Published in final edited form as:

Peptides. 2011 November ; 32(11): 2340–2347. doi:10.1016/j.peptides.2011.10.006.

Ghrelin in neuroendocrine tumors

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Abstract

Ghrelin is a 28 amino acid peptide, primarily produced by the oxyntic mucosa X/A like neuroendocrine cells in the stomach. It is also found in the small intestine, hypothalamus, pituitary gland, pancreas, heart, adipose tissue, and immune system. In gastrointestinal neuroendocrine tumors (NETs) ghrelin release has been well documented. Ghrelin is a brain-gut circuit peptide with an important role in the physiological regulation of appetite, response to hunger and starvation, metabolic and endocrine functions as energy expenditure, gastric motility and acid secretion, insulin secretion and glucose homeostasis, as well as in the potential connection to the central nervous system. Recently, there has been a significant interest in the biological effects of ghrelin in NETs. In this article, we present a comprehensive review of ghrelin's expression and a brief summary of ghrelin's physiological role in NETs patients with carcinoids, type A chronic atrophic gastritis (CAG), with or without MEN-1, and with and without liver metastases. We hope, with the research reviewed here, to offer compelling evidence of the potential significance of ghrelin in NETs, as well as to provide a useful guide to the future work in this area.

Keywords

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

1. Molecular characteristics of acylated ghrelin and desacyl ghrelin

Ghrelin is a 28 amino acid peptide, first identified in 1999 by Kojima et al. as the endogenous ligand of the growth hormone sec-retagogues receptor type 1a (GHS-R1a), and found predominately in the stomach [6,37]. Predominantly produced by the endocrine cells of the gastric oxyntic mucosa and hypothalamus, ghrelin has also been detected in the small intestine, hypothalamus, pituitary gland, pancreas, heart, adipose tissue, as well as in the immune system [11,23,37,68,70]. Ghrelin acts on the GHS-R1a and activates phospholipase C to generate inositol trisphosphate (IP₃) and diacylglycerol, resulting in an increase of intracellular Ca²⁺, indicating that ghrelin receptor is coupled to a G_q subunit [43]. The first

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recognized effect of ghrelin was the stimulation of growth hormone release from the anterior pituitary into the circulation. However, consistently with its wide distribution, ghrelin is also involved in the diverse biological actions of reproduction, tumor development, colonic motility, gastric acid secretion, glucose metabolism and immune response, in addition to increasing appetite and growth hormone secretion.

Currently, there are two known major forms of ghrelin: a 28 amino acid with an n-octanoylated serine in position 3 (acyl ghrelin), and a des-acylated (des-[Gln14]) ghrelin (desacyl ghrelin). Desacyl ghrelin, considered the inactive form of ghrelin, and constituting approximately 80–90% of total circulating ghrelin levels, does not activate the ghrelin receptor (GHS-R1a) and, therefore, cannot cross over with the endocrine activities of acyl ghrelin. In both the blood and the stomach, desacyl ghrelin exists at significantly higher levels than acylated ghrelin [30]. Desacyl ghrelin is not able to bind to GHS-R1a and has not been currently reported to possess endocrine activities or a specific receptor in humans. However, desacyl ghrelin may have some biological activities, in fact Baldanzi et al. showed that both ghrelin and desacyl ghrelin recognize common high affinity binding sites on H9c2 cardiomyocytes, that do not express GHS-R1a, and inhibit cell death through ERK1/2 and phosphatidylinositol 3-kinase/AKT pathways *in vitro* [8]. On food intake and appetite regulation, Asakawa et al. noted with central and peripheral administration of desacyl ghrelin in rodents, a significantly decrease of food intake in food-deprived mice and gastric emptying [7]. Transgenic mice, over-expressing desacyl ghrelin gene, exhibited a decrease in body weight, food intake and fat content. Most likely, the effects are inverse to those of the acylated ghrelin and mediated by neurons in the arcuate nucleus (ARC) and paraventricular nucleus (PVN) of the hypothalamus, as shown in Fig. 1. However in subsequent studies, intracerebroventricular administration of desacyl ghrelin to rats or mice, fed *ad libitum*, stimulated feeding during the light phase, but neither intraperitoneal, or intracerebroventricular, injection to fasting mice suppressed feeding [64]. Administration of desacyl ghrelin stimulated food intake independently from GHS-R1a and did not increase neuropeptides Y (NPY) expressing neurons of the ARC, but induced expression of Fos, a marker of neuronal activation in orexin-expressing neurons of the lateral hypothalamic area. Ghrelin and desacyl ghrelin, simulatenously injected intraperitoneally, abolished the stimulatory effect of ghrelin on food intake while activating nesfatin-1 immunoreactive neurons in the ARC of rats [33]. Furthermore, desacyl ghrelin has been shown to antagonize acyl ghrelin-induced effects on insulin secretion and blood glucose levels in humans and having cardioprotective, antiproliferative and adipogenic properties [31]. Therefore, although desacyl ghrelin may not be able to bind to GHS-R1a, it may have some biological activities and may even be an antagonist of acyl ghrelin's orexigenic effects. Currently, the physiological role of desacyl ghrelin has not yet been fully elucidated and further studies are required to determine whether desacyl ghrelin binds to a yet to be discovered receptor.

For years the enzyme responsible for octanoyl esterification of ghrelin into its active form, acyl ghrelin, remained unknown. Recently, two independent groups discovered that ghrelin is modified by a fatty acid, ghrelin O-acyltransferase (GOAT), which is essential for its binding and activity to GHS-R1a, by attaching the medium-chain fatty acid octanoate in mice [72] and humans [26]. GOAT is well conserved across vertebrates, and the amino acid sequence alignment of mouse, rat and human GOAT proteins show 74.5% identity and

98.4% similarity [10,26]. In human tissues, levels of GOAT transcripts are high in the gastrointestinal tract, especially in the stomach and pancreas, and highly correlated with ghrelin distribution [72]. Physiologically, GOAT appears to play a major role in metabolic adaptation during survival with severe caloric restriction. In studies with mice subjected to 60% caloric restriction, both GOAT deficient mice and wild-type (WT) mice lost 30% of body weight. However after 4 days, glucose readings stabilized in WT mice but continued to decline in GOAT deficient mice. After 7 continuous days of 60% caloric restriction, WT mice appeared to have normal physical activity, whereas GOAT deficient mice were moribund. Hormonal assays of both WT and GOAT deficient mice demonstrated growth hormone (GH) levels rising progressively in calories-restricted WT mice, while they were rising significantly less in GOAT deficient mice. Infusion of either ghrelin or growth hormone reversed the very low blood glucose levels in GOAT deficient mice [73]. Therefore, GOAT serves an essential role in preserving blood glucose levels and preventing mortality. After a 24 h fast in rats, GOAT protein levels were strongly increased in the plasma, possibly indicating extracellular acylation of ghrelin and increased capacity of ghrelin acylation in response to fasting [58]. Therefore, the detection of both GOAT and acyl ghrelin in NETs may be of importance in preventing mortality in patients.

2. Ghrelin and appetite stimulation in the central nervous system

Primarily produced in the gastrointestinal organs, ghrelin's role in the response to hunger and starvation is mediated through its connection to the central nervous system. Biological actions of ghrelin are predominantly, via the central nervous system, in the control of food intake; they modulate energy expenditure, gastric motility and acid secretion, as well as insulin secretion and glucose metabolism [16,71]. Studies with chronic central administration of ghrelin significantly increased food intake and body weight, thereby suggesting a role for ghrelin in the body metabolism and in the control of energy expenditure during starvation. Fat mass as a percentage of total body weight was also shown to be increased following chronic central administration of ghrelin. Since ghrelin binds to GHS-R1a, the orexigenic effect of ghrelin is thereby specifically modulated through this receptor. Consequently, in mice lacking GHS-R1a, ghrelin injection did not stimulate either GH release or food intake [60]. GHS-R1a is highly expressed in the hypothalamic arcuate nuclei, a key site in the modulation of energy balance. Furthermore, immunohistochemical analyses indicate that ghrelin containing neurons are located in the ARC of the hypothalamus, as demonstrated in Fig. 1 [25]. Moreover, intracerebroventricular injection of ghrelin increased both NPY and AgRP mRNA levels in the hypothalamus by 160% [55]. This area of localization of both GHS-R1a and ghrelin in the hypothalamus suggests a role for ghrelin in the regulation of food intake besides stimulating GH release. In the ARC, the hypothalamic genes neuropeptide Y and agouti-related peptide (AgRP), which are potent stimulators of feeding, were found to be increased upon chronic ghrelin administration [36,65]. NPY and AgRP are critical for ghrelin's role on feeding behavior, in fact mice lacking both NPY and AgRP fail to increase food intake [14]. Co-injection of NPY receptor 1 antagonist and ghrelin in rats blocked the appetite stimulatory effects [55]. These results suggest that ghrelin is a peripheral signal, released mainly from the stomach, to regulate food intake by acting on hypothalamic neuropeptides in the ARC.

Other hypothalamic sites including the paraventricular nuclei, later hypothalamus and non hypothalamic sites such as the hind-brain, also promote positive energy balance [21]. GHS-R1a is expressed in the ventral tegmental area (VTA), which contain a subpopulation of dopamine neurons projecting to forebrain regions, such as the amygdala, prefrontal cortex, hippocampus and ventral striatum [1]. Ghrelin has been shown to bind to the VTA and increase food take when injected directly into this region. Consistently, the opposite effect of decreased feeding response was observed, during blockade of GHS-1Ra in the VTA region, in course of rebound feeding behavior in fasted mice even when peripheral injections of ghrelin were given. Functional magnetic resonance imaging in humans, after ghrelin administration, stimulated activity of VTA target cells of the amygdala [40]. Furthermore, it is interesting to note that GHS-R1a expression has also been reported in other areas of the central nervous system, that affect biological rhythms, mood, cognition memory and learning, such as the hippocampus, pars compacta of the substantia nigra, ventral tegmental area, dorsal and median raphe nuclei and Edinger-westphal nucleus and pyriform cortex [24]. The diverse localization of GHS-1Ra and ghrelin in the central nervous system suggests an important role of ghrelin in the brain-gut axis.

3. Ghrelin in the gastrointestinal tract

The distribution of ghrelin producing cells in the gastrointestinal tract has been studied using immunohistochemical and *in situ* hybridization techniques in rodents. Ghrelin immunoreactive cells have been localized in all regions of the gastrointestinal tract: stomach, duodenum, jejunum, ileum, and colon [17,30,39]. Ghrelin producing cells are primarily located in the stomach and in decreasing concentration from the duodenum to the colon. The majority of ghrelin cells were observed in the gradular base, with few cells observed in the glandular neck of the stomach. Following a gastrectomy, plasma ghrelin concentrations in rats were decreased by 80%, whereas plasma ghrelin levels were also reduced by 65% in human gastrectomised patients, strongly suggesting the major source of circulating plasma ghrelin is the gastric mucosa. Structurally, ghrelin producing cells in the stomach appear morphologically small, round and closed-type, but in the duodenum, ileum and colon two ghrelin releasing cell types are present: closed-type with triangular or elongated shapes, and opened-type [53]. However, closed type cells with triangular or elongated shapes and opened type cells with apical cytoplasmic processes contacting to the lumen, were found in the duodenum, ileum, cecum and colon. Generally, hormones, neurons, and mechanical distension functionally regulate the closed-type cells, whereas the open-type cells are functionally regulated by pH and nutrients [39]. The diverse localization and morphology of ghrelin in the gastrointestinal tract suggests that this peptide plays a significant role in both metabolic and endocrine functions; therefore, its role in the regulation of these functions in the human physiology and in the gastrointestinal neuroendocrine tumors (NETs) is of scientific and clinical interest and it needs to be studied.

Ghrelin is predominantly produced by the oxyntic mucosa X/A like neuroendocrine cells, which account for approximately 20% of the neuroendocrine cell population of the oxyntic glands [37]. X/A like neuroendocrine cells are part of a population that includes: enterochromaffin-like (ECL), EC, and D cells. Being primarily produced in the gastrointestinal organs, ghrelin's role in the response to hunger and starvation as well as the

potential connection to the central nervous system, are being elucidated. By its multiple functions in the gastrointestinal tract, ghrelin stimulates food intake and modulates energy expenditure, gastric motility and acid secretion, as well as insulin secretion and glucose metabolism [16,18,71]. Since ghrelin containing neurons are located in the ARC of the hypothalamus, a region that regulates appetite, this suggests a role for ghrelin in the regulation of food intake. In both rats and in humans, plasma ghrelin levels are elevated in fasting state and are decreased after feeding. Rats, fasted for 48 h, showed an increase in ghrelin serum levels, and re-feeding blunted this steady increase [65]. This suggests that ghrelin is a peripheral signal, released mainly from the stomach, to regulate food intake by acting on hypothalamic neuropeptides. In the body, blood glucose levels are tightly regulated to maintain the human metabolic homeostasis. Observations of ghrelin levels, following intravenous administration of glucose, resulted in a reduction of ghrelin levels in obese and lean children [9]. These ghrelin functional effects further reinforce its role in maintaining metabolic homeostasis. In summary, all of these physiological observations suggest that ghrelin is involved in the neuroendocrine regulation of energy balance in physiological conditions. Ghrelin's regulation of energy balance as well as its role in the metabolism of NETs has not been yet fully elucidated.

The regulation of ghrelin induced strong metabolic activity mediated by somatostatin producing D cells [13]. Somatostatin is a peptide that exerts a broad spectrum of biological activities through neuroendocrine, endocrine, and paracrine mechanisms, and it is mainly produced in the gastrointestinal tract, brain, and pancreas. In the gastrointestinal tract, somatostatin is produced in the oxyntic mucosa by non-luminal cells, whose cytoplasmic processes are in close proximity to the ghrelin releasing cells. Given the localization of the ghrelin immunoreactive cells, accounting for approximately 20% of the endocrine population in the oxyntic glands, and the somatostatin immunoreactive cells, the role of D cells regulation on ghrelin release could be significant. In humans, somatostatin infusion lowered ghrelin levels by 70–80% in comparison to normal control values [38,45,54]. In subjects with pathophysiological conditions, such as Prader-Willi syndrome and in postoperative patients with acromegaly, high ghrelin levels associated with pathological obesity have been reported. In such patients somatostatin administration lowered the circulating ghrelin levels, thereby suggesting a role for somatostatin as a potentially effective ghrelin inhibitor [22,28]. In NETs patients, somatostatin-induced tumoral inhibitory effects have been well documented [5,19,32]. Clinical studies in patients with ghrelinomas revealed high body mass index (BMI). Currently, octreotide and lanreotide, which are somatostatin analogs, are available and used in the treatment of patients with acromegaly or neuroendocrine tumors. Approximately 50% of patients with neuroendocrine tumors showed an escape of response after 1 year of treatment, and 35% of acromegalic patients responded only partially, or not at all, to treatments with octreotide and lanreotide [19,32]. However, in rats, inhibition of ghrelin secretion was diminished after 14 days of somatostatin therapy, when desensitization and lower levels of ghrelin inhibition were observed [56]. Therefore, given previous research data [5,19,32] and ghrelin release by neuroendocrine tumors (NETs), the relationship between ghrelin and somatostatin in tumoral neuroendocrine cell metabolism need to be further explored.

4. Expression of ghrelin in NETs

Primary sites of NETs are the gastro-intestinal-pancreatic tract (62–67%) and the lungs (22–27%) [61]. The incidence of NETs is increasing at a rate of approximately 6% annually, due to the improved imaging techniques and greater awareness in the medical community [29,41,49]. Previously, the majority of these patients received elusive diagnoses, since they usually do not show the signs and symptoms of this clinical syndrome. In general, the five-year survival rates for patients with NETs are mainly associated with the tumor stage: 93% in local disease, 74% in regional disease, and 19% in metastatic disease [42]. Currently, the only curative treatment option for NETs is surgical, and the clinical outcome is heavily dependent upon the diagnostic stage of the tumor [25]. Among all types of NETs, the gastric carcinoids are probably the best studied. Most of the gastric NETs appear to be well differentiated, arising from the oxyntic mucosa of either the corpus or fundus of the stomach, and they are divided into three distinct types: Type I is normally associated with autoimmune chronic atrophic gastritis (CAG), Type II is associated with multiple endocrine neoplasia type 1 (MEN-1) and Zollinger-Ellison syndrome (ZES), Type III are sporadic tumors (Fig. 2). Ghrelin levels have been clinically detected in all three types of NETs [48,52,61,68]. The release of ghrelin by the neuroendocrine tumors may bypass the normal regulatory inhibitory pathways, as depicted in Fig. 2.

In NETs, ghrelin producing cells have been identified by immunohistochemistry, *in situ* hybridization, along with mRNA expression using reverse transcriptase PCR and Northern blotting techniques as described in Table 1 [48,52,68]. Pappoti et al., examining 36 gastric carcinoid patients, observed ghrelin expression in 12 of 16 cases [48]. Rindi et al., studied ghrelin expression in 37 gastric carcinoid patients and observed ghrelin immunoreactive cells in 25 of 33 gastric endocrine tumors [52]. In total, ghrelin expression in gastric carcinoids has been reported to be 75% by immunohistochemistry, and 81% by *in situ* hybridization, with a 94% concordance between the two procedures [48,52]. Ghrelin expression in intestinal endocrine tumors was reported to be 27% by immunohistochemistry and 72% by *in situ* hybridization [33]. Ghrelin mRNA expression by RT-PCR, paralleled immunoreactivity in the gastric carcinoids, indicating that the ghrelin-producing cell population is transcribing mRNA and subsequently synthesizing the protein [48,52]. However, 100% positive expression of mRNA by RT-PCR was observed in intestinal carcinoids, suggesting that some level of ghrelin expression is observed in almost all gastrointestinal tumors [68]. In pancreatic NETs, ghrelin immunoreactivity was observed in only 40% of cases, in 68% of these by *in situ* hybridization, and in 79% by RT-PCR [52,68]. Since RT-PCR is a highly sensitive method, the extent of transcription detected by RT-PCR is likely below the immunohistochemistry or *in situ* hybridization sensitivity threshold. Volante et al. [68], investigated ghrelin expression in pancreatic NETs and found that 11 of 28 tumors were immunoreactive for ghrelin: six insulinomas, three non-functioning tumors, one gastrinoma, and one VIPoma. In these studied tumors, there was no preferential association between ghrelin expression and any studied hormone, and no statistical correlation with age, sex, tumor size, or tumor grade [68]. In total, only two cases of digestive NETs have been reported in the literature as ghrelinomas, due to the extremely high levels of ghrelin in the gastric and pancreatic origin tumors [15,66]. The ghrelin levels

reported in NETs, showed no significant difference between tumors of gastrointestinal or pancreatic origin [15]. Since the expression of ghrelin mRNA and protein has recently been detected in the majority of gastric and intestinal carcinoids, and in pancreatic NETs, there has been an increase of interest in this field.

5. Measurement of serum ghrelin in patients with neuroendocrine tumors

Depending upon the primary tumor localization and cell origin, gastrointestinal NETs produce a variety of different amines and peptide hormones. Chromogranin A (CgA), used as general marker of NETs, is stored in the secretory granules of neuroendocrine cells and is detectable in the plasma [46,59]. Although CgA is an extremely sensitive marker of gastrointestinal NETs, it is also nonspecific; in fact all other NETs such as the pancreatic, the small cell lung neoplasia, and even the prostate carcinomas produce CgA. Furthermore, CgA has a relatively low sensitivity and specificity for detecting any increase in tumor size over time. Gastrin, a specific tumor marker in patients with gastrinoma, has also been found to have a relatively low sensitivity and specificity for detecting either an increase in gastrinoma size or tumor stabilization over time [1,69]. The identification of tumor size change is critical to the proper clinical management of NETs. Currently, only one single approach exists to reflect the tumor surface area: the observation of a single, large lesion to reflect the overall tumor growth. This approach can be problematic because of its potential to severely underestimate the volume of the examined tumor. Considering that it is impossible to accurately measure every single metastatic lesion, especially in those patients with multiple metastases, this approach remains the most commonly used.

Clinical features of patients with NETs, even those with advanced clinical disease, appear to be well preserved with no apparent signs of cachexia [62]. This observation is consistent with our clinical experience, at UCLA, in the management of patients with NETs. Cancer cachexia results from the relentless progression of nutritional state deterioration and body mass index wasting, that account for up to 30% of cancer related deaths [63]. Cachexia is characterized primarily by anorexia, weight loss, weakness, fatigue, impaired immunological function, and poor mental and physical performance. Ghrelin, a potent orexigenic peptide, is elevated in cancer cachexia, but weight loss and lack of appetite appear to persist, possibly due to the release of inflammatory cytokines, which would determine a partial resistance to ghrelin [51,67]. In a mouse model of cancer cachexia, using nude mice bearing human melanoma, the exogenous intraperitoneal administration of ghrelin stimulated anabolic effects, which reversed cachexia [27]. Also, exogenous ghrelin, given intravenously to patients with cancer cachexia, increased energy intake by 31% over the controls [44]. In a case of ghrelinoma reported by Tsolakis et al. [66], the patient had an extremely high ghrelin serum levels and maintained high BMI through a one year follow up after diagnosis. Although this tumor failed to respond to the applied chemotherapy treatment, the patient's appetite was preserved and the BMI was maintained [66]. Furthermore, Wang et al. [69] positively correlated serum ghrelin levels (pg/ml) with patient weight (lbs.) in patients with NETs and hepatic metastases (Fig. 3). Therefore, ghrelin induced physiological effects can be observed also in patients with clinically advanced NETs syndrome, and in those with hepatic metastases.

6. Measurement of serum ghrelin levels in patients with chronic atrophic gastritis

Autoimmune chronic atrophic gastritis (CAG), type A CAG, is characterized by chronic inflammation of the oxyntic mucosa, affecting the gastric corpus and resulting in achlorhydria and atrophy of oxyntic glands, with progressive disappearance of the chief and parietal cells. The underlying pathophysiology is an autoimmune reaction involving anti-parietal cell antibodies resulting in progressive disappearance of the parietal cells and resultant hypo- and achlorhydria. Ghrelin localization studies of ECL hyperplastic lesions and gastric carcinoids are strongly positive for ghrelin mRNA and protein expression in the setting of type A CAG [47,48]. Most studies on patients with non type A CAG have observed low ghrelin plasmatic concentrations, low ghrelin gastric mRNA, and low ghrelin positive cell numbers in the gastric mucosa; however, all of the studied patients possessed *Helicobacter pylori* (*H. pylori*) infection and biochemical markers of gastric mucosa atrophy [34,47]. A possible explanation for low ghrelin concentrations is the loss of ghrelin producing cells and in fact Osawa et al. reported lower ghrelin mRNA and protein levels in the gastric mucosa, as well as lower ghrelin serum levels in association with *H. pylori* infection [47]. In these patients, the loss of ghrelin producing cells can account for the low ghrelin plasmatic concentrations; whereas in type A CAG patients, immunohistochemical analyses showed ghrelin expression in the neuroendocrine cells contained in the hyperplastic mucosa lesions [48,57]. Alonso et al. [3], first reported elevated ghrelin concentrations in type A CAG patients with type 1 diabetes mellitus and increased prevalence of pernicious anemia. CgA is a marker of neuroendocrine cell hyperplasia, since it appears to be elevated almost exclusively in neuroendocrine cell hyperplastic lesions, compared to normal gastric mucosa [2]. Ghrelin expression was enhanced in patients with gastric neuroendocrine cell hyperplasia, compared to normal gastric mucosa. Patients with diabetes mellitus-CAG show high ghrelin concentrations, which can be due to hyperplastic ECL cells, as in patients with type A CAG [3]. Although plasma ghrelin concentration is not used as a biochemical marker in the management of type A CAG patients, it may be a useful indicator for predicting carcinoid tumor development. These observations may also explain the reason why the majority of patients with NETs maintain their BMI, despite having extensive tumor involvement.

7. Ghrelin expression in patients with multiple endocrine neoplasia type 1 (MEN I)

Ghrelin expression has been shown in multiple endocrine neoplasia type 1 (MEN-1) associated tumors both by Southern blot and immunohistochemistry. First established by Iwakura et al. [35], in a case of glucagonoma associated with MEN-1, ghrelin expression was detected by both Northern blot analysis and immunohistochemistry. In the same study, another case of glucagonoma, not associated with MEN-1 syndrome, did not show any levels of ghrelin expression. Therefore, Iwakura et al. proposed that ghrelin is involved in tumor formation of NETs in MEN-1 patients. However, another study examined 12 cases of gastrointestinal tract NETs in patients with MEN-1, using immunohistochemistry, and in only 3 cases ghrelin expression was observed [50]. The different observations can possibly

due to the different antibodies used for immunohistochemistry in the two studies. Ekeblad et al. [20] showed immunoreactivity in 8 out of 9 patients in pancreatic endocrine tumors with MEN-1 [20]. Plasma ghrelin levels were significantly higher in subjects with MEN-1 associated tumors, than in those patients with sporadic tumors or in the controls. These patients also had ghrelin mRNA levels 5 times higher than subjects with sporadic tumors and controls. Clinically, in these MEN-1 patients, no significant difference in survival rates was observed between the ghrelin immunoreactive and the nonimmunoreactive tumor bearing groups. Despite the fact that the studied patient showed significant size of the tumor masses, and most of them had multiple liver metastases, their mean BMI was within the same range of the non-tumor control patients. In many of these cases, patients that possessed multiple liver metastases, the mean BMI in the patients within the normal reference range and did not display symptoms of cachexia [20]. One patient with a ghrelin-producing tumor, a radical pancreatic surgery decreased ghrelin levels by 42%, and consequently BMI levels resulted 20% lower [20]. Therefore, ghrelin, influencing body weight, can potentially prevent the development of cancer cachexia in patients with ghrelin producing tumors.

8. Ghrelin in NETs patients with hepatic metastatic versus non-metastatic disease

In patients with an increased neuroendocrine tumor burden, manifested by the presence of hepatic metastases, we have demonstrated that elevated endogenous levels of ghrelin, presumably released from the neuroendocrine tumor cells, could exert an orexigenic effect in the patients and maintain their BMI [69]. In 35 patients with NETs, 85% of the subjects with hepatic metastases had elevated serum ghrelin levels compared to those without hepatic metastases [69]. In the same group of subjects, the analysis of CgA expression showed no positive correlation with the tumoral stage, confirming our previous published data [69], showing a low sensitivity and specificity for CgA as an indicator of tumor increase. Consequently, even though CgA is a biochemical marker for NETs, it cannot be used to follow up the growth of metastatic lesions. In the same patients [69], a greater mean BMI was attributed to the elevated ghrelin levels in those with hepatic metastases compared to those without. In NETs patients with hepatic metastases, or with non hepatic metastases, elevated levels of endogenous ghrelin appeared sufficient to overcome any partial ghrelin resistance seen in cancer cachexia by other authors. The two patient groups were well matched for all these relevant clinical characteristics: mean age, male/female ratio, subtype and primary location of NETs, and treatment history. Patients with hepatic metastases had a lower incidence of Zollinger-Ellison Syndrome (ZES) and lower mean levels of gastrin, compared to those without hepatic metastases. However, the effects of gastrin upon ghrelin release remain still unclear and need to be further elucidated. Srivastava et al. [57] have previously suggested that ECL cells produce ghrelin, or can acquire the capability to synthesize it, however this is only a speculative hypothesis at the present. Therefore, patients with-out hepatic metastases could have more elevated ghrelin levels, due to the additional release of ghrelin from transformed ECL cells present within the ZES lesions, compared to the subjects with hepatic metastases [11,69]. Therefore ghrelin, released by NETs, can exert a potent orexigenic effect in these patients, in such a way preventing cancer cachexia.

9. Summary

Ghrelin is widely expressed throughout the gastrointestinal tract and plays an important role in several physiological functions. The peculiar biological characteristic of NETs is the hypersecretion of amines and peptides, such as ghrelin, leading to a variety of unique clinical symptoms. Given the known secretion of ghrelin from NETs, it is thought that ghrelin may be responsible for the weight preservation observed in patients with cancer. In this review, we have discussed the expression of ghrelin and its role in chronic atrophic gastritis, MEN-1, and in NETs patients with hepatic compared to those with non-hepatic metastases. Given the powerful role of ghrelin as an orexigenic hormone and its presence in NETs, its role still needs to be elucidated. One of the clinical features of NETs is the patients' maintenance of BMI and the lack of apparent clinical signs of cachexia. Ghrelin has been shown to be upregulated in NETs, and to be able to maintain BMI in patients with hepatic metastases. Further research needs to be focused on the physiological effects of ghrelin in patients with different subtypes of NETs. Significant discussion in the literature remains on the function of measuring active or acylated ghrelin versus total ghrelin and therefore, it is beyond the scope of this review. The acylated form of ghrelin is believed to be the only form to exert endocrine activity, but it is also highly unstable and rapidly degrades [4]; however, deacylated ghrelin has also been shown to be potentially biologically active [12,33]. The majority of the current studies analyzing ghrelin in NETs have not examined acyl and desacyl ghrelin. It would be possibly of significant clinical interest to study the physiological effects of either acylated ghrelin versus total ghrelin in NETs.

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Fig. 1.

A proposed pathway of acyl ghrelin, its modification by GOAT and action through the GHS-R1a receptor in the hypothalamus contributing to increase in food intake, GH release, and fat oxidation. Currently, the receptor for desacyl ghrelin has not been discovered.

Fig. 2.

Proposed effects of ghrelin release from neuroendocrine tumors on the regulatory pathways for appetite control. Under normal physiology ghrelin is released during periods of low-protein diet and fasting states. Inhibitory signals such as somatostatin, high fat diet, while inhibited by somatostatin, feeding, high fat diets, feeding, vagal tone, growth hormone, and Interleukin 1 β inhibits this release of ghrelin. However, with neuroendocrine tumors, the release of ghrelin from the tumors is not subject to these same regulatory controls.

Fig. 3.

Plot of serum ghrelin against body weight in patients with NETs with (*diamond*) and without (*square*) hepatic metastases. Patients with hepatic metastases (*blue line*) had greater levels of ghrelin, correlating with increase in body weight, compared to patients without hepatic metastases (*gray line*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1

Comparison of published data of ghrelin expression detected in NETs by immunohistochemistry, *in situ* hybridization and/or by mRNA.

	Ekeblad et al.	Pappoti et al.	Rindi et al.	Tsolakis et al.	Volante et al.
Presence of NETS	+	+	+	+	0
Total number of patients	31	36	37	1	28
Patients with ghrelin expressing NETS					
Immunohistochemistry	21/31 (68%)	17/34 (50%)	25/33 (76%)	1/1 (100%)	11/28 (39%)
In situ hybridization	n/a	26/34 (76%)	n/a	n/a	19/28 (68%)
mRNA Level	19/20(95%)	14/14(100%)	n/a	n/a	22/28 (79%)
BMI (kg/mg ²)	High (24.3 kg/mg ²)	n/a	n/a	High (27 kg/mg ²)	n/a