastric emptying in PWS.

A possible mechanism for delayed GETs in PWS may be the obesity itself. Jackson *et al.* (13) found delayed gastric emptying in obese women using a noninvasive nonradioactive method, after adjusting for the effects of potential confounders, particularly for age, gender, and exercise. They concluded that this delay may be a consequence of a high fat diet, a sedentary lifestyle, or increased gastric distension associated with obesity, which also suggests that delayed gastric emptying is a contributory factor in the pathogenesis of obesity due to the inactivation of gastrointestinal satiety signals and an increase in food intake. It is known that obese persons have lower plasma ghrelin levels than healthy lean controls and delayed gastric emptying times (14). However, gastric motility in obese subjects with elevated ghrelin levels has not been previously reported.

In the present study PWS children with elevated ghrelin levels showed delayed gastric emptying. However, the present study is limited by the relatively small number of patients enrolled between 6 and 17 yr of age. Thus, a study of adult PWS patients may broaden the understanding of the relationship between ghrelin and gastric empting. Also, it is known that young PWS patients (even underweight children) have increased levels of body fat, and that BMI has its limitations as a comparable obesity index (15). Thus, our controls may not ideally match PWS patients, and tissue fat percentage measured by dual energy absorptiometry may be a better index to match. However, we did not measure the tissue fat percentage in controls.

Taken together, the above observations support the idea that gastric emptying is independent of plasma ghrelin levels and that gastric emptying is delayed in PWS children. We conclude that the voracious appetite of PWS patients is probably related to another mechanism, such as the action of

TABLE 2. Gastric emptying rates and ghrelin levels in PWS patients and controls

	No.	Age (yr)	Sex (M/F)	BMI (kg/m^2)	Fat (%)	$t_{1/2}$, median (min, range)	GET at 90 min, median (%, range)	Precibum ghrelin (ng/ml), median (range)	Postcibum ghrelin (ng/ml), median (range)
PWS	11	8.0 (6–17)	6/5	22.2 (16.6–47.1)	49.3 (30.4–67.9)	102.2 (52.3–254.8)[a]	57.1 (34.0–83.2)[b]	19.6 (6.49–52.1)[c]	11.8 (3.77–38.1)[d]
Control	11	9.0 (6–16)	6/5	23.3 (16.7–46.0)		62.8 (37.1–98.2)	40.2 (27.2–60.2)	6.30 (1.01–10.6)	3.06 (1.19–10.16)

M, Male; F, female.
[a] $P = 0.049$ *vs.* control.
[b] $P = 0.03$ *vs.* control.
[c] $P = 0.003$ *vs.* control.
[d] $P = 0.001$ *vs.* control.

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ghrelin on the central nervous system, but this speculation remains to be elucidated.

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