REVIEW

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Role of Ghrelin in the Pathophysiology of Gastrointestinal Disease

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Ghrelin is a 28-amino-acid peptide that plays multiple roles in humans and other mammals. The functions of ghrelin include food intake regulation, gastrointestinal (GI) motility, and acid secretion by the GI tract. Many GI disorders involving infection, inflammation, and malignancy are also correlated with altered ghrelin production and secretion. Although suppressed ghrelin responses have already been observed in various GI disorders, such as chronic gastritis, *Helicobacter pylori* infection, irritable bowel syndrome, functional dyspepsia, and cachexia, elevated ghrelin responses have also been reported in celiac disease and inflammatory bowel disease. Moreover, we recently reported that decreased fasting and postprandial ghrelin levels were observed in female patients with functional dyspepsia compared with healthy subjects. These alterations of ghrelin responses were significantly correlated with meal-related symptoms (bloating and early satiation) in female functional dyspepsia patients. We therefore support the notion that abnormal ghrelin responses may play important roles in various GI disorders. Furthermore, human clinical trials and animal studies involving the administration of ghrelin or its receptor agonists have shown promising improvements in gastroparesis, anorexia, and cancer. This review summarizes the impact of ghrelin, its family of peptides, and its receptors on GI diseases and proposes ghrelin modulation as a potential therapy. **(Gut Liver 2013;7:505-512)**

Key Words: Gastrointestinal tract; Ghrelin; Receptors, ghrelin; Ghrelin O-acyltransferase

INTRODUCTION

In the gastrointestinal (GI) tract, appetite regulation is mainly controlled by multiple anorectic hormones (e.g., cholecystokinin, peptide YY, and glucagon-like peptide-1) and orexigenic hormone (ghrelin) in the gut. Previous review by De Silva and Bloom[1] discussed the possibilities of two anorectic gut hormones peptide YY and glucagon-like peptide-1, released after meal as mediation of postprandial satiation, as potential therapeutic targets in obesity. In this review, we will focus on the role of ghrelin in GI diseases, the only identified orexigenic gut hormone so far.

The discovery of ghrelin has enriched our knowledge of the interaction between the GI tract and the brain. This discovery has shed new light on multiple physiologic functions including GI activity, glucose metabolism, insulin release, cardiovascular activity, regulation of pituitary hormone secretion, food intake, and energy homeostasis.

Ghrelin was first discovered in 1999 as a 28-amino acid acylated peptide endogenous ligand of growth hormone secretagogue receptor (GHS-R) with a unique posttranslational modification of the Ser3 residue.[2] This modification is essential for ghrelin activity. The amino-terminal 10 amino acids of ghrelin are highly conserved among mammals and suggested to have an important role in the protein's activity.[3] It is produced by A-like cells and localizes mainly to the oxyntic mucosa of the stomach. Total gastrectomy reduced the plasma concentration by 65% which is produced by the small and large intestine and pancreas.[4]

Ghrelin is actively involved in multiple physiological functions such as the regulation of growth hormone (GH) secretion,[5] adiposity,[6] gastric acid secretion,[7] and gut motility.[8] Ghrelin plays an important role in orexic behaviors on appetite stimulation and regulation of body weight.[9] Ghrelin expression in the stomach rises during fasting[10] and decreases within 1 hour of having a meal.[11] Postprandially, the decrease of plasma ghrelin levels is also proportional to the ingested calorie intake,[12] therefore underlining its role as a hunger signal. Ghrelin levels and hunger scores have been shown to be correlated.[13] Ghrelin is also associated with the interdigestive contractions of the stomach in rats and stimulation of gastric acid secretion and gastric

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motility.[7,14]

Age, lactation, and sex hormones can also influence ghrelin secretion and mRNA expression of octanoylating enzyme ghrelin O-acyltransferase (GOAT), which has a critical effect on ghrelin activity.[15] Studies have shown that ghrelin secretion is up-regulated in patients with anorexia[16] and cachexia,[17] and down-regulated in patients with hyperphagia and obesity.[18] Moreover, we recently reported that decreased basal and postprandial ghrelin levels were observed in age-matched female patients with functional dyspepsia (FD) compared to healthy controls. Suppressed ghrelin responses were significantly correlated with meal-related symptoms with higher bloating and satiety rating experienced by female FD patients.[19] Our findings also revealed abnormal ghrelin responses in functional GI disorders. However, the mechanisms by which it is regulated in these conditions remain unclear.

GHRELIN-FAMILY PEPTIDES AND RECEPTORS

Various derivatives of ghrelin also have crucial roles in the body. Table 1 summarizes the major GI functions of the family of ghrelin-derived peptides. Fig. 1 depicts the relationship between des-acyl ghrelin, GOAT, and acyl-ghrelin.[3]

1. Acyl-ghrelin

The ghrelin precursor, preproghrelin, is 117 amino acids in length and cleaved to produce the 28-amino acid ghrelin peptide. With alternative splicing, preproghrelin gives rise to a second form of ghrelin, 27 amino acid des-Gln14 ghrelin. Except for the deletion of Gln14, des-Gln14-ghrelin is identical to ghrelin and so retains the n-octanoic acid modification and has the same potency and activities as ghrelin. However the level of des-Gln14 ghrelin in the stomach is low.[20] Acyl-ghrelin is responsible for ghrelin's major functions. Acyl-ghrelin requires a medium-chain fatty acid (n-octanoic acid) at the Ser3 residue for complementary binding to the GHS-R type 1a (GHS-R1a).[21] The acyl modification of ghrelin is easily cleaved during sample extraction. However, acyl-ghrelin can be isolated from blood specimens by adding ethylenediaminetetraacetic acid with aprotinin or p-hydroxymecuribenzoic acid, separating the plasma by centrifugation and immediate acidification before freezing at -80°C to ensure stability of acyl-ghrelin during storage.

Table 1. Major Gastrointestinal Functions of Ghrelin-Family Peptides and Receptors

Ghrelin-family peptides	Major functions in gastrointestinal tract	Reference
Acyl-Ghrelin	↑ Appetite ↑ Gastric acid secretion ↑ Gastric motility	6-9,21
Des-acyl ghrelin	↓ Fasted motility in antrum but not in duodenum ↓ Food intake in food-deprived mice	25,26
Obestatin	↓ Orexigenic effect of ghrelin ↓ Motor activity in antrum and duodenum in rats after feeding	27,28
Motilin	↑ Gastric emptying in healthy volunteers ↑ Premature phase III contractions in stomach	7,34,35
Ghrelin-O-acyltransferase	• Essential for acylation of ghrelin for various gastrointestinal activities	36,37
Growth hormone secretagogue receptor	• Essential for binding and functional properties of ghrelin	22,38-40

2. Des-acyl ghrelin

The nonacylated form of ghrelin without octanoic acid modification at Ser3 residue, des-acyl ghrelin is also present at significant level in both stomach and blood.[20,22] Des-acyl ghrelin is the most abundant ghrelin-related molecule in the body, comprising 80% to 90% of the total circulating ghrelin, and has a longer half-life. Des-acyl ghrelin was first identified as the inactive form of ghrelin unable to bind to GHS-R. However, des-acyl ghrelin was later proposed to have nonendocrine functions including cardioprotective, antiproliferative, and adipogenic activities, and antagonizing octanoyl-ghrelin-induced effects on insulin secretion and blood glucose levels in humans.[23,24] Des-

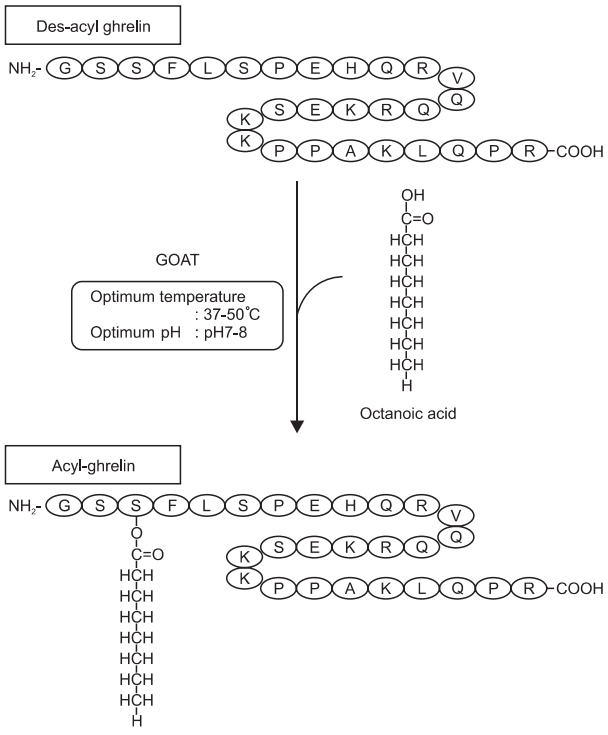

Fig. 1. Structure of human ghrelin and the modification process of octanoic acid by ghrelin O-acyltransferase (GOAT). Adopted from Sato *et al*. J Biochem 2012;151:119-128, with permission from Oxford University Press.[3]

acyl ghrelin was found to disrupt fasting-induced motility in the antrum[25] and oppose acyl-ghrelin-induced hyperphagic effects.[26]

3. Obestatin

Obestatin is a 23-amino acid ghrelin-related peptide encoded by the same gene that encodes ghrelin. Preproghrelin breaks into two peptides, ghrelin, and obestatin.[27] With peripheral and central administration, obestatin was found to antagonize ghrelin's effects on food intake, body weight, and gastric emptying, but not on GH levels.[28] Studies on obestatin/ghrelin ratio in the GI tract and plasma have been reported to be associated with some disease such as irritable bowel syndrome (IBS), obesity, and type II diabetes mellitus.[29-31]

4. Motilin

Significant homology also exists between ghrelin and motilin, which share eight identical amino acids. Similar functions between ghrelin and motilin were proposed due to their structure similarities. Motilin is a 22-amino acid peptide first isolated from the porcine intestine.[32] It was further discovered to be predominantly expressed by the endocrine cells of the duodenal mucosa.[33] Its concentration decreases distally in the small intestine. Motilin is also present in the thyroid and brain. In addition, ghrelin and motilin both stimulate gastric acid production and gastric movement.[7] Motilin inhibits the emptying of a liquid meal[34] but accelerates gastric emptying of standard breakfasts and oral glucose solutions, except for fatty cream.[35]

5. GOAT

The recently discovered enzyme GOAT octanoylates the peptide hormone ghrelin into the acyl-ghrelin peptide, confirmed by gene silencing of GOAT showing reduction of acyl-ghrelin production. GOAT is expressed in the stomach and pancreas. It was proposed that acylated ghrelin may mediate insulin regulation.[36] Zhao *et al*.[37] showed that GOAT is essential for ghrelin-mediated elevation of GH, necessary to prevent death from severe calorie restriction through preservation of blood glucose levels. New discovery of GOAT provided new understandings to the ghrelin modulation. Moreover, it also enables a new pathway of therapeutic development in altering ghrelin responses.

6. GHS-R

GHS-R was a G-protein coupled receptors that is expressed in pituitary, hypothalamus, and hippocampus.[38] In 1999, ghrelin was identified as the endogenous ligand of GHS-R.[21] There are two genes encoded in GHS-R. The first, GHS-R1a, encodes the seven transmembrane domains with binding and functional properties. The other GHS-R type 1b (GHS-R1b), is produced by alternative splicing. It is C-terminal (COOH) truncated from GHS-R1a and it is physiologically inactive.[13,38]

In the study by Sun *et al*.,[39] mice lacking growth hormone secretagogue receptor (GHSR) showed failure to induce food intake by ghrelin treatment. Body weights of these mice were modestly lower than the wild type controls. Insulin-like growth factor 1 levels were also suppressed despite no difference of food intake was observed.[39] Others reported that mice lacking ghrelin receptors also resisted the development of diet-induced obesity. Ghrelin administration failed to stimulate appetite in these mice and they eat less food and store less of their consumed calories. In particular, female mice showed less body weight and adiposity.[40] These important findings suggested the lack of ghrelin receptors may alter major physiological functions of ghrelin therefore subsequently influence the GI functions such as appetite stimulation and energy homeostasis.

GHRELIN AND GASTRIC DISORDERS

1. Gastritis

Chronic gastritis is characterized by chronic inflammatory changes in the gastric mucosa leading to extensive mucosal damage and eventual epithelial metaplasia. Destruction of oxyntic mucosa results in loss of gastric intrinsic factor-producing parietal cells, leading to pernicious anemia. Decreased serum ghrelin levels was found to be a sensitive marker of gastric atrophy regardless of *Helicobacter pylori* infection.[41]

2. *H. pylori* infection

H. pylori infection may result in gastritis, increased risk of peptic ulcers[42] and gastric carcinoma.[43] *H. pylori*-infected patients were shown to have lower gastric ghrelin mRNA expression than uninfected subjects.[44] Furthermore, the suppression of ghrelin mRNA expression is correlated with severity of glandular atrophy and chronic inflammation in the gastric corpus. Plasma ghrelin levels also decrease in *H. pylori*-infected patients.[45] After *H. pylori* eradication treatment, plasma ghrelin, and gastric preproghrelin mRNA levels increase.[46]

GHRELIN AND GI TRACT CANCER

Ghrelin expression was detected in many gastric carcinoids, even intestinal neuroendocrine tumors[47] and pancreatic neuroendocrine tumors.[48] Significant reduction of ghrelin mRNA and peptide expression in esophagogastric adenocarcinomas compared to adjacent nonneoplastic gastric mucosa was found. This finding suggested that ghrelin production may be suppressed due to damage to normal ghrelin-secreting mucosa from adenocarcinoma.[49]

In contrast, Huang *et al*.[50] reported that there was no significant difference in plasma ghrelin levels among patients with gastric or colorectal cancer and control subjects. Ghrelin levels in gastric cancer tissues were found to be significantly lower compared to normal tissue in patients with gastric cancer who had had radical subtotal or total gastrectomies. An *et al*.[51] also

reported that lower ghrelin levels were present in differentiated tumor tissue than in undifferentiated tissue. These findings suggested that the development of cancer may lead to an inability to produce ghrelin, which is also influenced by the state of differentiation.

In colorectal cancer studies, Waseem *et al*.[52] showed that colorectal cancer cells excessively secrete ghrelin *in vitro* to promote proliferation. Malignant colorectal tissue samples also showed enhanced stage-dependent expression of ghrelin. However, expression of ghrelin and its functional receptor (GHS-R1a) were suppressed in advanced grade, poorly differentiated tumors. GHS-R1a expression was lost in malignant colorectal cells, while GHS-R1b expression was enhanced.[52] Furthermore, this observation was abrogated by pretreatment of a GHS-R1a antagonist or ghrelin-neutralizing antibody.[52] The altered expression in GHS-R1b in these tumors was proposed to stimulate proliferative and invading/migrating action even in the absence of growth factors.[52]

GHRELIN IN FUNCTIONAL GI DISORDERS

1. Gastroparesis

Delayed gastric emptying is caused by gastric motility dysfunction that results in prolonged food retention in the stomach. Delayed gastric emptying is correlated with fullness, nausea, vomiting, impaired fundic accommodation with early satiety, weight loss, and pain. Animal studies have shown that ghrelin enhances GI motility and gastric emptying.[53,54] A study by Edholm *et al*.[53] showed that ghrelin stimulates motility *in vitro* and that ghrelin receptors are present in intestinal neuromuscular tissue through cholinergic neurons. Trudel *et al*.[54] showed that ghrelin increases gastric emptying and small intestinal transit in normal rats, and that ghrelin can be a strong prokinetic to reverse postoperative gastric ileus in rats. Ghrelin induces fasting motor activity in fed rats, suggesting its importance in physiological regulation of GI motility.

Acyl-ghrelin has a limited half-life. Small molecule ghrelin receptor[55] agonists had been developed to have enhanced stability and binding affinity to the ghrelin receptor in order to accelerate gastric emptying by antropyloric contractions in animal models of delayed gastric emptying.[56] Administration of ghrelin induces a premature gastric phase III without the mediation by motilin in humans.[8] In rodents, ghrelin also stimulates phase III-like contractions.[14,57] Through ghrelin infusion in conscious freely moving rats, Taniguchi *et al*.[58] showed an increase of motility index of antral phase III-like contractions in a dose-dependent manner. An intravenous (IV) injection of GHS-R antagonist in rats also blocks the effect of IV injection of acyl-ghrelin on gastroduodenal motility.[14] In dogs, IV injection of synthesized canine ghrelin stimulates GH production but not digestive tract motility.[59] Studies have suggested that acyl-ghrelin originating from the stomach may act on ghrelin receptors localizing to the vagal afferent nerve terminal and neuropeptide Y neurons in the brain in order to mediate gastroduodenal motility.

Des-acyl ghrelin could disrupt fasted motility in the antrum but not in the duodenum. However, des-acyl ghrelin does not alter motility in either the antrum or duodenum.[25,60] It was also proposed that ghrelin may have a role in many disorders involving abnormal gastric emptying rate such as Prader-Willi syndrome and dyspepsia.[18,61]

2. FD

FD is a common functional GI disorder. Epidemiological studies report that approximately 8% to 23% of Asians suffer from FD.[62] FD is characterized by chronic recurrent epigastric symptoms including pain, burning, and postprandial fullness.[63] The pathophysiological mechanisms of FD remain unclear, although visceral hypersensitivity, impaired fundic accommodation, and gastric dysmotility are common proposed mechanisms. FD is classified into two major subtypes according to Rome III classification. The first subtype involves meal-induced dyspeptic symptoms, and is known as postprandial distress syndrome (PDS). It is characterized by postprandial fullness and early satiation. The second subtype involves epigastric pain and burning, and is called epigastric pain syndrome.[63]

Lee *et al*.'s study[64] found that preprandial ghrelin levels are significantly lower in FD patients with delayed gastric emptying as predominant symptoms. Moreover, low preprandial ghrelin levels were observed in patients with dysmotility-like FD.[64] Shindo *et al*.[65] also revealed that the maximum gastric emptying time, T_{max}, for PDS is significantly higher with significant lower acyl-ghrelin levels in these patients. Lower acyl-ghrelin levels were also found in nonerosive reflux disease patients. There is an established correlation between PDS patients in acyl-ghrelin levels and T_{max}, suggesting acyl-ghrelin's role in gastric emptying of PDS patients.[65]

3. Celiac disease

Celiac disease is a chronic immune-mediated disorder. It is a T cell-mediated, gluten-sensitive enteropathy characterized by accumulation of intraepithelial $CD8^+$ and $CD4^+$ T cells sensitized to gliadin in the lamina propria, atrophy of small intestinal villi, intestinal malabsorption, and a negative energy balance. Elevated plasma ghrelin levels in patients with active celiac disease have been reported. However, plasma ghrelin levels return to normal or near normal levels after following a gluten-free diet.[66] Furthermore, Capristo *et al*.[67] showed no difference in ghrelin levels between untreated celiac patients and healthy controls and therefore suggested that the discrepancy may be affected by gender, age, and disease duration. Since ghrelin has high structural similarity to the duodenal motility peptide motilin, and exogenous ghrelin stimulates GI motility in normal human subjects,[8] ghrelin may play a role in abnormal gastric emptying in celiac disease.

4. IBS

IBS is a functional GI disorder characterized by abdominal bloating, altered bowel habits, pain, and discomfort without a clearly identifiable organic disease. Approximately 8.6% of Japanese and 9.8% of Singaporeans are affected.[68] The subtypes of IBS can be classified into constipation-predominant, diarrhea-predominant, or a mix of both.[69] Serotonin and motilin have been altered in IBS patients. A study showed enhanced motilin in diarrhea-predominant patients,[70] while another study showed increased motilin in diarrhea-predominant and constipation-predominant patients.[71] Despite finding no difference of plasma ghrelin concentration, Sjolund *et al*.[29] reported that the ratio of acyl-ghrelin to total ghrelin decreases. El-Salhy *et al*.[72] demonstrated that ghrelin-producing cells are suppressed in constipation-predominant IBS patients, while IBS patients have significantly higher ghrelin-positive cells in the oxyntic mucosa compared to normal controls. All these findings suggested that altered ghrelin modulators may subsequently affect gut motility, and may thus contribute to the pathophysiology of IBS.

GHRELIN IN GI INFLAMMATORY DISORDERS

1. Inflammatory bowel diseases

Crohn disease and ulcerative colitis are inflammatory bowel diseases (IBD) that are characterized by chronic inflammation of the GI tract. Symptoms include anorexia, malnutrition, altered body composition, and metabolic abnormalities. Ghrelin was suggested to be an important biomarker for activity determination in IBD patients. Serum ghrelin levels were found to be higher in ulcerative colitis patients and also higher in ileal Crohn disease patients compared to colonic disease patients.[73] Ghrelin is also significantly elevated in active IBD patients and positively correlated with serum inflammatory markers like tumor necrosis factor-α, C-reactive protein, erythrocyte sedimentation rate, and sedimentation fibrinogen.[74] Furthermore, increased plasma and colonic ghrelin expression was found in IBD patients.[75]

GHRELIN MODULATORS AS TREATMENTS FOR GI DISORDERS

1. Gastroparesis and gastric dysmotility

Ghrelin was found to stimulate gastric contractions in rats.[7] Ghrelin could accelerate gastric emptying and small intestinal transit of a liquid meal.[54] It was recognized to be a strong prokinetic agent that could reverse postoperative gastric ileus in conscious rats.[54] Trudel *et al*.[76] showed similar improvement of postoperative gastric ileus in dogs.

In human studies, ghrelin was found to stimulate motility and gastric emptying both in healthy individuals and gastroparetic patients. Ghrelin was found to stimulate a faster rate of gastric emptying and administration of ghrelin to healthy subjects also results in premature phase II motility and increased gastric tone.[8] Furthermore, administration of ghrelin also showed acceleration of liquid and solid gastric emptying and reduced meal-related symptoms in a study of patients with idiopathic gastroparesis.[77] IV administration of ghrelin receptor agonists (80 μg/kg of TZP101) also showed symptom improvement as evaluated both by patients and clinicians.[78]

2. Appetite stimulation in cancer patients

Ghrelin's role in appetite stimulation was first discovered as a side effect in a study of the effect of ghrelin injection on GH regulation in healthy controls.[5] Ghrelin was later identified as a peripheral orexigenic hormone that can stimulate food intake in a dose-dependent manner in rodents and humans.[9] IV and subcutaneous injection was shown to stimulate food intake in multiple studies.[6,9] Peripheral injection of ghrelin also stimulates food intake, although it cannot pass the blood-brain barrier. Plasma ghrelin levels are increased by fasting and decreased after ingestion of a meal or oral glucose, but remain unchanged after drinking water.[6]

In a rat cancer model, food intake improved after 6 days of twice daily intraperitoneal ghrelin injections.[79] Chronic intracerebroventricular injection of ghrelin increases overall food intake and also decreases the metabolic rate, leading to increased body weight. Similar results have been achieved in ghrelin-treated mice.

Rikkunshito is a kampo herbal medicine for treatment of upper GI symptoms of patients with FD, gastroesophageal reflux disease, dyspeptic symptoms of post-GI surgery patients.[80] Rikkunshito was proposed to potentiate the orexigenic action of ghrelin by various mechanisms.[81] From these emerging findings, we believe that increasing ghrelin availability especially in end-stage cancer patients may improve their appetite and energy homestasis.

3. Anti-inflammatory treatment in IBD

The effect of ghrelin treatment in IBD has only been studied in animal models of colitis. Gonzalez-Rey *et al*.[82] demonstrated a set of experiments on IBD and found that ghrelin treatment produced a near-total amelioration of multiple findings of colitis, inducing weight loss, histological colitis score, survival, and myeloperoxidase activity in the colon. In the dextran sulfate sodium model of colitis in mice, there was also body weight improvement and 67% decreased disease activity by ghrelin administration.[82]

CONCLUSIONS

Since the discovery of ghrelin, it has been found to have multiple functions. Besides its roles in growth and proliferation, ghrelin is also a potent prokinetic peptide that can stimulate

appetite and gastric motility. Recent discoveries have also suggested its involvement in various GI disorders such as gastritis, GI tract carcinoma, and functional GI disorders. Gastric disorders likely disrupt the morphological structure of the stomach, thus altering ghrelin production, since the stomach is its major source. These alterations may induce various GI disorders including functional GI disorders, eating disorders, abnormal energy homeostasis and growth. By understanding ghrelin secretion in the regulation of GI disorders, ghrelin levels may serve as a good diagnostic biomarker for early detection of GI disorders.

We strongly believe that ghrelin will be a new therapeutic target for various GI disorders, especially functional GI disorders, and useful for appetite stimulation in patients with cachexia.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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