Circulating Ghrelin Levels Are Suppressed by Meals and Octreotide Therapy in Children with Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is characterized by severe obesity, hyperphagia, hypogonadism, and GH deficiency. Unlike individuals with common obesity, who have low fasting-plasma ghrelin concentrations, those with PWS have high fasting-ghrelin concentrations that might contribute to their hyperphagia. Treatment with octreotide, a somatostatin agonist, decreases ghrelin concentrations in healthy and acromegalic adults and induces weight loss in children with hypothalamic obesity. This pilot study was performed to determine whether octreotide administration (5 μg/kgd) for 5–7 d lowers ghrelin concentrations and affects body composition, resting energy expenditure, and GH markers in children with PWS. Octreotide treatment decreased mean fasting plasma ghrelin concentration by 67% (P < 0.05). Meal-related ghrelin suppression (~35%; P < 0.001) was still present after intervention but was blunted (~11%; P = 0.19). Body weight, body composition, leptin, insulin, resting energy expenditure, and GH parameters did not change. However, one subject's parent noted fewer tantrums over denial of food during octreotide intervention. In conclusion, short-term octreotide treatment markedly decreased fasting ghrelin concentrations in children with PWS but did not fully ablate the normal meal-related suppression of ghrelin. Further investigation is warranted to determine whether long-term octreotide treatment causes sustained ghrelin suppression, changes eating behavior, and induces weight loss in this population. (J Clin Endocrinol Metab 88: 3573–3576, 2003)

The hypothalamic peptide ghrelin is an endogenous ligand for the GH secretagogue receptor (1) and is also a potent orexigenic in humans and other animals (2–5). Ghrelin has been implicated in the short- and long-term regulation of appetite and body weight, as well as in GH secretion (6). At least some of the orexigenic effects of ghrelin are mediated via activation of anabolic neurons in the hypothalamic arcuate nucleus that coexpress neuropeptide Y (NPY) and agouti-related protein (Agrp) (6).

Circulating ghrelin levels correlate negatively with body mass index (BMI) and are therefore low in people with common obesity, consistent with a compensatory rather than causal role for ghrelin in this condition (7–10). In an effort to identify subsets of subjects whose obesity might be caused by ghrelin overproduction, we predicted that the phenotype of such individuals would resemble that resulting from excessive activation of arcuate NPY/Agrp neurons, the central targets of ghrelin action. Phenotypic findings in animals receiving repeated central injections of NPY or Agrp include hyperphagia, obesity, GH dysregulation, and central hypogonadism (11–15).

Prader-Willi syndrome (PWS) is the most common form of human syndromic obesity, resulting from a disruption of several imprinted genes on chromosome 15 (16). Hallmark features include severe hyperphagia, obesity, GH deficiency, and hypogonadotropic hypogonadism (17), characteristics that overlap with the predicted phenotype of NPY/Agrp neuron overactivation and therefore might be compatible with ghrelin excess. Accordingly, we and others have measured ghrelin levels in children and adults with PWS and found levels to be three to five times higher than those in age- and BMI-matched controls (9, 10, 18). In contrast, all other forms of obesity (monogenic and polygenic) tested to date have been associated with low ghrelin levels. Hyperghrelinemia may therefore play a causal role in the hyperphagia, obesity, and dysregulation of hypothalamic hormone secretion of PWS. Consistent with the last of these hypotheses, ghrelin administration has been shown to suppress pulsatile LH secretion (19), which is also impaired in PWS (17). Finally, ghrelin was recently reported to influence slow-wave sleep (20), and people with PWS suffer abnormal sleep patterns (21, 22).

The hypothesis that hyperghrelinemia causes some of the features of PWS predicts that this disorder will be ameliorated (partially or completely) by lowering ghrelin levels. We have reported that ghrelin levels are lower after gastric bypass compared with obese controls (23); but this approach raises significant ethical issues for this group of patients who suffer from significant cognitive and emotional disturbances. Octreotide, a somatostatin agonist that inhibits secretion of

Abbreviations: Agrp, Agouti-related protein; BMI, body mass index; CV, coefficient(s) of variation; IGFBP, IGF binding protein; IRMA, immunoassay; LM, lean mass; NPY, neuropeptide Y; PWS, Prader-Willi syndrome; REE, resting energy expenditure.
many gut peptides, has recently been shown to suppress ghrelin levels by 5-fold in healthy adults and 2-fold in people with acromegaly (24, 25). Therefore, as a first step toward investigating whether octreotide might have therapeutic potential in PWS, we conducted a pilot study to determine whether short-term octreotide treatment could also suppress ghrelin levels in children with PWS.

**Subjects and Methods**

**Subjects**

All participating children had genetically confirmed PWS (two males, two females; mean age, 11.7 ± 4.6 yr) and were recruited from a larger, previously studied group (22). Of the four subjects recruited for the octreotide study, all had a deletion of paternal chromosome 15q11-13, two subjects were on GH therapy, and two had not received GH therapy previously (22). Of the four subjects recruited for the study, one had previously studied group (22). Of the four subjects recruited for the study, one had a mean age of 11.7 ± 4.6 yr and was recruited from a larger, previously studied group (22). Of the four subjects recruited for the octreotide study, all had a deletion of paternal chromosome 15q11-13, two subjects were on GH therapy, and two had not received GH therapy for at least 1 yr before the octreotide intervention. This study was approved by the Scientific Advisory Committee of the General Clinical Research Center and the Institutional Review Board of Oregon Health & Science University. A parent of each child gave written informed consent, and, when appropriate, each child provided assent before entry into the study.

**Protocol**

In 11 subjects, blood was drawn after an overnight fast between 0800 and 0900 h and again 1 h after a standardized breakfast meal (243 total calories; 83.8% carbohydrate, 4% fat, and 11.6% protein). In the four subjects treated with octreotide (5 μg/kg sc given at 0800, 1500, and 2100 h), fasting morning blood samples were collected at baseline and after 5–7 d of treatment. All samples were collected in plain (serum) and EDTA-containing (plasma) Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tubes, with and without aprotinin (0.6 TIU/ml of blood) added, immediately placed on ice, centrifuged within 30 min of sampling, and stored at −70 C until assayed. All samples were measured in duplicate.

**Hormone measurements**

Samples were assayed for total immunoreactive ghrelin concentration with a commercial RIA (Phoenix Pharmaceuticals, Belmont, CA). This assay uses a 125I-labeled ghrelin tracer and a rabbit polyclonal antibody against full-length, octanoylated human ghrelin that recognizes the acylated and des-acyl forms. Although only acylated ghrelin is bioactive (1), total ghrelin is a reasonable surrogate for the acylated form because the ratio of the two levels is constant under a wide variety of conditions (26, 27). The lower and upper detection limits were 80 and 2500 pg/ml, and the inter- and intrasay coefficients of variation (CV) were 5.9 and 9.8%, respectively.

Plasma samples were assayed for insulin using a modified double-antibody RIA (28). The lower and upper detection limits were 13 and 1680 pmol/liter, respectively, and the intraassay CV was less than 10%.

Plasma leptin levels were determined using a commercial RIA that uses a double-antibody/polyethylene glycol technique (Linco Research, St. Charles, MO). The lower and upper limits of detection were 0.5 and 100 ng/ml, and the intra- and interassay CVs were 5.0 and 5.5%, respectively.

IGF-I, free IGF-I, and IGF binding protein (IGFBP)-3 were all measured using commercial kits from Diagnostic Systems Laboratories, Inc. (Webster, TX). IGF-I concentrations were assessed in serum using an immunoradiometric assay (IRMA) with acid/ethanol extraction. The intra- and interassay CVs were 1.3–3.4% and 1.5–8.2%, respectively, and the sensitivity was 5 ng/ml. Free IGF-I assays were performed using an IRMA with intra- and interassay CV of 3.3–10.5% and 3.6–10.7%, respectively, and a sensitivity of 0.17 ng/ml. IGFBP-3 assays were performed using an IRMA with intra- and interassay CV of 1.8–3.9% and 0.5–1.9%, respectively, and a sensitivity of 2.0 ng/ml. Samples with values below the lowest or above the highest standard at the dilution recommended by the kit instructions were assayed again at appropriate dilutions to assure that values fell within the sensitive portion of the standard curve. Normal adult human serum from a pool of an equal number of 20- to 40-yr-old males and females was used as an internal standard in all IGF-I, free IGF-I, and IGFBP-3 assays.

**Metabolic and behavioral measurements**

Body composition was measured by bioelectrical impedance, and resting energy expenditure (REE) was determined by open-circuit indirect calorimetry using a ventilated hood collection system (Vmax 29n, SensorMedics Corp., Yorba Linda, CA) at baseline and again after octreotide administration. Measurements were conducted before blood was drawn, between 0600 and 0800 h, after an 8-h overnight fast, while subjects were awake but inactive and comfortably resting on a hospital bed. Each measurement lasted approximately 55 min or until subjects terminated the procedure. Food intake and eating behaviors were estimated by parent-reported recall of 24-h food intake.

**Statistical analysis**

Comparisons before and after meals and octreotide treatment were made by paired Student’s t tests, using SigmaStat, version 2.03 (SPSS Inc., Chicago, IL).

**Results**

All four subjects completed the octreotide treatment protocol. At baseline, subjects had a mean BMI of 36.1 kg/m² (range, 25–43 kg/m²) and 41.1% (37–47%) body fat. Fasting plasma ghrelin concentrations decreased by ~67% after 5–7 d of octreotide administration, from a mean ± sd of 277 ± 179 to 91 ± 73 pmol/liter (P < 0.05) (Table 1 and Fig. 1).

Ghrelin was suppressed 1 h after consumption of a standardized meal of fixed caloric content (243 calories, total; 83.8% carbohydrate, 4% fat, 11.6% protein) (~35%; P < 0.001) (Fig. 2). In the smaller group of octreotide-treated children, the average meal-related suppression before octreotide intervention was ~24% (preprandial vs. postprandial; 277 ± 179 vs. 210 ± 215 pmol/liter; P = 0.06) and ~11% after

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After octreotide intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (pmol/liter)</td>
<td>277 ± 179</td>
<td>91 ± 73*</td>
</tr>
<tr>
<td>Insulin (pmol/liter)</td>
<td>139.8 ± 18.6</td>
<td>175.2 ± 113.4</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>24.5 ± 6.6</td>
<td>24.3 ± 5.8</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>404 ± 149</td>
<td>455 ± 230</td>
</tr>
<tr>
<td>Free IGF-I (ng/ml)</td>
<td>1.2 ± 0.7</td>
<td>1.8 ± 2.2</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>3678 ± 497</td>
<td>3712 ± 944</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± sd.

* P < 0.05 vs. baseline.

**Fig. 1. Fasting ghrelin concentrations before and after octreotide treatment in four children with PWS.**
Body weight, body composition, and REE before and after octreotide intervention in 11 children with PWS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After octreotide intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>36.1 ± 9.9</td>
<td>35.9 ± 9.9</td>
</tr>
<tr>
<td>LM (kg)</td>
<td>39.6 ± 15.1</td>
<td>40.3 ± 15.0</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.0 ± 15.9</td>
<td>28.6 ± 15.7</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>41.1 ± 4.4</td>
<td>40.4 ± 5.5</td>
</tr>
<tr>
<td>REE (kcal/d)</td>
<td>1824.8 ± 338.5</td>
<td>1720.8 ± 186.3</td>
</tr>
<tr>
<td>REE/LM (kcal/kg LM)</td>
<td>49.3 ± 11.3</td>
<td>47.0 ± 15.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.

There was no significant difference between measurements of ghrelin concentration obtained in plasma samples with or without the protease inhibitor aprotinin (n = 4) [mean ± SD, with aprotinin, 240 ± 248 pmol/liter; without aprotinin, 277 ± 179 pmol/liter; P = 0.19].

Because octreotide suppresses secretion of a number of hormones, several other proteins and hormones were measured in this study. During this short-term octreotide treatment, no decreases in insulin or leptin were detected (Table 1). Likewise, serum concentrations of IGF-1, free IGF-1, and IGFBP-3 did not change with octreotide treatment (Table 1).

In addition, no significant changes in body weight, BMI, triceps skinfold, subscapular skinfold, midarm circumference, waist and hip circumferences, fat mass, lean mass (LM), or percentage of body fat, REE, and REE/LM occurred with octreotide intervention (Table 2). Parent-reported recall of energy intake did not change significantly after octreotide intervention (data not shown), although one subject’s parent noted fewer tantrums over denial of food during the octreotide intervention.

Three of four subjects reported mild diarrhea associated with octreotide therapy. No episodes of abdominal pain, flatulence, or other adverse events were observed during this short treatment duration.

**Discussion**

Circulating ghrelin concentrations are up to three times higher in individuals with PWS than in BMI-matched controls (9, 10, 18). It has been postulated that ghrelin, a possible long-term regulator of energy balance, may play a causal role in the severe hyperphagia and obesity of people with PWS. In this pilot study, we show that elevated ghrelin levels in children with PWS can be lowered to the normal range using a modest dose of octreotide. We also show that, in contrast to a recent study by DelParigi et al. (18), subjects with PWS demonstrate meal-related ghrelin suppression and this suppression is still present, albeit blunted, after octreotide therapy.

The mechanism underlying elevated ghrelin levels in PWS is unknown. One possibility is that decreased negative feedback from GH causes a compensatory increase in ghrelin. If this feedback mechanism exists, then GH treatment should decrease ghrelin concentrations. Others have shown, however, that ghrelin concentrations are not elevated in adults with GH deficiency, and GH therapy in these same individuals does not alter their ghrelin levels (29), a finding we have replicated in children with PWS (22). These findings argue against a physiologically important interdependence of ghrelin and GH regulation and strengthen our hypothesis that high ghrelin levels may be a primary consequence of the genetic lesion in PWS. Several deleted genes in PWS encode trans-acting proteins that could affect ghrelin expression, such as the SNURF-SNRPN, MAGEL2, and MKN3 loci. SNURF encodes a 71-amino acid nuclear protein that may bind to RNA. Small nucleolar proteins are methylation-guidance RNAs that are involved in the modification of rRNA and may play a role in the dysregulation of ghrelin in PWS (11). Such small nuclear proteins include SmN (encoded by exons 4–10 of SNRPN), a core spliceosomal protein involved in mRNA splicing. Finally, MKN3 encodes a polypeptide with a RING zinc finger and suggests that it is a ribonucleoprotein, which could alter gene expression. It should be emphasized, however, that the role, if any, of these genes in the dysregulation of ghrelin levels in PWS remains speculative at present.

Regardless of the mechanism leading to elevated ghrelin levels in PWS, our data demonstrate that ghrelin suppression can be achieved with a dose of octreotide that, in the short-term, does not adversely impact levels of insulin, leptin, or parameters of GH secretion. However, as mentioned in Subjects and Methods, half of the subjects treated with octreotide were receiving GH replacement. This, combined with the long half-life of some of the GH-associated proteins, could explain any lack of effect of octreotide on these parameters. Although we did not detect changes in body weight, body composition, or energy expenditure in this pilot study, meaningful conclusions about these outcomes are limited due to the small sample size and short duration. These data, however, set the stage for longer-term studies designed to determine whether octreotide treatment at optimal dosages can durably suppress ghrelin levels in people with PWS and, if so, whether this effect can ameliorate the hyperphagia and/or obesity of the condition.

In summary, the high fasting-plasma ghrelin concentrations of children with PWS are markedly suppressed by short-term octreotide administration. Meal-induced ghrelin suppression is present in children with PWS and is not fully attenuated by octreotide treatment. These findings indicate that octreotide may be a viable obesity treatment for patients with PWS. Long-term intervention studies are needed to
determine whether octreotide suppression of ghrelin will promote favorable changes in energy intake, body composition, energy expenditure, and biochemical parameters in these subjects.

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