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Original Article

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

Simona Perboni [a,*], Akio Inui [b]

[a] Unità Operativa Day-Hospital Area Medica, Ospedale di Manerbio, Azienda Ospedaliera di Desenzano del Garda, Brescia I-25025, Italy
[b] Department of Behavioral Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8520, Japan

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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

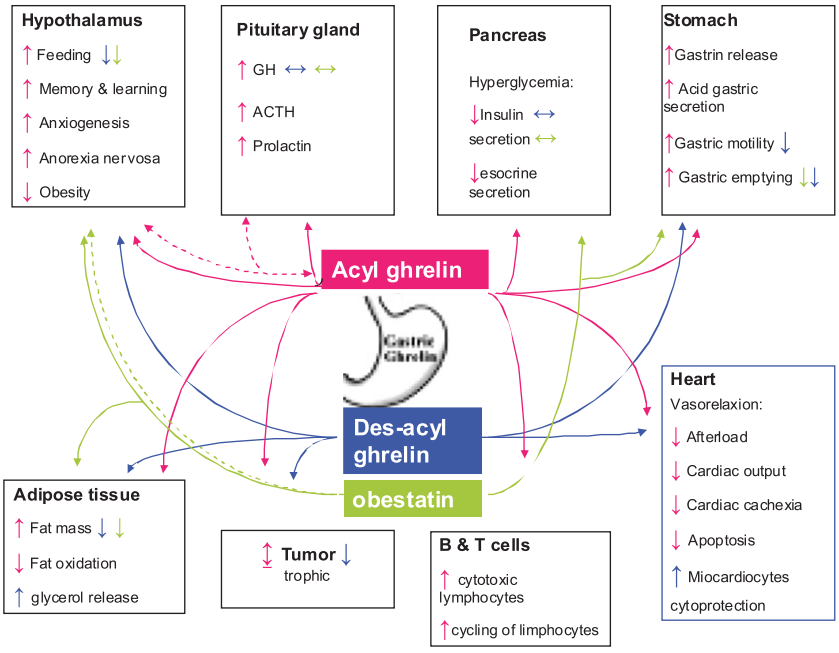


**Fig. 1.** Physiological functions of ghrelin-family peptide. Solid lines refer to blood stream, dashed lines refer to vagus nerve, up-arrows refer to stimulation, down-arrows refer to inhibition and bidirectional lines refer to no action.

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

levels. Insulin, CCK, peptide YY and glucagone-like peptide 1(GLP-1), for example, rise rapidly after food ingestion and circulating ghrelin begins to fall simultaneously.[45,71] Ghrelin and CCK exert opposite effects on feeding behaviour through the vagal afferent, thereby regulating food intake on a short-term basis as a meal initiator and terminator, respectively. Abnormalities in the release of or sensitivity to ghrelin and/or CCK may be involved in alterations of food intake.[20]

An especially large amount of attention was paid to the relationship between ghrelin and insulin. Although acute administration of ghrelin reduced insulin secretion and caused hyperglycaemia,[72] plasma ghrelin concentrations were not affected by glucose or insulin in healthy subjects.[73–76] However, the presence of at least circulating basal insulin concentrations is essential for prandial ghrelin suppression, as demonstrated in type 1 diabetic subjects. In absence of any insulin treatment, these diabetics did not manifest a postprandial ghrelin response to a standard breakfast meal. However, low basal dose of insulin, which maintains euglycemia during the hours before the meal, was sufficient to reduce circulating ghrelin.[77] Moreover, a recent work showed a relevant association between insulin-mediated glucose metabolism and the regulation of ghrelin secretion after food intake in children with different levels of overweight. It is possible that the maintenance of an adequate level of insulin sensitivity and glucose oxidation may affect appetite regulation by favouring a more efficient postprandial ghrelin reduction.[78] However, some authors showed that in type 2 diabetic subjects, hyperinsulinemia and insulin resistance were significantly associated with decreased plasma levels of acyl ghrelin.[79,80]

*2.1.7. Acyl ghrelin and the long-term regulation of food intake*

Ghrelin is considered as an "adiposity signal". Fluctuations of plasma ghrelin concentrations may reflect physiological adaptation to long-term alterations in the energy balance.[38] Ghrelin levels correlate inversely with adiposity at baseline.[76,81,82] Moreover, circulating ghrelin levels increase in response to weight loss resulting from multiple causes.[71,83–86] The increase in ghrelin concentration during starvation may promote eating and its fall in obesity may be a secondary response to over-eating.[87] Patients with Prader-Willi syndrome, who have profound obesity and voracious and uncontrollable appetite, have remarkably high level of ghrelin when compared with other obese individuals.[46] Patients with anorexia nervosa exhibit high plasma ghrelin concentrations compared with age- and sex-matched controls and weight gain decreases their elevated ghrelin concentrations.[85,88] Morbidly obese patients, who underwent Roux-en-Y gastric bypass operation (RYGB), experienced durably decreases in body weight by ~36%. RYGB is currently the most successful treatment for obesity.[89] Recent data suggest that neural and hormonal mechanisms may contribute to the decrease of appetite and greater efficacy of the bypass procedure compared with diet-induced weight loss.[90] It would expect the massive decrease of BMI achieved with RYGB to trigger an elevation of ghrelin levels; however, these patients had greatly reduced ghrelin concentrations and ghrelin pre-meal peaks.[86,91] The absence of the compensatory increase in ghrelin concentrations that usually occurs with diet-induced weight loss may contribute to maintain durably weight loss.[92] Some authors explain the paradoxical suppression of ghrelin levels with an "override inhibition". The uninterrupted diversion of food from contact with the distal stomach and the duodenum initially produces stimulatory signals to release gastric ghrelin and later paradoxically suppresses its release. Other authors suggest a neural based explanation. Since the effect of RYGB to reduce plasma ghrelin has been observed within a very short time after surgery, it is tempting to speculate that this effect is related to treatment of the vagal nerve in the procedure.[45,93] The influence of vagal nerve on ghrelin regulation has been described above in this review. However, other studies have shown no change in ghrelin levels [94,95] and one group has reported a rise in ghrelin levels after RYGB.[96]

*2.1.8. The relationship between acyl ghrelin and leptin in the long-term regulation of food intake*

Circulating ghrelin levels appears to track body weight. It would be of great interest to discern the means through which the ghrelin regulatory system detects changes in weight. It has recently shown that hypocaloric diet-induced weight loss induces a coordinated increase in circulating ghrelin levels and decrease in plasma leptin levels.[86] Because ghrelin is a potent orexigenic peptide and leptin is a satiety signal at the level of the CNS,[38] this coordinated change in hormone levels should elicit a strong compensatory increase in appetite that contributes to the poor long-term maintenance of weight loss achieved by caloric restriction.[97]

Plasma ghrelin concentrations show a diurnal variation, in phase with leptin, with highest levels in the morning and lower at night.[98] Various studies showed that reciprocal rhytmicities in 2 peripheral hormones are the major afferent signals for the timely activation of the NPY system in the arcuate nucleus of the hypothalamus.[99–101] It has been found that leptin inhibits both the secretion of gastric ghrelin and the stimulation of feeding by ghrelin.[102] It has been suggested that this dual leptin restraint is the major regulatory arm of the feedback communication between the periphery and the hypothalamus for weight homeostasis,[99,100,103] and the disruption in the rhythmic communication at any locus in the leptin–ghrelin–NPY feedback loop impels loss of hypothalamic control, leading to abnormal weight gain and obesity.[104]

The relationship between ghrelin and leptin has to be considered, in order to understand why the most popular diets are based on varying macronutrient distribution. In humans, low fat, high carbohydrate diet produced loss of body weight without a compensatory increase in plasma leptin and ghrelin concentrations.[97] Conversely, high-protein diet induces sustained reductions in appetite, *ad libitum* caloric intake and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. In this work, increased protein intake enhances the satiating effect of circulating leptin in the CNS. The anorexic effect of dietary protein, which may be due in part to increased CNS leptin sensitivity, is apparently stronger than any orexigenic effect of increased ghrelin concentrations accompanying weight loss with high-protein diet.[105]

However, a recent study has suggested that the leptin and the ghrelin systems play energy homeostasis function independently of each other in healthy humans. In these subjects, the circulating ghrelin concentrations were regulated by changes in adiposity independently by leptin levels.[106]

*2.2. Des-acyl ghrelin*

To date, little is known about the physiological role of des-acyl ghrelin. Some authors suggested that des-acyl ghrelin might have an anorexigenic activity that is contrary to the orexigenic activity of acylated ghrelin.[107,108] Conversely, a recent study showed that both ghrelin and des-acyl ghrelin function as orexigenic peptides in the hypothalamus.[109]

Since the acylation of ghrelin is required for the activation of the type 1 growth hormone secretagogue-receptor, it was assumed that des-acyl ghrelin was void of endocrine properties.[4] The des-acyl ghrelin showed no effect on the elevation of intracellular $Ca^{2+}$ concentrations in cells that express the GHS-R or on the increasing of plasma GH concentrations in rats.[110] Later, a paper reported that des-acyl ghrelin is able to antagonize the metabolic but not the

neuroendocrine response elicited by acylated ghrelin in humans.[111] Serum GH levels correlated closely with plasma acylated, rather than des-acylated ghrelin.[112] Transgenic mice overexpressing des-acyl ghrelin exhibited thin phenotype although their plasma GH levels showed no differences with non-transgenic littermates. This thin phenotype may be due, at least in part, to a decrease in food intake.[19] In another experiment, transgenic mice showed decrease body weight and nose-to-anus length associated with normal nutritional conditions. It has been suggested that des-acyl ghrelin may modulate the GH-insulin growth factor-I axis in the pituitary and in the hypothalamus of transgenic mice, resulting in the small phenotype.[113]

Moreover, recent studies indicate that ghrelin and des-acyl ghrelin exhibit similar GHS-R-independent biological activities, including a cytoprotective effect on cultured cardiomyocytes and endothelial cells,[114] the inhibition of cell proliferation in human breast and prostate cancer lines,[115,116] the reduction of glycerol released from rat epididymal adipocytes.[117] Des-acyl ghrelin promotes adipogenesis directly *in vivo* in bone marrow fat in rats.[13] Overall, these findings suggest des-acyl ghrelin plays its actions by a different receptor than GHS-R1a, as yet unknown.

#### 2.2.1. Des-acyl ghrelin as an anorexigenic peptide

Several studies showed that des-acyl ghrelin induces a state of negative energy balance. It reduces body weight by decreasing the food intake and delaying the gastric emptying in mice. These effects are mediated in the hypothalamus. The peripheral administration of des-acyl ghrelin increases the neuron c-Fos expression in the arcuate nucleus and in the paraventricular nucleus of the hypothalamus. The anorexigenic cocaine and amphetamine regulated transcript (CART) and the urocortin [19] as well as the corticotropin-releasing factor type 2 receptor, but not type 1, are involved in this action.[118] Peripheral des-acyl ghrelin may directly activate the brain receptor by crossing the blood-brain barrier [24] but not by the activation of vagal afferent pathways.[19] According to these results, the intracisternal administration of des-acyl ghrelin decreased food intake and inhibited gastric emptying without altering small intestine transit in food-deprived rats.[108]

#### 2.2.2. Des-acyl ghrelin as an orexigenic peptide

Interestingly, a recent work shows that des-acyl ghrelin stimulates feeding via a mechanism independent of GHS-R. In rats, the intracerebroventicular administration of des-acyl ghrelin increased feeding and locomotor activity by stimulating orexin neurons in the lateral area of the hypothalamus. Orexin-A and -B are involved in the hypothalamic regulation of feeding, energy homeostasis and arousal. It has been found that des-acyl ghrelin does not compete with ghrelin for binding to the GHS-R in orexin neurons. Thus, there are three possible subtypes of orexin neurons: those that express the GHS-R as a receptor for ghrelin, those expressing an as-yet unknown receptor or target protein of des-acyl ghrelin and neurons possessing both proteins.[109] Further studies examining the physiological and neuroanatomical interactions between des-acyl ghrelin and its targets will help to highlight the roles of ghrelin-related peptides in the regulation of feeding and energy homeostasis.

#### 2.2.3. Ghrelin system and glucose and lipid metabolism

The ghrelin system, using both the acylated and des-acylated molecules, is actively involved in the acute- and the long-term control of glucose metabolism and insulin concentrations.[111] It has been demonstrated that glucose-output by primary hepatocytes is time- and dose-dependently stimulated by acyl ghrelin and inhibited by des-acyl ghrelin. Furthermore, it has been reported that des-acyl ghrelin is able to antagonize acyl ghrelin induced glucose-output. These actions might be mediated by a different receptor than GHS-R1a, which is not expressed in the hepatocytes. Apparently, the two forms of peptides must be considered as separate hormones able to modify each other's actions on glucose handling, at least in the liver.[119]

In humans, the acute administration of acyl ghrelin induced a rapid rise of glucose and insulin levels. In healthy humans, physiological increments in plasma ghrelin concentrations do not change glucose flux and circulating concentrations of glucose, insulin, C-peptide and glucagons.[120] The acute administration of des-acyl ghrelin has no effect on insulin secretion in humans. However, des-acyl ghrelin prevented the acyl ghrelin-induced rise of insulin and glucose when co-administered. The combination of acylated and des-acylated ghrelins significantly improved insulin sensitivity.[121] This finding might lead to new therapeutic approach for many disorders in which insulin sensitivity is disturbed.

Ghrelin as well as des-acyl ghrelin promotes bone marrow adipogenesis *in vivo* by a direct peripheral action, in rats. This action is mediated via a receptor other than GHS-R1a. The ratio of ghrelin and des-acyl ghrelin production could help to regulate the balance between adipogenesis and lipolysis in response to nutritional status.[13,117]

### 2.3. Obestatin

Obestatin, a 23 amino acid peptide recently isolated from the rat stomach, is encoded by the same gene of ghrelin. Similarly to ghrelin, which requires post-translational process by acylation, the biological activity of obestatin requires the amidation at its conserved glycine residue at the carboxyl terminus.[5] Obestatin is expressed in cells of gastric mucosa and myenetric ganglion cells and this peptide is biologically active on central neurons.[122]

First reports have showed that obestatin plays opposite actions to ghrelin on food intake,[5,123] body weight and gastric emptying.[5] Obestatin injection suppressed food intake and decreased body weight gain in rats. These activities are induced whether obestatin is administered intraperitoneally or intracerebroventricularly. Serum leptin concentrations were not affected after treatment with either obestatin or ghrelin, suggesting minimal modulation of body fat content. Furthermore, treatment with obestatin led to a sustained suppression of gastric emptying activity. The contractile activity of jejunal muscle were decreased and antagonized by the stimulatory effects of ghrelin. The observed inhibition of jejunal contraction may trigger an afferent vagal signal to induce a central satiety response.[5]

Zhang et al.[5] reported that obestatin activates the orphan G protein-coupled receptor GPR39, which is a member of a family including the receptors for ghrelin and motilin. Real-time reverse-transcription polymerase chain reaction analyses indicate that GPR39 is expressed in jejunum, duodenum, stomach and other peripheral tissues. It is also expressed in the amygdala,[124] the hippocampus and the auditory cortex.[125] Low levels of GPR39 mRNA were found in several other brain regions. Surprisingly, there is no expression of GPR39 mRNA in the hypothalamus, expected to be the site of the anorexigenic action of obestatin.[125] It has been hypothesized that obestatin may simply suppress appetite by triggering nausea or visceral illness.[126] Unlike ghrelin, neither intravenous nor intracerebroventricular administration of obestatin affects the secretion of GH, PRL, TSH and ACTH, despite the presence of GHRP39 in the pituitary of rats.[127]

Recent works do not support a role of the obestatin/GPR39 system in the regulation of energy balance. Several *in vivo* and *in vitro* studies failed to confirm that obestatin reduces food intake and inhibits gastrointestinal motility.[128–132] In mice, obestatin did not show any effect on food intake, body weight, body composition, energy expenditure, locomotor activity, respiratory quotient or

hypothalamic neuropeptides involved in energy balance regulation. In agreement with the first reports, it has been found no effect on GH secretion *in vivo*.[130] In rodents, the peripheral administration of obestatin did not affect gastric emptying nor inhibited the prokinetic effects of ghrelin. Moreover, the intestinal contractility was not affected.[131] Zizzani et al.[133] confirmed that exogenous obestatin *per se* did not modify food intake in fasted and fed mice. However, they found that obestatin inhibited the orexigenic effects as well as the stimulation of GH levels by ghrelin administration. It remains to be clarified whether obestatin modulates endogenous ghrelin actions.[133] Taken together these results suggest that peripheral obestatin is not a satiety signal that play a role in the regulation of gastric emptying and do not support the concept that it is a physiological opponent of ghrelin.

Interestingly, the intracerebroventricular administration of obestatin inhibited water drinking in rats. This effect preceded and was more pronounced than any on food intake and it did not appear to be the result of altered locomotor/behavioural activity. It has been suggested that obestatin not acts in the pituitary to regulate GH secretion but may act in the brain to alter thirst mechanisms. From this point of view, the effects of obestatin on food intake may be secondary to an action of the peptide to inhibit water drink in rats.[134]

Convergent reports affirm that obestatin is not the cognate ligand for GPR39 receptor.[128,135,136] It has been found that obestatin does not bind GPR39. It has been observed no effects of obestatin on GPR39-transfected cells in various functional assays (cyclic adenosine monophosphate production, calcium mobilization and GPR39 internalization). Similarly observations have been reported by other researches.[128,136] No specific binding of obestatin could be detected in two different types of GPR39-expressing cells using three radioiodinated forms of obestatin.[128]

GPR39 appears to be involved in gut motor functions,[124] since in GPR39 knockout mice gastric emptying is accelerated. However, food intake, body weight and adiposity were similar between GPR39 (+/+) and GPR39 (−/−). Obestatin injection did not affect food intake in both phenotypes. It has suggested that the role of GPR39 should be conducted independently of the function of obestatin.[137] Taken together, existing reports curtail the initial promise that obestatin is a new regulator of appetite and digestive motility.

Despite the sequence homologies between rodent and human obestatin is 87% and 93% for GPR39, the effects of obestatin have yet to be determined in humans.[126] Some studies were performed in subjects suffering from Prader-Willi Syndrome with contradictory results. Park et al.[138] reported that plasma obestatin levels are not elevated and are not regulated by insulin in children both suffering from Prader-Willi Syndrome and from obesity. On the contrary, Butler and Bittel[139] found higher levels of obestatin in infants affected by Prader-Willi syndrome compared to controls.

Obestatin, like ghrelin, is secreted in a pulsatile manner, although their levels were not strictly correlated.[133] Obestatin appears to have an extremely fast influx rate to the brain. Absence of the blood-brain barrier permeation by obestatin was in contrast to the saturable transport of human ghrelin reported previously. Obestatin lacked specific bindings and endocytosis, and the small amount internalized showed rapid intracellular degradation. The differential interactions of obestatin and ghrelin with the blood-brain barrier illustrate their distinctive interactions with the CNS.[140] Further studies are necessary to highlight the physiological role of obestatin and GPR39 in both animals and humans.

## 3. Conclusion

The identification of obestatin, a novel peptide hormone derived from the same gene as ghrelin, has recently added further complexity to ghrelin physiology. Three peptide hormones (acyl ghrelin, des-acyl ghrelin and obestatin) derive from the same precursor act through distinct receptors and exert opposing physiological actions. This finding highlights the importance of post-translational regulatory mechanisms. Despite the rapid progress, many questions remain unanswered, including the regulation of acyl ghrelin, des-acyl ghrelin and obestatin secretion, the downstream pathways that mediate their effects, and their precise physiologic endocrine and paracrine roles. Further investigations are required to highlight the intricate balance of energy homeostasis and body weight control and to provide a successful treatment of eating disorders, obesity and cachexia.

## Conflict of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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