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Plasma Cholecystokinin Levels in Prader-Willi Syndrome and Obese Subjects

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Abstract

The cardinal feature of individuals with Prader-Willi syndrome (PWS) is severe hyperphagia-mediated obesity resulting from a faulty satiety mechanism. PWS is the most common genetic cause of marked obesity. Cholecystokinin (CCK) is a 33-amino-acid peptide found in high levels in the gut and brain involved in mediating the satiety response to meals. Free fatty acids (FFA) are responsible for the stimulation of CCK release after a fatty meal, and CCK and plasma FFA levels rise in tandem in normal individuals. Fasting plasma CCK levels were measured by radio-immunoassay in 33 PWS subjects with a mean age of 22.2 years $\pm$ 8.1 years and 24 obese control subjects without a known cause of their obesity with a mean age of 28.7 years $\pm$ 12.9 years. Consistent with previous findings, neither fasting plasma FFA levels (617.5 versus 486.8 μ m/L) or CCK levels (21.0 versus 19.1 pg/mL) were significantly different in PWS or control subjects, respectively. However, there was a significant correlation between fasting plasma FFA and CCK levels in obese subjects ($r = 0.64$, $P < 0.01$), this correlation was completely lacking in PWS subjects ($r = -0.06$, $P = 0.79$). This difference in correlation coefficients constitutes a large effect. There were no significant effects observed for genetic subtypes (15q11-q13 deletion or maternal disomy 15), body mass index, percentage of fat, plasma levels of insulin, C-peptide, glucagon or leptin, age, or gender on CCK levels in our PWS subjects. These results suggest that differences in the peripheral CCK response to FFA levels may be a factor contributing to the altered satiety response in PWS subjects. *Am. J. Med. Genet.* 95:67–70, 2000.

Keywords

Prader-Willi syndrome; obesity; free fatty acid levels; cholecystokinin (CCK); genetic subtypes; satiety

INTRODUCTION

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, hypogonadism, hyperphagia, early onset childhood obesity, small hands and feet, and a characteristic face [Cassidy, 1984; Butler, 1990]. In 60% of subjects with PWS, a de novo paternally derived

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chromosome 15q11-q13 deletion is found, and the remaining subjects have either maternal disomy of chromosome 15 (both 15s from the mother) or a genetic imprinting defect [Butler et al., 1986; Butler, 1990; Cassidy, 1997].

PWS is the most common genetic cause of marked obesity with an incidence of 1:15,000 individuals [Butler, 1990]. A cardinal feature of individuals with PWS is severe hyperphagia-mediated obesity resulting from a faulty satiety mechanism. Cholecystokinin (CCK) is a 33-amino peptide found in high levels in the gut and brain involved in mediating the satiety response to meals through stimulation of the vagal nerve, which has a direct negative influence on the brain and feeding center of the hypothalamus. CCK is released by the duodenal mucosa in response to food intake through stomach distention and a high-fat content meal [Reidelberger, 1992, 1994; Hildebrand et al., 1998; McLaughlin et al., 1998]. Free fatty acids (FFA) are responsible for the stimulation of CCK release after a fatty meal, and CCK and plasma FFA levels rise in tandem in normal individuals [Marchini et al., 1993]. Previous reports indicate that baseline plasma CCK levels are not altered in PWS [Tomita et al., 1989; Holland et al., 1993, 1995]. Tomita et al. [1989] also reported a small increase in CCK levels after a protein meal in all groups of adults studied, including Prader-Willi, lean, and obese subjects. CCK release in response to eating a mixed meal was reportedly higher in PWS subjects compared with obese controls, but PWS subjects consumed 3 times more food [Holland et al., 1993]. In addition, a blunted or absent pancreatic polypeptide secretion in response to protein meals compared with normal weight controls have been reported in PWS subjects [Zipf et al., 1983]. Adults with PWS also have a smaller and delayed rise in plasma insulin levels and a relatively smaller than expected and delayed pancreatic polypeptide elevation. Whereas obese controls are reported to have hyperglycemia, markedly higher insulin, moderately higher pancreatic polypeptide, cholesterol, and triglyceride levels than PWS subjects [Tomita et al., 1989].

The intent of our study is to further characterize and analyze CCK levels and other peptides and biochemical markers in PWS and compare with obese subjects. Herein, we report fasting plasma CCK levels, other peptides and biochemical markers, and anthropometrics in 33 PWS subjects and 24 obese comparison subjects selected from a large series of subjects recruited for a program project study on PWS at Vanderbilt University (Nashville, TN).

MATERIALS AND METHODS

Each subject was fasting overnight in the Clinical Research Center at Vanderbilt University prior to collection of the blood sample into a tube containing aprotinin, and the plasma was immediately separated and frozen at -70°C for later use. Blood specimens were collected following informed consent. The CCK levels were measured commercially by Inter Science Laboratories (Englewood, CA) using radio-immunoassay and a specific antibody directed against CCK [Chang and Chey, 1983; Nakano et al., 1989]. Cross reactivity was determined by the laboratory at 50% inhibition of binding level. Cross reactivity for total CCK was 100%, gastrin 4%, secretin 2%, and combined reactivity for other peptides was less than 1%. The sensitivity determined as the least amount of CCK that can be distinguished from zero is 5 pg/mL. The normative range reported by their laboratory for CCK for humans was less than 80 pg/mL. Other peptides and biochemical markers directly or indirectly involved in the

regulation and control of appetite, including leptin, C-peptide, insulin, glucose, triglyceride, cholesterol, glucagon, and FFA levels were measured routinely on the same plasma specimen for comparison with CCK levels and with demographic data for each subject.

Twenty-four comparison obese subjects (16 women and 8 men) were evaluated in this study with an average age of 28.7 ± 12.9 years and an age range of 11 to 50 years. Thirty-three PWS subjects (20 women and 13 men; 24 with 15q11-q13 deletion and nine with maternal disomy 15) who were not treated with growth hormone or other hormone replacement were evaluated with an average age of 22.2 ± 8.8 years and an age range of 10 to 45 years. Age, gender, CCK, C-peptide, leptin, insulin, glucagon, glucose, cholesterol, triglyceride, and FFA levels for the subjects are shown in Table I.

RESULTS AND DISCUSSION

Statistical analyses included comparisons of group means by Student's *t*-tests and bivariate correlation coefficients. Table II shows correlation data for CCK and other variables in our obese and PWS subjects. Consistent with the previous findings, neither fasting plasma FFA or CCK levels were significantly different in PWS and obese subjects, respectively. There was a significant positive correlation between fasting plasma FFA and CCK levels in obese subjects ($r = 0.64$, $P < 0.01$) as reported in previous studies [Marchini et al., 1993]; however, this correlation was completely lacking in our PWS subjects ($r = -0.06$, $P = 0.79$). This difference between correlation coefficients constitutes a large effect. Figure 1 shows the scatter plot of CCK and FFA data for the obese and PWS subjects. Hence, a possible explanation for obesity and hyperphagia in PWS may be the lack of responsiveness of CCK release to rising plasma FFA levels following a high-fat meal or from stomach distention during eating. In healthy non-PWS individuals the correlated release of CCK from the gut occurs in this setting. The CCK release would provide vagal nerve stimulation directly influencing the brain and feeding center in the hypothalamus. Similarly, CCK circulation to the brain impacts eating behavior at the hypothalamus level leading to satiety in normal controls. However, this neuro/biochemical process may not function appropriately in PWS subjects. This process of regulatory control of appetite and satiety by CCK appears altered in PWS such that the relationship between plasma FFA concentrations and CCK release is abnormal in PWS compared with obese controls. A blunted CCK response to a high-fat meal in PWS subjects may contribute to their lack of satiety and hyperphagia. Our data imply that the more the PWS individual consumes food (e.g., high-fat meal or through stomach distention), then the more he or she is stimulated to eat due to the lack of FFA influence on CCK release and its effect on satiety. There were no significant effects observed for genetic subtypes (15q11-q13 deletion or maternal disomy 15), body mass index, percentage of fat, plasma levels of insulin, C-peptide, glucagon or leptin, age, or gender on CCK levels in our PWS subjects. Figure 2 shows the scatter plot of CCK data from the obese control and PWS genetic subtype groups.

In summary, the lack of correlation between fasting FFA and CCK levels in PWS subjects compared with obese subjects suggests that differences in the peripheral CCK response to FFA levels may be a factor contributing to the altered satiety response in PWS subjects.

Additional research is needed to further characterize (and clarify) the eating behavior and neuropeptide control of feeding and lack of satiety in PWS subjects.

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REFERENCES

- Butler MG. 1990 Prader-Willi syndrome: current understanding of cause and diagnosis. *Am J Med Genet* 35:319–332. [PubMed: 2309779]
- Butler MG, Meaney FJ, Palmer CG. 1986 Clinical and cytogenetic survey of 39 individuals with the Prader-Labhart-Willi syndrome. *Am J Med Genet* 23:793–809. [PubMed: 3953677]
- Cassidy SB. 1984 Prader-Willi syndrome. *Curr Probl Pediatr* 14:1–55.
- Cassidy SB. 1997 Prader-Willi syndrome. *J Med Genet* 34:917–923. [PubMed: 9391886]
- Chang T, Chey WY. 1983 Radioimmunoassay of cholecystokinin. *Dig DisSci* 28:456–468.
- Hildebrand P, Petrig C, Burckhardt B, Ketterer S, Lengsfeld H, Fleury A, Hadváry P, Beglinger C. 1998 Hydrolysis of dietary fat by pancreatic lipase stimulates cholecystokinin release. *Gastroenterology* 114:123–129. [PubMed: 9428226]
- Holland AJ, Treasure J, Coskeran P, Dallow J. 1995 Characteristics of the eating disorder in Prader-Willi syndrome: implications for treatment. *J Intellect Disabil Res* 39:373–381. [PubMed: 8555713]
- Holland AJ, Treasure J, Coskeran P, Dallow J, Milton N, Hillhouse E. 1993 Measurement of excessive appetite and metabolic changes in Prader-Willi syndrome. *Int J Obes Relat Metab Disord* 17:527–532. [PubMed: 8220655]
- Marchini G, Persson B, Uvnäs Moberg K. 1993 Metabolic correlates of behaviour in the newborn infant. *Physiol Behav* 54:1021–1023. [PubMed: 8248367]
- McLaughlin JT, Lomax RB, Hall L, Dockray GJ, Thompson DG, Warhurst G. 1998 Fatty acids stimulate cholecystokinin secretion via an acyl chain length-specific, Ca^{2+} -dependent mechanism in the enteroendocrine cell line STC-1. *J Physiol* 513:11–18. [PubMed: 9782155]
- Nakano I, Funkakoshi A, Shinozaki H, Sakai K. 1989 Plasma cholecystokinin and pancreatic polypeptide responses after ingestion of a liquid test meal rich in medium-chain fatty acids in patients with chronic pancreatitis. *Am J Clin Nutr* 49:247–251. [PubMed: 2916444]
- Reidelberger RD. 1992 Abdominal vagal mediation of the satiety effects of exogenous and endogenous cholecystokinin in rats. *Am J Physiol* 263: 1354–1358.
- Reidelberger RD. 1994 Cholecystokinin and control of food intake. *J Nutr[Suppl]* 124:1327S–1333S. [PubMed: 8064379]
- Tomita T, Greeley G Jr, Watt L, Doull V, Chance R. 1989 Protein meal-stimulated pancreatic polypeptide secretion in Prader-Willi syndrome of adults. *Pancreas* 4:395–400. [PubMed: 2668931]
- Zipf WB, O'Dorisio M, Cataland S, Dixon K. 1983 Pancreatic polypeptide responses to protein meal challenges in obese but otherwise normal children and obese children with Prader-Willi syndrome. *J Clin Endocr Metab* 57:1074–1080. [PubMed: 6352724]

Fig. 1. Scatter plot data from obese and PWS subjects showing plasma FFA and plasma CCK levels. The number of subjects, correlations, and P values are presented for each subject group.

Fig. 2. Scatter plot of CCK data from the obese control and PWS (deletion and maternal disomy) groups. There were no significant differences in the CCK levels among the three groups of subjects.

TABLE I.

Biochemical Data for PWS and Obese Subjects

Variables	PWS subjects					Control subjects					P value
	# Subjects	Mean	Standard deviation	Minimum	Maximum	# Subjects	Mean	Standard deviation	Minimum	Maximum	
Age (years)	33	22.2	8.8	10.3	44.8	24	28.7	12.9	11.0	49.6	<0.05 *
Sex			13 men; 20 women					8 men; 16 women			
CCK (pg/mL)	33	19.1	12.7	8.8	50.0	24	21.0	13.2	8.9	52.0	>0.05
C-peptide (ng/mL)	28	2.0	1.0	0.8	4.8	17	2.6	0.9	0.9	4.2	= 0.05
Leptin (ng/mL)	28	44.1	18.9	20.2	91.0	18	41.4	25.2	6.9	91.8	>0.05
Insulin (μ units/mL)	28	16.6	11.5	2.8	44.7	18	28.5	16.1	3.4	69.8	<0.01 *
Glucagon (pg/mL)	25	72.4	26.1	39.0	144.0	17	66.1	22.8	38.0	132.0	>0.05
FFA (μ M)	19	486.8	272.9	99.0	1,087.0	15	617.5	350.6	80.0	1,355.0	>0.05

* Statistically significant.

TABLE II.

Correlation Data for CCK and Other Variables in PWS and Obese Subjects

Variables	PWS subjects		Control subjects	
	Correlation	P value	Correlation	P value
Age versus CCK	0.21	0.24	0.38	0.07
Insulin versus CCK	-0.01	0.97	-0.01	0.97
C-peptide versus CCK	-0.03	0.87	0.18	0.48
Glucagon versus CCK	0.16	0.45	0.27	0.30
Leptin versus CCK	-0.12	0.54	0.34	0.16
FFA versus CCK	-0.06	0.79	0.64	0.01