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Changes in Acyl Ghrelin, Des-acyl Ghrelin, and Ratio of Acyl Ghrelin to Total Ghrelin with Short-term Refeeding in Female Inpatients with Restricting-type Anorexia Nervosa

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Key words
- eating disorders
- energy intake
- hospitalization
- gut hormone

Abstract

Restricting type of anorexia nervosa (AN-R) is a serious disorder affecting adolescents and young adults and decreases quality of life over a long period. Successful weight restoration is an important prognostic factor for disease outcome; however, the underlying mechanism of refeeding resistance, a core psychopathology relevant to 'ambivalent' eating behaviors, remains unclear in this disorder. Ghrelin plays an important role in the regulation of growth hormone release, appetite, and energy metabolism. However, the early progress of these patients and changes in the levels of acyl ghrelin and des-acyl ghrelin during treatment were not reported. The purpose of this study was to determine the changes in ghrelin levels (acyl and des-acyl) during early treatment. As a result, des-acyl ghrelin in AN-R patients is higher than in control subjects before the therapy, but it decreases with treatment. The plasma des-acyl ghrelin level in AN-R patients started decreasing more rapidly and in early stage of the hospitalization than ever reported, and after 8 weeks, it is significantly lower than in control subjects. It means that des-acyl ghrelin is sensitive and changeable with their nutrition state. Furthermore, the ratio of the acyl ghrelin to total ghrelin increases with 8 weeks treatment. Eight weeks after, energy intake of the AN-R patients is recovered near the normal range with a daily energy intake of 1700±93.54 kcal. These findings may be valuable for future AN-R treatments in order to increase acyl ghrelin and decrease des-acyl ghrelin, thereby influencing the refeeding outcome.

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Introduction

Anorexia nervosa (AN) is a serious disorder affecting adolescents and young adults, and decreases the quality of life in persons affected for long periods [1]. Restricting-type AN (AN-R) is characterized by severe emaciation with chronic food restriction secondary to an inordinately strong desire for being thin and fear of obesity [2]. Successful weight restoration is an important prognostic factor for disease outcome [1]; however, the mechanism underlying refeeding resistance, a core psychopathology relevant to 'ambivalent' eating behaviors, remains unclear in this disorder [3–7]. Therefore, studies to determine the physiological and psychological effects of AN refeeding are very important.

Ghrelin plays an important role in the regulation of growth hormone release [8,9], appetite [10,11], and energy metabolism [12]. AN-R patients have been found to have increased circulating ghrelin levels [13]. Furthermore, ghrelin is involved in stimulating gastrointestinal motility and emptying [14]. It is secreted mainly by the stomach, and two major molecular forms are found in plasma: acylated ghrelin, with an *n*-octanoylated serine residue in position 3 and des-acyl ghrelin [15]. The acylated form of ghrelin (acyl ghrelin) is quite unstable and rapidly changes to the des-acylated form (des-acyl ghrelin) or smaller fragments [2]. It has been reported that plasma acyl ghrelin levels were not significantly different between AN patients and healthy control subjects, but plasma des-acyl ghrelin levels were significantly higher in AN patients than control subjects [16]. The administration of acyl ghrelin induces gains in body weight (BW) and fat mass via increased food intake and decreased fat oxidation for energy expenditure [17]. In contrast to acyl ghrelin, it has been reported that the peripheral administration of des-acyl ghrelin in mice decreases food intake and disrupts fasted stomach motility [18,19]. Very recently, Inhoff

Table 1 Clinical characteristics of the subjects

	Cont.	AN-R Patients		
		OB 0 week	1 week	8 weeks
Age	25.20±2.62	22.40±11.72	22.42±11.72	22.72±11.62
Body height	155.90±3.28	152.80±2.59	152.80±2.59	152.80±2.59
Body weight	50.96±4.72	29.81±5.84*	30.31±5.59†	32.65±5.90‡
BMI	20.97±1.90	12.71±2.07*	12.93±1.97†	13.93±2.09‡
Acyl ghrelin (pg/ml)	24.4±16.04	34.60±11.59	35.80±8.29	37.60±12.22
Des-acyl ghrelin (pg/ml)	281.50±144.20	503.20±19.60*†	372.40±103.48	281.80±138.58
Acyl ghrelin/total ghrelin (%)	7.51±3.20	6.40±1.03	9.58±4.64	12.60±3.96‡#

Results are expressed as mean±S.E.M. Analysis of variance (ANOVA) and post hoc LSD test were used to compare clinical data and hormone levels among the groups
* Control subjects vs. 0 week of AN-R. † Control subjects vs. 1 week. ‡ Control subjects vs. 8 weeks. # 0 week vs. 8 weeks
AN-R: restricting type of anorexia nervosa; Cont.: control subjects; BMI: body mass index; OB: observation period

et al. reported that des-acyl ghrelin inhibits the orexigenic effect of acyl ghrelin [20].

To date, majority of the studies have not stated whether AN patients had increasing, decreasing, or stable weight. Ghrelin levels were only significantly raised in AN patients who had lost more than 5% of their BW during the preceding four weeks, whereas ghrelin levels in weight-stable as well as weight-gaining patients did not differ significantly from those of the healthy controls [21]. Recently, it has been reported that total ghrelin plasma levels in AN-R patients were significantly lower than in the control group after 3 and 6 months of treatment [22]. However, the early progress of these patients and changes in the levels of acyl ghrelin and des-acyl ghrelin during treatment were not reported. The purpose of this study was to determine the changes in ghrelin levels (acyl and des-acyl) during early treatment.

Materials and Methods

A total of 15 women participated in the study: 5 inpatients with AN and 10 age-matched control subjects (mean age, 22.40±11.72 and 25.20±2.62, respectively). All AN-R patients with amenorrhea were diagnosed according to *DSM-IV* criteria [23]. The body mass indexes (BMI) of the patients were significantly low when compared with that of the control subjects (mean BMI, 12.17±2.07 and 20.97±1.90, respectively). Control subjects, who were healthy volunteers recruited by local public advertisement, had no history of psychiatric illness or metabolic disease, were unrestrained eaters, and were within the range of 10% above or below their ideal BW. In accordance with the principles of the Declaration of Helsinki, all subjects gave informed written consent prior to participation. This study was approved by the Institutional Committee of Kagoshima University. Blood sampling was conducted at 8:00 AM, after an overnight fast. Blood samples were drawn into chilled tubes containing ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) (1 mg/ml) and aprotinin (500 U/ml). Aliquots of plasma for quantification of acyl ghrelin were separated after centrifugation into tubes containing aqueous 1 N hydrochloric acid (HCl) and stored at −80 °C until assay. The entire sampling and separation procedure was conducted in less than 10 min. Plasma acyl ghrelin and des-acyl ghrelin were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mitsubishi Kagaku Iatron, Tokyo, Japan), and the ratio of acyl ghrelin to total ghrelin was calculated as described previously [14].

During the first hospital week [observation (OB) period], no therapeutic intervention was done [3,4,24]. A baseline assessment of each patient was made on the first day. A second assessment of each patient was made on the eighth day, the end of the OB period. Thereafter, all patients started the same 8-week treatment program consisting of nutritional rehabilitation and cognitive behavior therapy [3,4,24]. Total daily energy intake (EI) at the start was 1000 kcal and was increased by 200 kcal/week. Only after the patients ate complete meals for 1 week were they allowed to start the next EI stage [3,4,24]. The third assessment was performed after 8 weeks.

Statistical analyses were performed using SPSS software (version 17.0). Analysis of variance (ANOVA) and post hoc LSD test were used to compare clinical data and hormone levels among the groups.

Results

Clinical characteristics of the subjects are shown in **Table 1**. AN patients had a daily energy intake of 1700±93.54 kcal for eight weeks according to the program.

There were significant group effects with regard to BW [$F(3, 21)=28.34$, $p<0.001$], and BMI scores [$F(3, 21)=31.01$, $p<0.001$]. BW and BMI scores were significantly higher in control subjects than in AN-R patients at 0, 1, and 8 weeks ($p<0.001$), but there were no significant differences between AN-R groups.

There was no significant group effect with regard to fasting plasma acyl ghrelin (**Fig. 1a**). There were significant group effects in des-acyl ghrelin levels [$F(3, 21)=3.64$, $p=0.029$] and the ratio of acyl ghrelin to total ghrelin [$F(3, 21)=3.45$, $p=0.035$] (**Fig. 1b,c**). Des-acyl ghrelin levels were higher in AN-R patients during the OB period than the levels in the control subjects and the levels in AN-R patients 8 weeks after hospitalization ($p=0.006$ and $p=0.015$, respectively). The ratio of acyl ghrelin to total ghrelin was significantly higher in AN-R patients 8 weeks after hospitalization than the ratio in control subjects and the ratio in AN-R patients during the OB ($p=0.013$ and $p=0.009$, respectively).

Discussion and Conclusion

AN-R is one of the most refractory and severe diseases; however, only few treatments have been effective due to lack of understanding of its etiology.

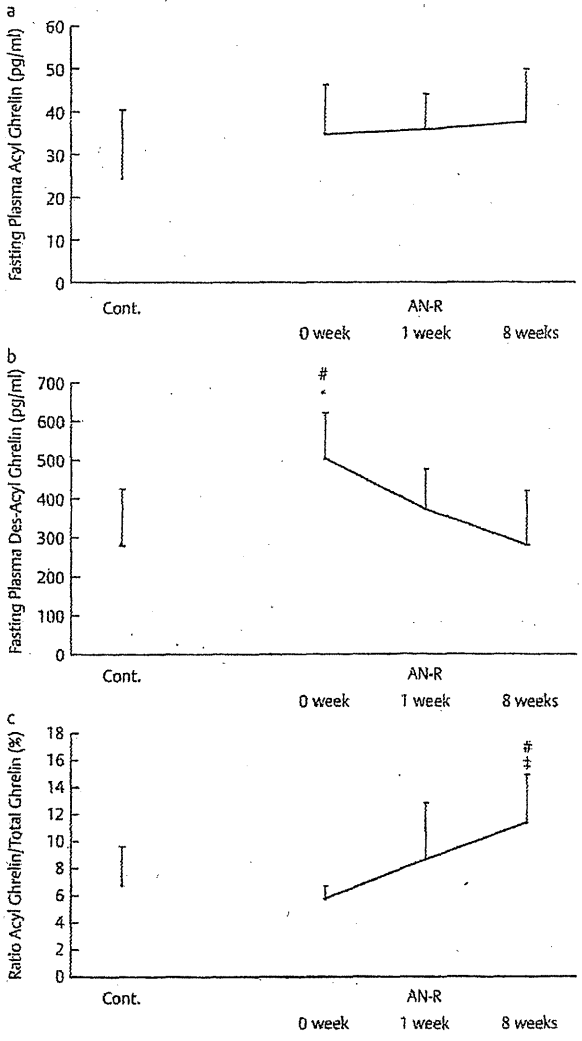

Fig. 1 Comparison of plasma acyl ghrelin (a), des-acyl ghrelin (b), and ratio of acyl ghrelin to total ghrelin (c) levels of anorexia nervosa (AN-R) patients in observation period (0 week), 1 week, 8 weeks after hospitalization and control subjects. Results are expressed as mean±S.E.M. Analysis of variance (ANOVA) and post-hoc LSD test were used to compare hormone levels among the groups. * Control subjects versus 0 week of AN-R. ‡ Control subjects versus 8 weeks. # 0 week versus 8 weeks. AN-R: restricting type of anorexia nervosa; Cont.: control subjects.

In this study, we measured acyl and des-acyl ghrelin levels separately in female AN-R inpatients in the OB period as well as 1 and 8 weeks after hospitalization. Fasting plasma des-acyl ghrelin levels of AN-R patients in the OB period were significantly higher than the levels in control subjects and the level in AN-R patients 8 weeks after hospitalization. However, after 1 week of hospitalization and in the absence of any special medical intervention, no significant difference was observed between the AN-R patients and control subjects.

It was already reported that after treatment for 3 and 6 months, total ghrelin levels were significantly lowered in AN-R patients as compared to the controls [22]. However, our study suggests that plasma des-acyl ghrelin levels in AN-R patients started decreasing very rapidly, and in the early stages of hospitalization and after 8 weeks the levels were significantly lower than those of the controls. Moreover, these findings indicate that des-acyl ghrelin is sensitive and is altered depending on the nutritional state. Despite the fact that no special medical intervention was provided to the patients during the OB period, we noticed that their BWs did not decrease, and this finding is consistent with a previous report [21]. This indicates that hospitalization only with minimum energy control and adequate rest is valuable for the treatment of AN. Recently, it was reported that acyl ghrelin is suppressed during resistance and aerobic exercise and also that hunger is suppressed during and for a short while after resistance and aerobic exercise [25].

Our study also suggests that plasma acyl ghrelin levels did not differ significantly between the AN-R patients and controls, but the ratio of acyl ghrelin to total ghrelin in AN-R patients 8 weeks after hospitalization was significantly higher than the ratio seen in these patients during the OB period.

It has already been reported that the administration of acyl ghrelin increases BW and fat mass via increased food intake and decreased fat oxidation for energy expenditure [17]; on the other hand, the peripheral administration of des-acyl ghrelin decreased food intake and disrupted fasted stomach motility [18,19]. Kawai et al. recently reported that des-acyl ghrelin and maturity fears play important roles in the prolonged malnutrition state seen in AN patients [26]. Furthermore, Inhoff et al. very recently reported that des-acyl ghrelin inhibits the orexigenic effect of acyl ghrelin [20]. These findings indicate that the ratio of acyl ghrelin to total ghrelin is an important factor in the diagnosis and treatment of AN-R. In our study, des-acyl ghrelin was higher in AN-R patients than in control subjects before therapy but decreased with treatment. In addition, the ratio of acyl ghrelin to total ghrelin increased after 8 weeks of treatment. Eight weeks after hospitalization, energy intake of the AN-R patients was near the normal range at 1700±93.54 kcal/day. These findings may be valuable for future AN-R treatments in order to increase acyl ghrelin and decrease des-acyl ghrelin, thereby influencing the refeeding outcome. Recently, ghrelin *O*-acyltransferase (GOAT), a polytopic membrane-bound enzyme that attaches octanoate to Ser-3 of ghrelin was found [27]. Therefore, studies on GOAT may provide insights into AN-R treatment.

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Editorial
Update on Ghrelin

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Few peptide hormones have attracted as much attention of the scientific community as ghrelin, the natural secretagogue for growth hormone (GH) identified by M. Kojima et al. in 1999 [1], resulting in more than 4000 PubMed citations over the last ten years. The initial interest can be attributed to the ability of ghrelin to stimulate feeding in mammals, suggesting it as a potential target for the development of antiobesity drugs. Many studies investigating this issue have however revealed the complexity of the ghrelin system including the differential physiological effects of the three peptide products of the ghrelin gene: *ghrelin*, which is peri-translationally modified via acylation with the octanoic acid; *des-acyl ghrelin* that is not acylated or has lost its fatty acid residue; and *obestatin*, another bioactive peptide derived from the preproghrelin precursor. Most importantly, it has been realized that beside stimulation of GH secretion and increased feeding, ghrelin has multiple biological effects that would be important to preserve if aiming to antagonize ghrelin-mediated positive energy balance. Among these functions, ghrelin was found to regulate gastrointestinal motility and associated sensory functions, to modulate the reproductive and stress axes, mood and emotion, glucose metabolism, as well as affecting the cardiovascular system and renal function.

The present special issue "Update on ghrelin" includes nineteen reviews and one original study and starts with the review by J. D. Veldhuis and C. Y. Bowers who "integrate the GH axis into the ghrelin system" and discuss the biological background of the multiplicity of ghrelin's functions with their relevance to drug development [2]. Other articles, as introduced below, provide in more details some specific aspects of the ghrelin system, altogether providing a comprehensive overview of the current knowledge in the field and showing how ghrelin research has helped advance our understanding of both physiology and disease.

Ghrelin is abundantly synthesized by specialized mucosal cells in the stomach accounting for about 80% of the serum ghrelin production in rats and for 65% in humans. Some ghrelin producing cells are also found in lower parts of the gastrointestinal (GI) tract, where ghrelin expression can be increased, for example, after gastrectomy [3]. Interestingly, gastric ghrelin cells are of the closed type, whereas those in the lower GI tract are of both the closed and the open type, suggesting their regulation preferentially by hormonal or luminal factors, respectively. These cellular and also phylogenic and ontogenic aspects of ghrelin production in the GI tract are reviewed by I. Sakata and T. Sakai [4]. Moreover, Fujimiya and colleagues show that the closed type gastric cells contain ghrelin, des acyl-ghrelin, and obestatin, while the open type cells contain only des-acyl ghrelin supporting a role of luminal pH on des-acyl ghrelin secretion [5].

Although the role of ghrelin to stimulate feeding is evident in mammals, it is possible that this function of ghrelin is evolutionary late and redundant, and that ghrelin has evolved as a messenger mediating other vital functions such as activation of the somatotropic axis. In fact, in fish, as reviewed by S. E. Schwandt et al., the orexigenic effect

of ghrelin is an inconsistent finding and can be species dependent while the GH stimulating effect is preserved [6]. This phenomenon is even more evident in birds, where ghrelin stimulates GH secretion and the hypothalamo-pituitary adrenal stress axis but inhibits feeding and drinking. The avian ghrelin system is discussed in details in the review by M. P. Richards and J. P. McMurtry [7].

The integrative role of ghrelin in homeostatic regulation is evident from its function in the regulation of reproduction versus feeding. Ghrelin inhibits the activity of the hypothalamo-pituitary-gonadal axis acting both centrally and peripherally, similar to hypothalamic neuropeptide Y (NPY), a down-stream target of ghrelin. In addition, ghrelin also acts directly to inhibit reproductive function during conditions of energy deficit. The effects of ghrelin in modulation of the activity of the gonadal axis in both males and females are reviewed by J. Dupont et al. [8].

Although ghrelin stimulates feeding, it inhibits drinking and activates neurons of the hypothalamic subfornical organ usually associated with dehydration. This suggests that circulating ghrelin may signal to the brain via the circumventricular organs not necessarily associated with the median eminence. However, how peripheral ghrelin enters the brains is still an unresolved issue discussed in details by M. Fry and A. V. Ferguson who propose the relaying roles of the circumventricular organs such as the subfornical organ and area postrema [9].

In its role in food intake regulation, ghrelin interacts with gastrointestinal hormones signaling satiety such as cholecystokinin, bombesin, peptide YY, glucagon-like peptide, pancreatic polypeptide, and amylin. As discussed by A. S. Wisser et al., these peptides may inhibit ghrelin secretion or antagonize its action in the appetite regulatory neurons in the brain [10]. Since plasma levels of ghrelin normally fall after a meal, it is possible to use it as a satiety marker to evaluate the satietogenic properties of different macronutrients for the development of various nutritional antiobesity approaches. How different types of nutrients affect ghrelin secretion is reviewed by C. Koliaki et al. [11].

In addition to the appetite-related neuronal pathways activated by ghrelin, ghrelin receptors are present in many brain areas that can affect mood and emotion, and, indeed, it was found that ghrelin may interfere with the regulation of the stress response, mood, and anxiety. Again, in analogy to the orexigenic NPY, which also regulates these behavioral modalities, the situation with ghrelin is not simple, and both anxiolytic and anxiogenic effects have been attributed to ghrelin as discussed by J. C. Chuang and J. M. Zigman [12]. Further elucidation of the brain ghrelin system should help to clarify the role of ghrelin, which will be important for the designing of drugs targeting the ghrelin system.

Next, five reviews provide full coverage of the important role of ghrelin in influencing gastrointestinal contractility and motility in normal and pathological conditions, as well as showing its relevance to both new and old therapeutic approaches. H. S. Sallam and J. D. Z. Chen introduce the subject with a meticulous review of the experimental and clinical data accumulated during the last ten years related to ghrelin's prokinetic effects [13]. T. Ohno et al. discuss the possibility that changes of plasma ghrelin concentration are not relevant to the prokinetic effect of ghrelin because of the decreased plasma levels found after a meal, similar to the situation observed for motilin, a hormone structurally related to ghrelin [14]. Further, the complexity of the ghrelin system in influencing gastric motility is presented in the review by M. Fujimiya et al. who discuss the differential effects of ghrelin, des-acyl ghrelin, and obestatin, providing evidence for the implication of distinct central pathways in these effects involving hypothalamic NPY, corticotropin-releasing hormone, and urocortin [5]. As gastric motility is altered in subjects with functional dyspepsia who also experience abdominal discomfort, nausea, and decreased appetite, exploring a role of ghrelin in these patients may be worthwhile. Indeed, as discussed by T. Akamizu et al., plasma levels of ghrelin have been found to correlate with reduction of symptoms in these patients and excitedly, a preliminary study using synthetic ghrelin showed a therapeutic potential in functional dyspepsia [15]. Thus, ghrelin or its analogs may become new drugs for the treatment of functional dyspepsia.

Similarly, improvement in physiological ghrelin secretion may underlie the beneficial effects of other therapeutic approaches, for example, traditional medicine. Such an example is given in the review by T. Hattori who discusses the mechanisms behind the beneficial effects of Rikkunshito, a traditional Japanese medicine based on a mixture of eight herbs. In fact, Rikkunshito was shown to alleviate dyspepsia, for example, during cancer chemotherapy, and this improvement was associated with increased ghrelin secretion [16].

Ghrelin is also expressed in the pancreatic islets and has an intricate relation with insulin in regulation of the glucose metabolism. These effects of ghrelin with relation to the pathophysiology of type 2 diabetes are reviewed in great detail by S. Sangiao-Alvarellos and F. Cordido [17]. The authors also discuss the results obtained in ghrelin- and ghrelin receptor-knockout mice, suggesting that absence of ghrelin signaling is more relevant to glucose homeostasis than to food intake control, supporting the phylogenetic data mentioned above.

Interestingly, although ghrelin stimulates feeding, low plasma levels of ghrelin are commonly present in obesity whereas levels usually increase with weight loss. These data do not support the causative role of ghrelin in increased appetite in obesity but may suggest a link via the role of ghrelin in glucose homeostasis. L. Pulkkinen et al. in their review make an emphasis on the relation between ghrelin and insulin resistance and human genetics in the development of obesity and the metabolic syndrome [18]. Further data of the putative role of ghrelin in obesity come from studies of obese subjects who have undergone bariatric surgery and hence have reduced gastric ghrelin production. As reviewed by D. J. Pournaras and C. W. le Roux [19], ghrelin plasma levels are altered after the surgery, and both decreased and increased levels were documented. In addition, similar results have also been reported in rats [3]. As bariatric surgery is accompanied by reduced appetite and body weight, the serum ghrelin data do not support a causative role of ghrelin in these beneficial effects. However,

bariatric surgery is consistently associated with an improved insulin resistance and diabetes, taken by some as a reason to rename the surgery "metabolic" and pointing to that the surgery-associated changes of ghrelin serum levels might still have beneficial effects on the glucose metabolism.

Expression of ghrelin and the ghrelin receptor was found in vascular endothelial cells providing the background for the vascular effects of ghrelin. In fact, ghrelin was shown to decrease blood pressure, although this effect might also involve the modulation of the central sympathetic tone. The cardiovascular effects of ghrelin are discussed by M. Tesauro et al. showing that activation of the ghrelin system might be a new therapeutic approach for chronic heart failure and cardiac cachexia [20]. A. Laviano et al. review the relevance of ghrelin in another form of cachexia associated with chronic renal failure both in regard to its pathophysiology and to its putative therapeutic role [21]. The authors propose the use of exogenous ghrelin which should be able to overcome endogenous ghrelin resistance present in renal cachexia, that might improve the nutritional status in cachectic patients. In fact, synthetic ghrelin or ghrelin analogs might be considered as a new therapy for a variety of pathological conditions characterized by anorexia or cachexia. For instance, a state of ghrelin resistance is present in anorexia nervosa, and a recent pilot study showed that administration of ghrelin is accompanied by improved appetite in these patients [22]. Another possible indication of "ghrelin therapy" can be anorexia associated with gastrectomy as suggested by the experimental study in Yada's laboratory [3].

To conclude this editorial, ten years of ghrelin research indicate that this peptide initially identified as a GH secretagogue is an universal hormone. As guest editors, we thank all the authors who have contributed to this special issue for preparing excellent articles and we wish the readers a good time in exploring the ghrelin world, which we find ourselves most exciting and promising for the new discoveries and potential therapeutic spin offs.

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The role of ghrelin in energy homeostasis and its potential clinical relevance (Review)

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Abstract. The novel gastric hormone ghrelin, a 28-amino acid peptide, has been identified as a potent growth-hormone secretagogue. Ghrelin production is regulated by nutritional and hormonal factors. Besides stimulating growth hormone secretion, studies show that ghrelin exerts a number of central and peripheral actions such as the regulation of food intake, the control of energy balance, glucose metabolism and insulin release, cardiovascular actions, the stimulation of gastric acid secretion, and motility. The broad spectrum of biological activities associated with ghrelin continues to expand. In the future, the diverse functions of ghrelin raise the possibility of its clinical application in a large number of pathological conditions.

Contents

1. History

Ghrelin was first reported by Kojima *et al* in 1999, as an endogenous ligand for the 'orphan' growth hormone (GH) secretagogue (GHS) receptor (GHS-R) (1,2). A group of peptidyl and non-peptidyl synthetic compounds with potent *in vitro* growth hormone-releasing activity, called GHSs, have since been developed. The receptor binding sites for GHSs have been found to be distinct from the GH-releasing hormone (GHRH) receptor. Radiolabeled GHSs have been displaced by other GHSs, but not by GHRH or somatostatin. GHSs and GHRH have shown synergistic effects on the GH release, suggesting that they act, in part, via different mechanisms (3-5). GHRH increased intracellular cyclic AMP via its receptor, while GHSs increased the concentration of free intracellular calcium (6). The existence of an endogenous substance that can activate the GHS-R could be considered.

Initially, the Kojima research team conducted studies to identify the GHS-R ligand. When they assayed for GHS-R-expressing cells in stomach extract, they observed abundant levels of intracellular calcium release in all the fractions. On the basis of this phenomenon and the results from other experiments, they speculated that an active peptide in the stomach could have damaged the cells. Then, in an assay using a small amount of stomach extract, activity was observed in the fractions of molecular weights between 3000 and 4000 Da. They synthesized the ligand peptide but it was inactive. The structures of the purified and synthetic ligands were compared. Their HPLC elution positions were different, and the molecular weight of the purified peptide was greater than that of the synthetic peptide. A functioning peptide with an octanoyl-modified structure was synthesized and purified by HPLC. The structure of ghrelin is a peptide hormone in which the serine at position 3 is n-octanoylated, a modification essential to the activity of the hormone (1,7). The name, ghrelin, is based on its role as a GH-releasing peptide, with reference to the Proto-Indo-European root 'ghre', meaning to grow, and 'lin', a common suffix for certain hormones (8).

2. Various forms

The ghrelin gene consists of 4 exons and 3 introns (9). This gene encodes the 117 amino acid, pre-proghrelin, both in rats and humans. In addition to ghrelin, des-Gln14-ghrelin is also produced, although in smaller quantities, by stepwise enzymatic processing (10). The cleaved protein consists of 28 amino acids with serine octanoylated at position 3. The N-octanoyl group is essential for the induction of GH secretion (1) and critical for the development of active peptides and peptide-like substances (11).

Ghrelin is the endogenous ligand for GHS-R and has been implicated in the regulation of food intake and energy homeostasis. Ghrelin is produced primarily by the cells in the oxyntic glands of the stomach or intestinal wall (12-15). Two molecular forms of ghrelin are found in the stomach:

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The 28-amino acid ghrelin, with the n-octanoylated serine at position 3, and the 27-amino acid, des-Gln14-ghrelin, produced by alternative splicing of the ghrelin gene (1,10). The acylation appears to be essential for the GH-releasing activity of both natural forms of ghrelin, although des-acyl ghrelin could have some additional biological activities, such as acting as a survival factor in the cardiovascular system (16). Other minor forms of ghrelin are present in human plasma and the stomach and these forms have been determined as total immunoreactivity by conventional radioimmunoassay based on the carboxy terminal fragment of ghrelin (2), Structural activity studies have shown that the 5 amino-terminal residues retain full functional activity (11).

Obestatin, a novel 23-amino acid peptide identified in the rat stomach, was also found by comparative genomic analysis to be a derivative of the mammalian pre-proghrelin gene, which also encodes ghrelin (17). It was originally suggested that obestatin binds to the orphan G protein-coupled receptor (GPR), named GPR39 (17). Increased obestatin, decreased ghrelin levels and a decreased ghrelin/obestatin ratio characterize obesity in women (18), and plasma obestatin levels are low in patients with type 2 diabetes mellitus and impaired glucose intolerance (19). Both obestatin and ghrelin levels are increased in anorexic subjects and decreased in human obesity, suggesting that obestatin is a nutritional marker reflecting body adiposity and insulin resistance.

3. Mechanisms of action

Ghrelin acts as an orexigenic endocrine signal that communicates peripheral caloric intake to the brain centers for energy homeostasis. The pathways that mediate the effects of ghrelin on appetite and food intake have been widely studied. In the hypothalamus, GHS-R mRNA is expressed in neurons containing neuropeptide Y (NPY; which stimulates the NPY-Y1 and Y5 receptors), agouti-related protein (AGRP; which blocks the melanocortin MC3 and MC4 receptors), pro-opiomelanocortin (POMC), and GHRH-containing neurons. These NPY/AGRP-containing neurons express ghrelin receptors (20,21) and respond to ghrelin by increasing their firing rate. Moreover, local projections from NPY/AGRP-containing neurons terminate on the POMC/CART-containing neurons and release γ-aminobutyric acid when activated by ghrelin. Thus, anorectic neurons are inhibited indirectly by ghrelin (14). Both the NPY/AGRP- and the POMC/CART-containing neurons are targets for several inhibitory signals for food intake. Importantly, on a long-term basis, the hormones, leptin and insulin, act through their respective receptors on NPY/AGRP-containing neurons (and on POMC-containing neurons) to inhibit food intake (15). Additionally, the L-cell-derived hormone, PYY3–36 (co-released with glucagon-like peptide 1 from the lower GI tract following food intake), has been proposed to inhibit food intake by acting on the pre-synaptic Y2 receptors (22). Thus, for the NPY/AGRP-containing neurons, the ghrelin receptor provides the only hormonal, appetite-stimulatory input that counterbalances a large number of inhibitory afferent inputs (10).

Vagal neurons could be an alternative and important target for the stimulatory effect of ghrelin on food intake (23). Appetite-regulating signals from the gut are conveyed to the hypothalamus through the afferent vagus and the nucleus of the solitary tract in the brain stem (23). Ghrelin receptors are also found in the dorsal vagal complex, making this another target for circulating ghrelin (15). Thus, although the major focus has been on the hypothalamic ghrelin receptor, several other sites that are important in appetite regulation could be involved in mediating ghrelin-induced food intake. It should be noted that the ghrelin receptor is co-expressed with the leptin receptor in these locations (Fig. 1) (24-26).

4. Major functions

Ghrelin is an endogenous peptide described as a strong physiological GHS that interacts with the GHS-R1a (27). Despite its strong GH-releasing activity, ghrelin also exhibits other prominent endocrine and non-endocrine functions (Table I) (26).

Ghrelin and metabolism

Ghrelin and food intake. Ghrelin was the first hormone to be identified as a food intake stimulatory signal originating from the stomach. The peripheral or intracerebroventricular administration of ghrelin induces adiposity and weight gain in rodents (28,29). The GH-independent effects of ghrelin on food intake and energy homeostasis can be viewed from an evolutionary perspective as a physiological survival mechanism. Due to its appetite-increasing effect, ghrelin helps stimulate food consumption and fat storage, thereby increasing chances for survival during times of famine (30). In human subjects, plasma ghrelin levels increase nearly 2-fold immediately before meals and drop within 1 hour after eating, in a pattern similar to that of serum insulin (31). Plasma ghrelin levels in fasting human subjects display a circadian pattern with spontaneous rises and declines at customary mealtimes (32). Prandial changes in plasma ghrelin levels occur in association with changes in hunger scores, even when external cues related to time of day have been removed from the environment (33). These findings, together with the findings of a rapid decrease in plasma ghrelin after oral glucose load, suggest that plasma ghrelin plays a role in meal initiation and that it reflects the short-term energy balance (34).

In addition to its role in meal initiation and short-term energy balance, ghrelin is involved in the regulation of long-term energy homeostasis. Several human studies have focused on the changes in plasma ghrelin levels in abnormally thin and obese subjects. Plasma ghrelin levels were found to be higher in patients with anorexia nervosa compared to healthy subjects, and weight gain reduced plasma ghrelin levels to normal in these patients (35). Post-prandial ghrelin suppression was also normal in anorexic patients (36). The reason why anorexic patients eat less than the required quantity in spite of their high fasting plasma ghrelin concentration, is not entirely understood. One possible explanation could be a decreased sensitivity or resistance to ghrelin. Alternatively, the cortical overexpression of ghrelin hormonal effects is also possible.

Ghrelin and insulin secretion. Ghrelin inhibits the effects of insulin on glycogen synthesis and gluconeogenesis *in vitro*

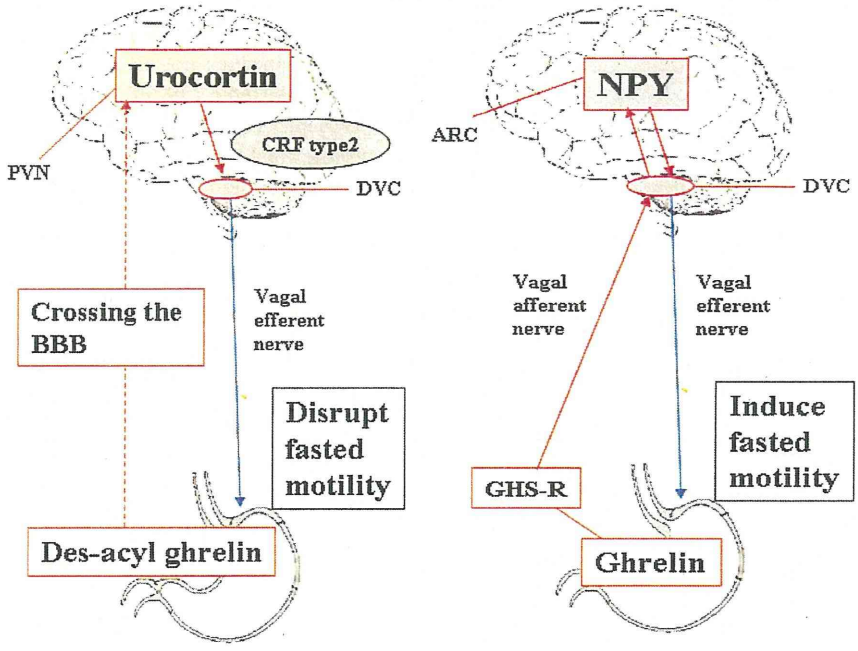

Figure 1. Summary diagram of ghrelin and des-acyl ghrelin on the gastroduodenal motility and brain mechanisms mediating the action of hypothalamic peptides.

Table I. Effects of ghrelin.

Target	Physiological or pathological role
GH secretion	Stimulates GH secretion
Food intake and energy homeostasis	Stimulates appetite and food intake Increases body weight
Insulin secretion, glucose and lipid metabolism	Inhibits insulin secretion and action Increases blood glucose Stimulates lipogenesis and proliferation of adipocytes, inhibits lipolysis
Gastrointestinal system	Stimulates gastric secretion and motility Protects against mucosal damage
Cardiovascular system	Decreases blood pressure Improves endothelial function Increases stroke volume and cardiac index Suppresses sympathetic activity Improves cardiac cachexia Decreases apoptosis of cardiomyocytes Protects against ischemic/reperfusion injury
Immune system	Stimulates immune cell proliferation Decreases pro-inflammatory cytokines

(37). In human subjects, acute ghrelin administration induces hyperglycemia and reduces insulin secretion (37). Changes over time in plasma glucose and serum insulin concentrations after acute ghrelin administration suggest that ghrelin could have a direct glycogenolitic effect (38). In healthy obese males, the 2-month treatment with oral GHS failed to influence fasting concentrations of plasma glucose and serum insulin, but an impaired glucose tolerance was observed after an oral glucose load (39). Substantial amounts of non-acylated ghrelin can also be found in human plasma. The simultaneous administration of ghrelin and non-acylated ghrelin prevents the acylated ghrelin-induced rise in serum insulin and plasma glucose levels, and non-acylated ghrelin alone, or in combination with acylated ghrelin, improves

insulin sensitivity (40). As non-acylated ghrelin does not bind to GHS-Rs, there could be an as yet unidentified receptor, which mediates the effects of non-acylated ghrelin (41).

Ghrelin as an immunomodulator. Ghrelin stimulates the thymus and T cells during aging in humans and rodents. The thymus involutes and T-cell production declines. However, the chronic administration of a ghrelin mimetic to old mice restored GH/insulin-like growth factor I levels and stimulated growth, differentiation and cellularity of the thymus, in addition to increasing T-cell production (42).

Ghrelin also modulates the production of pro-inflammatory cytokines. In rodents, ghrelin attenuated endotoxin-induced anorexia, reduced cytokine production, and improved mortality associated with lipopolysaccharide (LPS)-induced endotoxic shock (43-45). In a well-designed series of studies, Dixit *et al* showed that ghrelin and the GHS-R are found in human T cells and monocytes, where ghrelin specifically inhibited the chronic synthesis of pro-inflammatory anorectic cytokines such as leptin, interleukin 1ß (IL-1ß), IL-6, and tumor necrosis factor α (46). Ghrelin also inhibited the LPS-induced production of these cytokines in spleen and liver. Remarkably, ghrelin reduced IL-1ß and IL-1α levels in sham (non-LPS)-treated mice (46). Furthermore, ghrelin treatment inhibited leptin-induced cytokine synthesis, and leptin-augmented GHS-R expression in human T cells. The regulation of this network could have widespread implications for the development of wasting diseases, aging and frailty (46).

Ghrelin and gastrointestinal motility. Plasma ghrelin levels have been shown to correlate with gastric expansion. In rats, intravenously administered ghrelin stimulates gastric motility and secretion in a dose-dependent manner. The Intracerebroventricular administration of ghrelin also stimulates gastric acid secretion in anesthetized (47), but not in conscious rats (48). Furthermore, ghrelin appears to exert a gastro-protective effect via nitric oxide- and capsaicin-sensitive neurons (49).

The peripheral and central administration of acyl ghrelin induces faster motor activity in the duodenum of conscious freely-fed rats, while it increases phase III-like contractions in both the antrum and duodenum of conscious, fasted rats (50). These actions occur through brain mechanisms that involve GHS-R and NPY (51). Acyl ghrelin also stimulates gastric phase III of the migrating motor complex and solid gastric emptying in healthy human volunteers (52). To date, studies using canine and rodent models have indicated that acyl ghrelin is the only agent that could reverse post-operative gastric ileus (53,54). All the evidence suggests that acyl ghrelin can potentially be used as an exogenous prokinetic for treating slow transit in the upper gastrointestinal tract, such as in patients with gastroparesis and post-operative ileus (55). In contrast, the peripheral and central administration of des-acyl ghrelin disrupts the motor activity of the antrum in conscious, fasted rats, whereas it does not affect either the antral or duodenal motor activity in conscious, freely-fed rats (56).

The effects of obestatin on upper gut motility are controversial. Peripheral obestatin inhibits gastric emptying. Obestatin has clearly been shown to act via the corticotropin-releasing factor (CRF) receptor subtypes 1 and 2 in the brain to inhibit motor activity in the antrum and duodenum in conscious fed rats (57). However, other studies have shown that obestatin does not affect gastric and intestinal motility *in vivo* or *in vitro* (58-60). Obestatin immunoreactive cells are present in the pancreas and throughout most of the rat gastrointestinal tract, including the colon. Further studies are warranted in order to investigate the effects of central obestatin administration on gastrointestinal motility and secretory functions.

Ghrelin and lung development. The developing lung seems to be a major source of ghrelin. Significant ghrelin expression during the pseudoglandular stage of human fetal lung development (7-18 weeks of gestation) has been reported, suggesting that ghrelin can act as a regulator of fetal lung development via autocrine/paracrine mechanisms (61). In adult rats, it has been demonstrated that ghrelin modulates pulmonary vascular remodeling and hypertension (62).

Ghrelin expression was characterized in human and rat normal and hypoplastic lungs. Human lung tissue was derived from congenital diaphragmatic hernia (CDH) patients with severe pulmonary hypoplasia, as well as from available controls. For rat studies, the nitrofen-induced CDH model was used, as it best mimics the human disease (63). Nitrofen administration results in the equal interference in the growth of both lungs, even before diaphragm development occurs. After the establishment of the diaphragmatic defect, presumably 16 days after conception, ipsilateral lung development is impaired due to the herniated abdominal organs (63). The ghrelin effect appeared to be lung-specific, since the heart-to-body weight ratio of newborn rats was not altered. The lung-to-body weight ratio in normal fetuses was not significantly changed by ghrelin either. The ghrelin overexpression in hypoplastic lungs and the effect of exogenous ghrelin administration to nitrofen-treated dams, suggest a role for ghrelin in attenuating CDH-associated lung hypoplasia (63).

Cardiovascular effects of ghrelin. Increasing evidence supports a functional role for ghrelin in myocardial growth associated with improved cardiac function. Both ghrelin and GHS-R have been identified in the aorta and myocardium, indicating that ghrelin can modulate cardiovascular parameters through GH-independent mechanisms (64). Evidence has shown that ghrelin inhibits apoptosis in cardiomyocytes and endothelial cells through the activation of extracellular signal-regulated kinase 1/2 and Akt serine kinases. It should be noted that, although cardiomyocytes bind to ghrelin with high affinity, they do not express GHS-R1a. Taken together, it is reasonable to suggest the existence of other new GHS-R subtype(s) distinct from GHS-R1a in the cardiovascular system (65).

5. Role of ghrelin in disease

Ghrelin and obesity. Obesity is characterized by blunted GH secretion that could help to maintain the obese state and is reversed by weight loss (66). Fasting plasma ghrelin concentrations in obesity are significantly lower and are negatively correlated with body mass index, percentage body fat, and/or fasting insulin and leptin concentrations (67). Low levels of

Table II. Clinical conditions in which ghrelin agonists or antagonists could have therapeutic potential[a] (93).

Ghrelin agonists	Ghrelin antagonists
Growth hormone deficiency (therapy)	Simple obesity
Hypopituitarism or growth hormone deficiency (diagnosis)	Prader-Willi syndrome
Cachexia (associated with aging, gastrectomy, chronic organ insufficiency, malignancy, or HIV infection)	
Anorexia or eating disorders	
Dilated cardiomyopathy or CHF	
Atherosclerosis	
Gastroparesis	
Ileus	
Osteoporosis or metabolic bone disease	

[a]Conditions in which ghrelin agonists can serve as useful diagnostic agents are indicated. CHF, congestive heart failure; HIV, human immunodeficiency virus.

ghrelin could contribute to the decreased GH secretion observed in obese patients (67). Plasma ghrelin levels are also negatively correlated with plasminogen activator 1 levels, which are elevated in insulin-resistant subjects and are associated with increased cardiovascular risk of atherothrombosis (68). Decreased ghrelin secretion in established obesity could be a physiological adaptation to long-term positive energy balance, although a particular subset of obesity could be associated with high levels of ghrelin (69).

Despite its low levels, like NPY, ghrelin can act to maintain increased body weight (70,71), since weight loss increases ghrelin levels at a rate proportionate to the amount of weight loss (69). The lack of suppression of plasma ghrelin after a meal in obese subjects could contribute to increased food consumption (72).

Ghrelin and eating disorders. Anorexia nervosa is a psychopathological disorder that presents with neuroendocrine alterations reflecting starvation (73). Patients experience hunger, but are prevented from eating by an intense fear of losing control over their eating and becoming overweight. Plasma ghrelin concentrations in patients with anorexia nervosa are markedly elevated in comparison to those of healthy controls, although there could be some patients with normal (not increased) ghrelin levels (64-77). Plasma ghrelin levels do not drop after food intake, suggesting that a single meal is insufficient to suppress the drive to eat in the subjects (78). NPY concentrations in the cerebrospinal fluid are also increased in anorexia nervosa (75,79). Ghrelin levels return to normal after partial weight recovery, suggesting a physiological effect of ghrelin to compensate for the lack of nutritional intake and energy stores. However, the similarity of the ghrelin resistance model to the leptin resistance model remains unknown (36). The increased ghrelin levels observed in anorexia nervosa patients could explain the relatively high plasma GH concentrations also observed in these patients. Although there could be no difference in ghrelin secretion between restrictive vs. bulimic anorexia nervosa (77), plasma ghrelin is markedly elevated in patients with bulimia nervosa (80), suggesting that abnormal eating behaviors with habitual binge eating and purging can affect ghrelin secretion. It remains to be examined whether binges in bulimia nervosa are the consequence of elevated ghrelin.

Ghrelin and cardiovascular disease. The discovery of ghrelin as an endogenous GHS immediately prompted research on its hemodynamic effect, as GH is known to play a role in the maintenance of cardiovascular health (81). However, the possibility that GHSs can have direct cardiovascular effects, independent of GH release, has been strongly supported by different experimental approaches. Ghrelin can be synthesized by the cardiomyocytes of both human and murine origin, and it is secreted by HL-1 cells (a cultured line derived from murine atrial cardiomyocytes showing a heart phenotype) (82) and is widely used as an *in vitro* model of cardiac biology (83), as well as by human cardiomyocytes in primary culture (84).

The administration of ghrelin has been found to reduce cardiac afterload and increase cardiac output without increasing heart rate in healthy volunteers (64), to induce vasodilation (85,86), and to improve the hemodynamics of patients with chronic heart failure (87). Chronic heart failure-associated cachexia is attenuated by ghrelin in rats (88), and in humans is accompanied by above normal ghrelin levels, possibly as a compensatory mechanism in response to a catabolic–anabolic unbalance (87). Ghrelin also regulates cardiovascular function in rats suffering from septic shock and exerts a protective effect against ischemic injury in rat hearts (89). Similar cardiovascular effects have been observed in rabbits (90). As stated above, in both humans and experimental animals, the cardiovascular effects of ghrelin seem to not be mediated by GH (82-90). This indicates that one of the multiple mechanisms by which obesity favors cardiac pathology could be its association with low ghrelin levels, which can reduce cardioprotection.

Ghrelin and cachexia. Cancer patients treated with cytotoxic drugs experience a number of adverse effects, including delayed gastric emptying, early satiety, anorexia, nausea and vomiting, known as cancer chemotherapy-induced dyspepsia. In a mouse model of cisplatin-induced chemotherapy-associated dyspepsia, the administration of ghrelin (1 mg/kg i.p., bid) significantly increased food intake at 24 and 48 h after cisplatin treatment and improved the cisplatin-induced delay of gastric emptying (91). It has also been suggested that an adaptive up-regulation of ghrelin and GHS-R mRNA expression occurs in response to toxic challenges in the gut during cancer chemotherapy dyspepsia. Levels of plasma ghrelin were enhanced in patients with functional dyspepsia (92). Moreover, the ability of ghrelin to activate vagal afferent pathways, as well as central hypothalamic and peripheral enteric nerve-mediated responses, suggests that the ghrelin

effect could be similar to the anti-emetic effects of ondansetron, a 5-HT3 receptor antagonist with both central and peripheral sites of action, and could be used in the treatment of chemotherapy-induced nausea and vomiting (Table II) (93,94).

6. Conclusion

Ghrelin is a novel gastric hormone that was first recognized in 1999 as a mediator of GH release. Since GHs are anabolic, an important function of ghrelin could be to co-ordinate energy needs with the growth process. Later discovered biological roles of ghrelin imply that it could have many physiological functions as well. Ghrelin, a peptide hormone produced primarily in the stomach, has potent GH-releasing, orexigenic and adipogenic activities, and exerts important effects on the cardiovascular and gastrointestinal systems. In addition, ghrelin plays important roles in glucose metabolism and insulin secretion. Therefore, ghrelin appears to be involved in the pathophysiological mechanisms of several human disorders, including disturbances of appetite, energy homeostasis and glucose metabolism. In addition, the diverse functions of ghrelin raise the possibility of its clinical application in a large number of pathological conditions. It could be used, for example, for the diagnosis of GH deficiency or for the treatment of cachexia, wasting syndrome, heart failure and gastric motility disturbances. Ghrelin antagonists could also be useful in the treatment of obesity.

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Original Article

Appetite and gastrointestinal motility: Role of ghrelin-family peptides☆

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SUMMARY

Eating disorders, obesity and cachexia endanger the lives of millions of people worldwide. Fortunately, in last decade, there has been a rapid and substantial progress toward uncovering the molecular and neural mechanisms by which energy imbalance develops. In 1999, ghrelin was identified as the first orexigenic gut-derived peptide. It stimulates appetite and controls the gastric motility and the acid secretion through the activation of the growth hormone secretagogue-receptor. After the discovery of ghrelin, other forms of ghrelin-related proteins were isolated from the rat stomach. The unmodified des-*n*-octanoyl form (des-acyl ghrelin) and the recent obestatin act through distinct receptors and contrarily to acyl ghrelin, show an anorexigenic activity. The finding that these three peptide hormones derive from the same precursor exert opposing physiological actions, highlights the importance of post-translational regulatory mechanisms. Further investigations are required to highlight the complexity of ghrelin physiology in order to better understand the mechanisms regulating the energy balance and provide a successful treatment of eating disorders, obesity and cachexia.

1. Introduction

Eating disorders, obesity and cachexia endanger the lives of millions of people worldwide. Fortunately, in last decade, there has been a rapid and substantial progress toward uncovering the molecular and neural mechanisms by which energy imbalance develops. Energy balance is regulated in part by peptide hormones produced in brain or gut or both.[1] Earlier studies on synthetic and peptidyl growth hormone secretagogue led to the identification of the growth hormone secretagogue-receptor (GHS-R) and subsequently to the discovery of ghrelin, the first orexigenic gut-derived peptide ("ghre" is the Proto-Indo-European root of the word "growth").[2] After the discovery of ghrelin, other forms of the protein were isolated from the rat stomach. The first was the des-Gln[14]-ghrelin,[3] the second was the unmodified des-*n*-octanoyl form (des-acyl ghrelin) [4] and the latter was the recent obestatin, from the Latin "obedere" meaning to devour and "statin" denoting suppression.[5] Cumulative evidence indicates that rapid gastric emptying is closely related to over-eating and obesity, as delayed gastric emptying to anorexia and cachexia [6–8] This review aims to summarize recent data on ghrelin-family peptides, paying attention to appetite and gastrointestinal motility (see Fig. 1).

2. Ghrelin

In 1999, acyl ghrelin was discovered in the stomach of rats as an appetite stimulatory signal.[2] Its structure resembles motilin.[9] The human ghrelin gene is located on chromosome 3p26-p25, encoding a 117 amino acid peptide termed preproghrelin. Ghrelin circulates in two major molecular forms: acyl ghrelin, which has *n*-octanoylated serine in position 3 and des-acyl ghrelin, which is the major circulating isoform.[10] Despite the acylated residue of serine was supposed to be essential for its biological activity,[11] recent works showed that des-acylated form of ghrelin is active, playing a role in various metabolic activities.[12,13] Both the molecular forms are produced in the arcuate nucleus of the hypothalamus [14–17] as seen for the stomach.[18–20]

Deacylation of ghrelin to des-acyl ghrelin, which rapidly occurs in the plasma, is responsible for the reduced half-life of ghrelin. Two enzymes involved in the deacylation of ghrelin have been identified. The high-density lipoprotein (HDL)-associated paraoxonase functions in the plasma whereas the lysophospholipase I, a thioesterase active against palmitoyl-Gsα and palmitoyl-CoA, functions in the stomach.[21] In contrast, the enzyme that catalyzes the acyl modification of ghrelin has not been identified. It has been seen that medium-chain fatty acids are directly utilized for the acylation of ghrelin.[22] The increased hydrophobicity of the acyl side chain may explain why acyl ghrelin circulates bound to large plasma proteins, particularly HDL species, whereas des-acylated ghrelin circulates as free peptide. This fact may influence the

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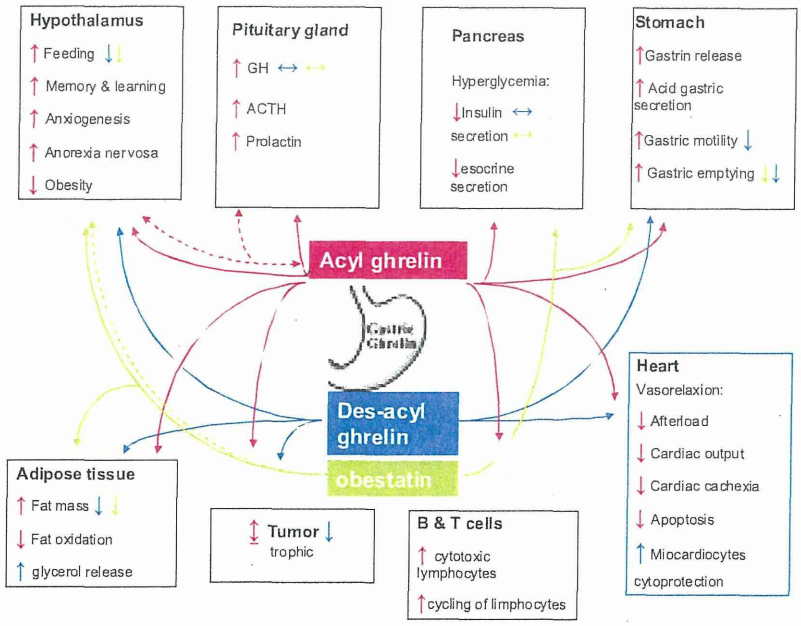

Fig. 1. Physiological functions of ghrelin-family peptide. Solid lines refer to blood stream, dashed lines refer to vagus nerve, up-arrows refer to stimulation, down-arrows refer to inhibition and bidirectional lines refer to no action.

transport of the different ghrelin forms to centres of appetite control in the central nervous system (CNS).[23] Acylated ghrelin crosses the blood-brain barrier in both directions using a saturable transport system that requires the presence of the unique octanoyl residue of the ghrelin molecule.[17] In contrast, des-acyl ghrelin crosses the blood-brain barrier by non-saturable passive mechanisms and is retained by the brain once within the CNS.[24]

2.1. Acyl ghrelin

Acyl ghrelin is presently considered as the first known circulating orexigenic hormone. It is a 28-amino acid peptide originally identified as the endogenous ligand of the growth hormone secretagogue-receptor (GHS-R).[10] It is secreted primarily from X/A-like enteroendocrine cells of the stomach[2] and secondarily from the small intestine and the colon.[18] Ghrelin may also be expressed in the hypothalamus,[2,25] the pituitary,[26] and several tissues in the periphery.[27] GHS-Rs are widely expressed in the CNS.[28] They are found in the pituitary,[26] the brainstem and the hypothalamus,[2,25] whereas peripheral receptor expression has been described in the myocardium, the gastrointestinal tract, the adipose tissue, the liver, the kidney, the placenta and the T cells.[29] Acyl ghrelin, besides having a strong growth hormone (GH)-releasing activity, as its name implies,[30,31] has several actions.[32,33] It plays an important role in the short-term regulation of appetite, determining food intake from meal to meal.[34] It is also involved in the long-term regulation of energy balance, playing as an adiposity signals.[16,35] Moreover, it controls glucose homeostasis as well as the gastric motility and the acid secretion.[9,36]

2.1.1. The mechanisms of action of acyl ghrelin

Exogenous ghrelin affects body weight and food intake more than 1000-fold more potently following central administration rather than intravenously or intraperitoneally. For this reason it has been suggested that ghrelin influences energy homeostasis predominantly via the modulation of central mechanisms.[37] In the hypothalamus, ghrelin exerts its effects on food intake independently by the growth hormone release. It activates the neurons expressing GHS-R in the arcuate nucleus of the hypothalamus that co-secrete the orexigenic neuropeptide Y (NPY) and Agouti-related protein (AgRP).[38–40] In particular, its satiety-reducing effect is related to the antagonism of the inhibitory effect of leptin on the hypothalamic NPY production, in rats.[41]

Although strong evidence supports the hypothalamic mode of action, there is growing body of findings suggesting that ghrelin may also work via hindbrain. The vagal nerve, which innervates most visceral and abdominal organs, relays information about nutrients and distension in the gut to the brain. In addition to its afferent fibres, vagal efferent signals influence the secretion of hormones, such as insulin. Given that ghrelin is produced in the gastrointestinal tract and is responsive to changes in metabolic state, it may be argued that peripheral ghrelin acts by effects on gastric vagal afferents in the CNS and that these afferent vagal fibres eventually alter the activity of hypothalamic NPY/AgRP circuits via hindbrain relay.[20,42] The critical role of the afferent vagus nerve as a mediator of feeding behaviour is consistent with the findings of early satiety, lack of hunger and stable weight reduction in obese patients following truncal vagotomy. It has been demonstrated that the blockade of gastric vagal afferent abolished ghrelin-induced feeding in both rodents and humans,[43–45] GH secretion and activation of NPY-producing and growth hormone releasing hormone (GHRH)-producing neurons.[32] It is interesting to note that the highest and lowest reported ghrelin concentrations, respectively, have been found in subjects with Prader-Willy syndrome, who are known to have low parasympathetic nervous activity and in Pima Indians, who are known to have high parasympathetic nervous activity.[46]

2.1.2. Acyl ghrelin is a signal for hunger

Acyl ghrelin is considered a short regulator of food intake in both animals and humans.[47–49] This finding derives originally from animal models. In rats, acute and chronic administration of ghrelin enhances food intake and weight gain.[16,35] Systemic studies pointed out the influence of exogenous ghrelin administration on appetite and eating in humans. Peripheral administration of ghrelin produced a 28% increase of food intake in normal weight

volunteers.[50] The subjects who received exogenous ghrelin reported an increase in appetite and showed a higher caloric intake than after placebo.[32,34] In addition to the augmentation of the appetite, subjects had vivid imagination of their favourite meal. It is well known that GHS-receptors are present in the hippocampus that is thought to be a site of visual imagination. The imagined, preferred flavour of the meal differed between females and males. These observations suggest that the role of ghrelin is more complex that a signal for hunger.[16,51]

2.1.3. The pre-meal peak of ghrelin

Ghrelin is considered as a meal initiator hormone. Accordingly to its role of short-term regulator of food intake, the greatest amount of ghrelin is produced by stomach and duodenum, organs that are well positioned to sense the presence or absence of recently ingested food.[47,52] In the rat stomach mucosa, ghrelin concentration decreased significantly after fasting caused by an increased secretion in the blood. Therefore, stomach tissue and the systemic circulation present inverse pattern of ghrelin concentrations.[53,54] In both animals and humans, ghrelin plasma levels are increased in response to fasting [38,55] and are suppressed by food intake.[32,35,47,52,56] Subjects receiving meals on a fixed schedule showed a pre-meal elevation in circulating ghrelin concentrations.[55] The pre-meal peak was confirmed also in subjects freely requested a meal, in absence of external time- or food-related cues.[57] In these subjects, hunger scores and ghrelin plasma concentrations showed similar temporal profiles and similar relative differences in magnitude between lunch and dinner. The increasing of ghrelin plasma concentrations generally precedes increase in the hunger sensation by a short interval.[57] The assertion that similar preprandial increases might affect human meal initiation is strengthened by observations that three different single-nucleotide polymorphisms in the human gene encoding either ghrelin or its receptors are associated with abnormal meal pattern characterized by excessive nibbling.[58]

However, a recent work has failed to show a relationship between plasma ghrelin concentrations and meal initiation, suggesting a possible role in physiological preparation for a meal.[51] A recent study supported its role in the regulation of anticipatory processes involved in food intake and nutrient disposition. Drazen et al.[59] found that the anticipation of eating, as well as fasting/feeding status, influences pre- and postprandial ghrelin plasma concentrations in rats.

2.1.4. Acyl ghrelin influences gut motility

In humans, ghrelin stimulates gastric motility[38] and acid secretion,[60] both of which increase in anticipation of meals. This fasted motor activity of the gastrointestinal tract has considered a mechanical cleansing of the stomach and the intestine in preparation for the next meal. In healthy volunteers, the peripheral administration of ghrelin induces the occurrence of phase III of the migrating motor complex after about 20 min. Moreover, it induces a premature phase III originating in stomach about 14 min after its injection.[61] A positive correlation was reported between preprandial ghrelin concentration and gastric emptying time. The duration of gastric emptying is considered as an important factor for the duration of satiety.[11,62]

Ghrelin induces the fasted motor activity in the gastrointestinal tract by the activation of NPY neurons in the hypothalamus both via central pathways and via vago–vagal reflex.[37] The intracerebroventricular and intraperitoneal injections of ghrelin stimulate gut motility as indicated by shortened colonic transit time in freely moving rats in the physiological fed status. It has been found that NPY-Y1 receptor is primarily involved in the modulation of colonic ghrelin-induced fasted motor activity.[63] The immune-neutralization of NPY in the brain completely blocked the fasted motor activity induced by both intracerebroventricular and intravenous injection of ghrelin.[64] Ghrelin also acts on GHS-R on vagal afferent nerve fibres in the stomach,[65] which transmit this signal to the nucleus of the solitary tract. From the nucleus of the solitary tract the information are projected to the arcuate nucleus of the hypothalamus, where NPY neurons are activated. From the arcuate nucleus, the signal is transmitted to the dorso-motor nucleus of vagus nerve and via vagal efferent fibres, the fasted motor activity is induced in the gut.[61] Once the brain mechanism is eliminated by truncal vagotomy, ghrelin receptors in stomach and duodenum might be primarily involved in the regulation of fasted motor activity. The effect of GHS-R antagonists, which block the fasted motor activity in both stomach and duodenum in vagotomized rats but not in normal rats, supports this hypothesis.[62]

Modulation of intra-gastric pH and the effects of ghrelin on the gastrointestinal motility are tightly related. Intra-gastric pH at 0–30 min after meal decreases into pH 2; ghrelin does not induce fasted motor activity in the gut at such low pH. It induces the fasted motor activity when intra-gastric pH becomes higher than pH 5.[62] From a teleological point of view, the decrease in acid secretion during fasting is relevant for the maintenance of gastric mucosal integrity.[66] In patients suffering from idiopathic gastroparesis, it has been found that the administration of ghrelin enhances gastric emptying and improves meal-related symptoms. These observations suggest a potential for ghrelin receptor agonists in the treatment of gastroparesis.[60,65]

2.1.5. The nutritional control of ghrelin: macronutrients

The nutritional control of ghrelin has not been fully clarified yet. The ways in which the various macronutrients affects specific component of the appetite regulation system is controversial. Understanding this point is very important in light of the focus of the most popular diets on varying macronutrient distribution. It has known that carbohydrates, proteins and fats differentially affect the secretion of some gastrointestinal hormones, such as CCK.[67] However, little is known about the specific effects of the different nutrients on ghrelin production and secretion.

The postprandial suppression of plasma ghrelin has been well studied. It has been suggested that the suppression of ghrelin plays a role in the satiating effect of ingested nutrients. The degree of postprandial ghrelin suppression is a function of the quantity of calories ingested.[16,35,68] Postprandial ghrelin suppression was initially reported in rodents and humans ingesting meals of mixed macronutrient content [49,59,68] and in rodents receiving intra-gastric glucose infusions.[35] In the rodent stomach, the ghrelin expression is decreased in response to glucose and amino acids ingestion more rapidly and strongly than lipids.[49,52] Similar results were found in humans. Plasma ghrelin levels were substantially suppressed after the ingestion of isovolemic and isocaloric beverages consisting of 80% carbohydrate, proteins or fats.[45] The 80% carbohydrate beverage was the most effective, either after acute enteral or parenteral administration [69] in both rodents and humans.[45,52] These data may explain the high-fat dietary promotion of weight gain and the known higher capacity of the satiating described for carbohydrates compared with fats.

2.1.6. The nutritional control of ghrelin: gut hormones

Animal studies showed that nor gastric distension neither presence of nutrients in stomach lumen is required for influencing ghrelin plasma concentrations.[55,70] It has been hypothesized that ghrelin may regulate feeding interacting with gut hormones or neural signals. The pattern of ghrelin suppression by food is broadly consistent with the idea that other gut hormones released in response to food may contribute to the reduction in plasma ghrelin