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Elevated Serum Ghrelin Exerts an Orexigenic Effect that May Maintain Body Mass Index in Patients with Metastatic Neuroendocrine Tumors

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Abstract

Ghrelin is a potent orexigenic peptide principally produced in the stomach by a distinct population of neuroendocrine cells in the oxyntic mucosa of the fundus. Exogenous ghrelin given as an intravenous infusion has been shown to increase caloric intake in patients with cancer cachexia. In this study, we hypothesized that elevated endogenous ghrelin, produced by increased neuroendocrine cell tumor burden, also exerts an orexigenic effect helping to maintain body mass index. To evaluate the effect of elevated endogenous ghrelin, 35 patients with neuroendocrine tumors were enrolled, assigning them to one of two groups depending on the presence of hepatic metastases. Following an overnight fast, serum was collected and sent for ghrelin measurement by an outside laboratory. The two groups were well matched for all other relevant clinical variables including subtype of tumor, primary location of tumor and tumor treatment history. Nearly all patients with hepatic metastases had elevated levels of ghrelin compared to the standard reference range given for matched controls. The presence of hepatic metastases was associated with significantly elevated ghrelin levels ($p < 0.05$) and a greater mean body mass index. In addition, we report a positive correlation between serum ghrelin and total tumor surface area and between serum ghrelin and body mass index, suggesting that elevated endogenous ghrelin may be sufficient to overcome any partial ghrelin resistance typically seen in cancer cachexia. These results support

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the possibility that ghrelin is co-released from neuroendocrine tumors and exerts an orexigenic effect in these patients, helping to maintain their body mass index despite widely disseminated disease.

Keywords

Ghrelin; Metastatic neuroendocrine tumors; Endogenous; Orexigenic; Cancer cachexia; Somatostatin; Gastrin

Introduction

Ghrelin, a potent orexigenic peptide, is known to correlate negatively with body mass index (BMI) so that levels are elevated in cancer cachexia and decreased in obesity (Tschop et al. 2001; Shiiya et al. 2002). In cancer cachexia, it has been postulated that weight loss and lack of appetite persist—despite elevated ghrelin levels—due to a state of partial ghrelin resistance possibly mediated by inflammatory cytokines (Ramos et al. 2004). Patients with lung cancer cachexia, for instance, have elevated plasma ghrelin levels compared to those with lung cancer without cachexia, a likely compensatory mechanism reflecting negative energy balance (Shimizu et al. 2003). In a cancer cachexia mouse model, exogenous intraperitoneal administration of ghrelin stimulated anabolic effects including increased food intake and adipose tissue deposition, reversing cachexia in nude mice bearing a human melanoma cell line (Hanada et al. 2003). Similarly, exogenous ghrelin given intravenously to patients with cancer cachexia increased energy intake by 31% in one trial, suggesting that any partial ghrelin resistance may be overcome with supplemental levels of ghrelin (Neary et al. 2004). In contrast, the effect of elevated endogenous ghrelin levels has not been evaluated to date.

Though first described in 1999 as an endogenous growth hormone receptor ligand, ghrelin was subsequently found to be primarily produced in the stomach by a distinct population of neuroendocrine cells, X/A like cells, found in the oxyntic mucosa of the fundus (Date et al. 2000). Typically, these cells account for 20% of the endocrine cell population in the stomach (Kojima and Kangawa 2005), and it has been shown that gastrectomy decreases serum ghrelin levels by 55–72% (Cummings et al. 2002; Popovic et al. 2005). The discovery that ghrelin is primarily produced by the neuroendocrine cell population of the stomach has raised nearly as many questions as it has answered. Principal among these is whether neuroendocrine cell hyperplasia, including neuroendocrine tumors (NETs), stimulate increased ghrelin production.

Previous research has established ghrelin production by NETs. In gastric carcinoids, ghrelin expression has been reported to be 75% by immunohistochemistry and 81% by *in situ* hybridization (ISH), with 94% concordance between the two techniques (Papotti et al. 2001; Rindi et al. 2002). Ghrelin expression in intestinal carcinoids is reported to be less by immunohistochemistry (27%) and comparable by ISH (72%; Papotti et al. 2001). However, 100% of intestinal carcinoid cases had positive expression of ghrelin mRNA using highly sensitive RT-PCR, suggesting that some level of ghrelin gene expression is present in most, if not all, gastrointestinal endocrine tumors (Papotti et al. 2001). In pancreatic

neuroendocrine tumors, ghrelin expression was observed in 40% of cases by immunohistochemistry, 68% by ISH and 79% by RT-PCR (Rindi et al. 2002; Volante et al. 2002). Only one study to date has evaluated serum ghrelin in patients with gastroenteropancreatic neuroendocrine tumors reporting no significant difference in ghrelin levels between these patients and BMI-matched controls (Corbetta et al. 2003). In addition, two ghrelinomas, a gastrointestinal neuroendocrine tumor expressing ghrelin as its main hormone, have been identified (Corbetta et al. 2003; Tsolakis et al. 2004).

It has long been observed that patients with neuroendocrine tumors, even those with extensive disease and hepatic metastases, generally appear well-preserved without signs or symptoms of cancer cachexia (Thompson et al. 1985). In this study, we sought to establish serum ghrelin values for the first time in a population of patients with neuroendocrine tumors in the US and to evaluate the effect of hepatic metastases on serum ghrelin values. We hypothesized that elevated endogenous serum ghrelin, produced by increased neuroendocrine cell tumor burden, exerts an orexigenic effect in these patients, helping to maintain BMI despite the presence of widely metastatic disease.

Materials and Methods

The study was conducted from 2003 to 2005 as a single-center, prospective trial enrolling 35 patients with histologically determined NETs. All patients provided their informed consent to participate in the study. This study was approved by the Office for Protection of Research Subjects at the David Geffen School of Medicine at UCLA. Patients with one or more of the following conditions were excluded: recent antineoplastic therapy including chemotherapy, radiation, therapy or surgery; previous gastrectomy; history of cancer other than NETs; cachexia or cachectic states including thyroid disease, severe liver disease, anorexia or a history of eating disorders; concomitant tube feedings or parenteral feedings or a history of dysphagia; use of progesterone, testosterone or other orexigenic agents; or any other clinically significant medical condition including congestive heart failure, drug or alcohol abuse, chronic obstructive pulmonary disease, or any other psychiatric illnesses.

Throughout the study period, patients were allowed to continue concurrent medications including somatostatin analogs and antisecretory agents including proton pump inhibitors. All medications and their doses were monitored during the course of the study. They were encouraged to eat and exercise as usual and no patient dieted at any time while on the study. Following referral to the UCLA Medical Center, patients were evaluated for multiple clinical parameters including age, gender, race, weight (in kilograms), cancer diagnosis including primary location, presence of hepatic metastasis, multiple endocrine neoplasia type 1 (MEN1) status, and current and previous medications.

Following an overnight fast (10–12 h), a morning blood sample was obtained between 0700 and 0900 h for measurement of baseline laboratories as well as total ghrelin, chromogranin A (CgA), and gastrin. CgA is a 49-kDa glycoprotein released from neuroendocrine cells commonly used as a biochemical marker for neuroendocrine tumors. Physical measurements including height and weight were recorded and the patients were evaluated and treated as part of a standard clinic visit.

To measure total ghrelin, a 5 mL sample of serum was separately collected in a chilled tube at 4°C and immediately centrifuged in a refrigerated centrifuge. The supernatant was then separated, stored at -80°C and shipped in dry ice via overnight courier to an outside laboratory, Inter-science Institute. Ghrelin was measured by radioimmunoassay utilizing a specific antibody directed against ghrelin conjugated to bovine serum albumin. Cross-reactivity was determined at the 50% inhibition of binding level (Ghrelin= 1.00). The sensitivity was determined as the least amount of ghrelin that can be distinguished from zero. The lower and upper limits of detection are 85 and 2,400 pg/ml, respectively. Intraassay coefficient of variation is reported to be 11.8% by Interscience institute.

To evaluate the effect of tumor size on serum ghrelin values, all 35 patients had recent imaging completed (within 1 month of enrollment) via either magnetic resonance imaging or computed tomography scan. In all patients, either the primary lesion or the largest single hepatic metastasis was measured by an academic radiologist. The dimensions of the lesion were then recorded and used to calculate total tumor surface area (cm²) that would reflect total tumor burden in patients with and without hepatic metastases.

Quantitative results were recorded as means with their standard deviations. Statistical analysis was performed using a paired t test to assess for differences between patients with and without hepatic metastases. Differences were considered to be statistically significant when the calculated *p* value was 0.05 or less.

Results

Thirty-five patients with histologically determined NETs with and without hepatic metastases were enrolled. Table 1 lists clinical characteristics regarding these patients. Seventeen patients with hepatic metastases and 18 patients without hepatic metastases were well matched regarding all clinical characteristics assessed including, mean age, male to female ratio, subtype and primary location of neuroendocrine tumors, and treatment history including number of patients with a history of primary resection and with a history of previous somatostatin therapy. The two groups did differ in the number of functional gastrinomas identified as seven were reported in patients without hepatic metastases while two were reported in patients with hepatic metastases. Mean surface area of hepatic lesions measured in patients with hepatic metastases was 12.3 m² compared to a mean surface area of 4.3 cm² measured in primary lesions in patients without metastases.

Mean ghrelin, BMI, chromogranin A (CgA) and gastrin values are listed in Table 2. Nearly all patients with hepatic metastases (85%) had elevated levels of serum ghrelin compared to the standard reference range (up to 420 pg/ml) for matched controls. In contrast, only half of all patients without hepatic metastases had elevated levels of ghrelin compared to the standard reference range. Patients with hepatic metastases had statistically greater levels of serum ghrelin values compared to patients without hepatic metastases. These patients also had a greater mean body mass index as compared to patients without hepatic metastases, suggesting a positive correlation that can be easily visualized in Fig. 1. No significant differences were identified between mean serum CgA and mean serum gastrin measured.

In Fig. 1, we plot ghrelin values against BMI and plot a regression line for both patients with (solid line) and without hepatic metastases (dashed line). At nearly every BMI observed, patients with hepatic metastases had greater levels of ghrelin than those without hepatic metastases. In addition, in both subgroups, we report a positive correlation between BMI and ghrelin, that is with increasing BMI observed, we measured greater levels of serum ghrelin.

In Fig. 2, ghrelin is plotted against CgA and a regression line for both patients with (solid line) and without hepatic metastases (dashed line) is shown. In patients with hepatic metastases, no significant correlation is reported between ghrelin and CgA whereas in those without hepatic metastases, a mildly positive correlation is seen. In Fig. 3, ghrelin is plotted against mean tumor surface area, with regression lines, in patients with (solid line) and without hepatic metastases (dashed line). A positive correlation is seen between ghrelin and tumor surface area for both the primary tumor and the largest hepatic lesion measured.

Discussion

In this study, for the first time, we evaluated 35 patients with NETs and report elevated serum ghrelin levels in nearly all patients (85%) with NETs with hepatic metastases when compared to the standard reference range given for matched healthy controls. Given recent findings suggesting ghrelin production by neuroendocrine cells, we sought to further evaluate the effect of hepatic metastases, reflecting increased neuroendocrine cell tumor burden, on serum ghrelin levels. Briefly four major types of cells have been identified to date in the oxyntic mucosa of the stomach. These cells include enterochromaffin-like cells, X/A-like cells, D cells and enterochromaffin cells which account for 30, 20, 22 and 7%, respectively of the cells of the oxyntic mucosa of the stomach (Kojima and Kangawa 2005). Ghrelin is thought to be principally produced by X/A-like cells that have been shown to contain round, compact electron-dense granules filled with ghrelin (Dornonville de la Cour et al. 2001). In addition, however, ghrelin immunoreactive cells are found in the duodenum, jejunum, ileum and colon in gradually decreasing concentrations (Inui et al. 2004). As well, previous studies in the rat pancreas have also shown the pancreas to be a ghrelin-producing organ though it is unclear what specific cell type in the pancreatic islets, if any, is responsible for ghrelin production (Wierup et al. 2002).

We hypothesized that patients with increased neuroendocrine tumor burden, manifested as hepatic metastases, would have elevated levels of serum ghrelin compared to patients without hepatic metastases. We therefore assigned 35 patients with NETs to one of two groups depending on the presence of hepatic metastases. In matched patients, the presence of hepatic metastases is associated with significantly elevated ghrelin levels ($p < 0.05$). Elevated endogenous levels of ghrelin in these patients were also associated with greater mean BMI though this difference was not statistically significant.

We have also shown that in all patients with NETs, a positive correlation exists between ghrelin and BMI. Previously multiple studies have illustrated a negative correlation between ghrelin and BMI both in cachectic states (anorexia nervosa, cancer) and in obesity (Rigamonti et al. 2002). In these patients with NETs with and without hepatic metastases,

elevated levels of endogenous ghrelin production appears sufficient to overcome any partial ghrelin resistance normally seen in cancer cachexia (Garcia et al. 2005).

In contrast, in Fig. 2, no positive correlation is seen between ghrelin and chromogranin A, a biochemical marker for NETs, in patients with hepatic metastases. In previous studies, CgA has been shown to have low sensitivity and specificity for detecting tumor increases and stabilization (Abou-Saif et al. 2003). It therefore is not surprising to see poor correlation here. Further studies following ghrelin serially in patients with both tumor progression and tumor regression are needed to evaluate its efficacy as a potential biochemical marker.

Finally, in Fig. 3, we report a positive correlation between serum ghrelin and calculated tumor surface area of either the largest hepatic lesion or the primary lesion in patients with and without hepatic metastases, respectively. Like the use of chromogranin A, the use of a single, large hepatic lesion to reflect overall tumor burden can be problematic, potentially severely underestimating the true tumor volume present. However, because it is often impossible to accurately measure every single metastatic lesion, especially in patients with innumerable metastases, this approach is often the only one possible. Furthermore, this approach has been previously used to assess hepatic metastasis response to chemotherapy.

To evaluate the effect of hepatic metastases on serum ghrelin levels alone, we matched clinical characteristics between the two groups so that essentially all other possibly relevant variables were controlled. One noticeable difference, however, was present. Patients with hepatic metastases had lower mean levels of gastrin (Table 2) compared to those without hepatic metastases due to more cases of Zollinger-Ellison Syndrome (ZES) in the latter group. The effects of gastrin on ghrelin remain unclear. The trophic effect of gastrin on ECL cells, enhanced further in MEN1 positive patients, is well established (Bordi et al. 1995). In addition, Srivastava et al. (2004) has previously reported high immunoreactivity for ghrelin in ECL carcinoids and ECL hyperplasia, concluding that ECL cells are either the ghrelin producing cells of the gastric mucosa or acquire the capability to synthesize ghrelin. In this study, however, patients without hepatic metastases had greater mean levels of gastrin so that measured serum ghrelin levels in that group may have been falsely elevated via ECL cell hyperplasia, suggesting that differences between the two groups may be even greater than reported here.

In contrast, both groups had patients with similar treatment histories, neuroendocrine tumor subtypes and primary locations. In both groups, seven carcinoid tumors were identified with four tumors primarily localized to the small intestine. Previous studies have shown that carcinoid tumors, of gastric or intestinal primary, have high rates of ghrelin expression as assessed by immunohistochemistry, ISH or RT-PCR (Papotti et al. 2001; Rindi et al. 2002). Similarly, pancreatic neuroendocrine tumors, have been shown to have moderately high rates of ghrelin expression by immunohistochemistry and ISH with greater rates seen by RT-PCR (Rindi et al. 2002; Volante et al. 2002). The extent of transcription detected by RT-PCR is likely below the threshold of sensitivity for either immunohistochemistry or ISH, explaining lack of concordance in detecting ghrelin expression between the three techniques. Since both groups were well matched regarding subtypes and primary locations of neuroendocrine

tumors, it is highly unlikely that either variable acts as a confounder in this analysis between patients with and without metastasis.

Somatostatin has been shown to suppress ghrelin secretion in the rat stomach (Shimada et al. 2003) and in patients with acromegaly (Freda et al. 2003) and Prader–Willi syndrome (Haqq et al. 2003). The inhibitory actions of somatostatin have been well documented in rat and human ECL cells (Fykse et al. 2004) and other neuroendocrine cells including human gastric carcinoids associated with MEN1 and ZES (Aparicio et al. 2001; Ducreux et al. 2000; Imtiaz et al. 2000). However, since both subgroups were well matched for numbers of patients with a history of somatostatin therapy, this variable is unlikely to have affected our results. On average, patients with and without metastasis had been on somatostatin therapy for at least 6 months, at nearly equivalent doses, prior to enrollment. In addition, in rats, inhibition of ghrelin secretion diminished after 14 days of somatostatin therapy when desensitization was observed, leading to lower levels of ghrelin inhibition with subsequent treatments (Silva et al. 2005). Such tachyphylaxis to somatostatin may render treatment history irrelevant when assessing drug effect on serum ghrelin levels.

While the two groups were adequately matched so as to evaluate the effects of metastasis on serum ghrelin alone, some limitations of this study bear mentioning. Foremost, no tissue samples were obtained from enrolled patients to assess for ghrelin expression by immunoreactivity, ISH or by RT-PCR. Given extensive time from diagnosis to enrollment, the majority of patients did not have tissue readily accessible for analysis. Nevertheless, data reported in the literature reveals that nearly all, if not all, NETs have detectable levels of mRNA by RT PCR and moderately high levels of ghrelin immunoreactivity; therefore, we would expect a similar percentage of patients in this cohort to yield similar results if tissue had been available. In addition, it would have been useful to quantify appetite in the enrolled patients with a validated appetite scale in order to distinguish weight gain due to hunger versus weight gain due to inactivity, though all patients were encouraged prior to enrollment to exercise per regular routine. Finally, significant discussion in the literature surrounds the utility of measuring active or acylated ghrelin versus total ghrelin (acylated and deacylated). Intact active ghrelin is considered highly unstable and rapidly degrades to its inactive form (Akamizu et al. 2004). While some believe acylated ghrelin to be the only form able to exert endocrine activity, deacylated ghrelin has also been shown to be biologically active (Broglio et al. 2004). In this study, we can only best estimate levels of active ghrelin as previous studies have shown acylated ghrelin to be present in the serum at 2.5-fold lower concentrations than deacylated ghrelin (Broglio et al. 2004). In a future study, it may be worthwhile to assess both active and total ghrelin in patients with NETs as a biomarker of disease activity.

In summary, we report for the first time in the US that nearly all patients with NETs with metastases had elevated levels of ghrelin compared to the standard reference range given for matched healthy controls. Elevated levels of endogenous ghrelin, sufficient to overcome any partial ghrelin resistance in cancer cachexia, may help to maintain BMI in these patients. Patients with increased neuroendocrine cell tumor burden, manifested as hepatic metastases, had statistically greater mean levels of ghrelin and greater mean BMI compared to well matched controls. This data supports the possibility that ghrelin is co-released from NETs

and exerts an orexigenic effect in these patients, helping to maintain weight despite widely disseminated disease. Future studies in patients with NETs should be directed at evaluating the role of hypergastrinemia and NET subtype on serum ghrelin levels in a multivariable analysis.

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Correlation of Ghrelin Levels with Body Mass Index in NETs with and without Hepatic Metastases

Figure 1.

Plot of serum ghrelin against BMI in patients with NETs with (*triangle*) and without (*square*) hepatic metastases. Regression analysis shows that patients with hepatic metastases (*solid line*) had greater levels of ghrelin at every BMI observed compared to patients without hepatic metastases (*dashed line*)

Correlation of Ghrelin Levels with Chromogranin A Levels in NETs with and without Hepatic Metastases

Figure 2.

Plot of serum ghrelin against CgA in patients with NETs with (*triangle*) and without (*square*) hepatic metastases. Regression analysis shows that patients with hepatic metastases (*solid line*) had greater levels of ghrelin at most CgA levels observed compared to patients without hepatic metastases (*dashed line*)

Correlation of Ghrelin Levels with Tumor Surface Area in NETs with and without Hepatic Metastases

Figure 3. Plot of serum ghrelin against tumor surface area (cm²) in patients with NETs with (*triangle*) and without (*square*) hepatic metastases. A positive correlation is seen between tumor surface area measured of the largest primary tumor or the largest hepatic metastasis and serum ghrelin

Clinical characteristics of 35 enrolled patients with neuroendocrine tumors with and without hepatic metastases

Table 1

	Hepatic metastases (<i>n</i> = 17)	No hepatic metastases (<i>n</i> = 18)
Mean age	53 years ± 14	57 years ± 14
Sex	11 females 6 males	11 females 7 males
NET subtype	10 pancreatic NETs 7 carcinoid tumors	11 pancreatic NETs 7 carcinoid tumors
Carcinoid tumor primary location	4 small bowel 2 unknown 1 rectal	4 small bowel 1 gastric 1 unknown
Pancreatic NET functional status	7 non-functioning NETs 2 gastrinomas 1 glucagonoma	4 non-functioning NETs 7 gastrinomas
MEN1 status	4 MEN1 positive	4 MEN1 positive
Zollinger-Ellison Syndrome (ZES)	2 ZES	7 ZES
Previous surgery	5 primary resections	4 primary resections
Previous somatostatin therapy	7 previous treatments	5 previous treatments
Mean primary NET surface area (cm ²)	10.0 ± 17.1	4.3 ± 5.7
Mean hepatic metastases surface area (cm ²)	12.3 ± 14.4	N/A

Comparison of mean ghrelin, BMI, CgA and gastrin levels between patients with NETs with and without hepatic metastases

Table 2

	Hepatic metastases (<i>n</i> =17)	No hepatic metastases (<i>n</i> =18)
Mean ghrelin (pg/ml)	600±264	411 ±229
Two-tailed unpaired <i>t</i> test	<i>p</i> =0.03	
Mean BMI (kg/m ²)	27.0±6.7	26.3±5.6
Two-tailed unpaired <i>t</i> test	<i>p</i> =0.73	
Mean chromogranin A (ng/ml)	472± 1,459	127± 176
Two-tailed unpaired <i>t</i> test	<i>p</i> =0.33	
Mean gastrin (pg/ml)	136±335	318±448
Two-tailed unpaired <i>t</i> test	<i>p</i> =0.19	