Ghrelin – Physiological Functions and Regulation

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Abstract

Ghrelin is an orexigenic peptide predominantly secreted from the stomach and stimulates appetite and growth hormone (GH) release. Studies have provided evidence that ghrelin exercises a wide range of functions, including regulation of food intake and energy metabolism, modulation of cardiovascular function, stimulation of osteoblast proliferation and bone formation and stimulation of neurogenesis and myogenesis. In the gastrointestinal system, ghrelin affects multiple functions, including secretion of gastric acid, gastric motility and pancreatic protein output. Most of these functions have been attributed to the actions of acylated ghrelin. The balance among its secretion rate, degradation rate and clearance rate determines the circulating level of ghrelin. This review explains what ghrelin is, its physiological functions and the factors that influence its level.

Keywords

Ghrelin, food intake, obesity, lipolysis, ghrelin receptors, regulation

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Ghrelin is a 28-amino-acid peptide predominantly secreted in the stomach and stimulates appetite and growth hormone (GH) release. The name ghrelin is based on 'ghre' a word root in Proto-Indo-European languages meaning 'grow' in reference to its ability to stimulate GH release. In 1976, Bowers and co-workers discovered opioid peptide derivatives that did not exhibit any opioid activity, but had weak GH-releasing activity, and were referred to as GH secretagogues (GHSs).¹ GHSs act on the pituitary and hypothalamus to release GH, not through the GH-releasing hormone receptor (GHRH-R) but through an orphan receptor, the GHS-R.² Synthetic GHSs and the GHS-R indicated that an unknown endogenous ligand for GHS-R should exist. In December 1999, Kojima et al. were the first to purify and identify ghrelin from rat stomach as the endogenous ligand for the GHS-R.¹

Expression and Synthesis of Ghrelin

Approximately 60–70 % of circulating ghrelin is secreted by the stomach, with most of the remainder originating in the small intestine. Low-level ghrelin expression also occurs in several tissues outside the gut, including hypothalamus (arcuate nucleus and paraventricular nucleus), pituitary, lung, adrenal cortex, kidney, bone, testis, placenta and pancreatic islet cells.³ In the stomach, the ghrelin-containing cells are more abundant in the fundus than in the pylorus originally termed X/A-like cells. These X/A-like cells account for approximately 20 % of the endocrine cell population in adult oxyntic glands. However, the number of X/A-like cells in the foetal stomach is very low and increases after birth. As a result, the ghrelin concentration of foetal stomach is also very low and gradually increases after birth until 5 weeks of age.⁴

The human preproghrelin gene is located on chromosome 3p25-26 and consists of five exons with four introns. Spliced ghrelin messenger

(mRNA) is translated to a 117-amino acid preproghrelin precursor, which is subsequently cleaved to yield ghrelin. In addition, obestatin, a 23-amino acid peptide is a putative proteolytic fragment of the preproghrelin precursor purified from rat stomach extracts. In contrast to the appetitestimulating effects of ghrelin, treatment of rats with obestatin suppressed food intake, inhibited jejunal contraction and decreased bodyweight gain.⁵ However the appetite suppressing effect of obestatin failed to be demonstrated in.⁶ The amino acid sequences of mammalian ghrelin are well conserved, particularly the 10 amino acids in their NH₂ termini, which are identical. This structural conservation and the universal requirement for acyl-modification of the third residue indicate that this NH₂-terminal region is of central importance to the activity of the peptide. Rat and human ghrelin differ in only two amino acid residues.²

Plasma Level of Ghrelin

The measurement of ghrelin levels is affected by blood collection and storage conditions.⁷ To ensure ghrelin stability, it is strongly recommended to collect blood samples with EDTA-aprotinin (or other proteases inhibitors) under cooled conditions and proceed to the sample acidification and dilution prior to ghrelin measurement.⁸ Recently, an addition of esterase inhibitor to the ghrelin measurement guidelines was found to enhance ghrelin stabilisation.⁹

The plasma ghrelin-like immunoreactivity concentration in normal humans measured by a specific radioimmunoassay (RIA) was 166.0 \pm 10.1 fmol/ml. Serum ghrelin concentrations increase with age with no sex difference and vary widely throughout the day, with higher values during sleep.¹⁰ Other studies have shown differences in gastric ghrelin cells and ghrelin levels in serum between women and men, indicating that secretion of the hormone can be under control of sex hormones

or other unknown factors.¹¹ Novel methods permit accurate means to detect the different forms of circulating ghrelin and determine how various manipulations influence the levels of these different forms.¹² The sandwich enzyme-linked immunosorbent assays (ELISAs) were found to be more sensitive in measuring ghrelin levels and are also available for both forms of ghrelin.¹³

The two major different circulating forms of ghrelin that exist in rats and man are the acylated (or n-octanoylated, AG) and unacylated (or des-octanoylated or des-acylated, UAG). AG has a unique feature that is a post-translational esterification of a fatty (n-octanoic or, to a lesser extent, n-decanoic) acid on serine residue at position 3. Ghrelin O-acyltransferase (GOAT) is a membrane-bound enzyme responsible for this octanoylation by attaching an 8-carbon medium-chain fatty acid (MCFA) (octanoate) to serine 3 of ghrelin. This acylation is necessary for the activity of ghrelin. Animal data suggest that MCFAs provide substrate for GOAT and an increase in nutritional octanoate increases acyl-ghrelin.¹⁴

Ghrelin acylation is necessary for its actions via GHS-R1a. Normally, AG accounts for less than 10 % of the total ghrelin in the circulation. The majority of circulating ghrelin is UAG.¹⁵ UAG was first considered an inactive form of ghrelin, although accumulating evidence indicates that UAG can modulate metabolic activities of the ghrelin system either independently or in opposition to those of AG.¹⁶

Ghrelin Receptors and their Distribution

The GHSR mRNA is expressed as two splice variants encoding the cognate receptor GHS-R1a and the apparently non-functional receptor GHS-R1b. Unlike GHS-R1a, GHS-R1b is not activated by ghrelin or synthetic GHS and it is unclear whether it is a functional receptor.¹⁷ It has been shown that GHS-R1b is likely not correctly transported and accumulates in the nucleus, and is thus very likely inactive.¹⁸ GHS-R1a consists of 366 amino acids. It is a seven transmembrane G-protein-coupled receptor with a high degree of constitutive, ligand-independent signalling activity as it signals with approximately 50 % of its maximal signalling, depending on the signal transduction pathway, without the presence of any hormone. The constitutive activity of GHSR is based on *in vitro* overexpression experiments. The level of expression is so low *in vivo* that constitutive activity is unlikely to be of consequence.¹⁹ This constitutive activity of the ghrelin receptor is of physiological importance.²⁰

GHS-R1b mRNA is as widely expressed as ghrelin, whereas GHS-R1a gene expression is concentrated in the hypothalamus–pituitary unit, although it is also distributed in other central and peripheral tissues. Centrally, in areas of the central nervous system (CNS) that affect biological rhythms, mood, cognition, memory and learning, such as the hippocampus, pars compacta of the substantia nigra, ventral tegmental area (VTA), dorsal and medial raphe nuclei, Edinger–Westphal nucleus and pyriform cortex.²¹ However, published descriptions of the distributions of ghrelin-like immunoreactivity in the CNS are inconsistent.²²

Peripherally, the GHS-R1a gene is expressed in the stomach, intestine, pancreas, thyroid, gonads, adrenal, kidney, heart and vasculature, as well as several endocrine tumours and cell lines, and have been found to express GHS-R1a with negligible binding found in the parathyroid, pancreas, placenta, mammary gland, prostate, salivary gland, stomach, colon and spleen.²³ This wide distribution of GHS-R1a indicates that the ghrelin and synthetic GHS possess broader functions beyond the control of GH release and food intake.²⁴

Physiological Functions of Ghrelin

Ghrelin exercises a wide range of functions including, regulation of food intake and energy metabolism, stimulation of gastric acid secretion, motility and pancreatic protein output,¹ modulation of cardiovascular function (reviewed in reference 24), stimulation of osteoblast proliferation and bone formation,²⁵ stimulation of neurogenesis²⁴ and myogenesis,²⁷ learning and memory,²⁸ thymopoiesis,²⁹ sleep/wake rhythm,³⁰ ageing³¹ and a neuroprotective role in neurodegenerative diseases (e.g., Parkinson's disease).³²

Growth Hormone-releasing Effect

The GH-releasing effect of the ghrelin occurs through direct effect of ghrelin on pituitary somatotroph cells,³³ synergistic effect with GHRH³⁴ and through stimulation of vagal afferents.³⁵ In high doses, ghrelin may also stimulate prolactin, corticotropin and cortisol secretion in humans.³⁶

Orexigenic Effect (Appetite-stimulating Effect)

Ghrelin is the only known orexigenic gut peptide. The pre-prandial elevation of ghrelin levels and its fall after meals led to the notion that ghrelin was a 'hunger' hormone responsible for meal initiation. Ghrelin is involved in short-term regulation of food intake and long-term regulation of bodyweight through decreasing fat utilisation.³⁷ The effect of ghrelin on feeding is mediated through the GHS-R1a, as indicated by the lack of its orexigenic effect in GHS-R knocked out mice.³⁸ GHS-R1a is highly expressed in hypothalamic cell populations that regulate feeding and bodyweight homeostasis.³⁹

In the arcuate nucleus (ARC), the ghrelin-containing neurons send efferent fibres onto neuropeptide Y(NPY) and agouti related peptide (AgRP)-expressing neurons to stimulate the release of these orexigenic peptides. Ghrelin has also been reported to inhibit the firing of proopiomelanocortin (POMC) neurons by increasing the frequency of spontaneous synaptic γ -aminobutyric acid (GABA) release onto them in a pattern representing a functional antagonism to leptin, without affecting POMC mRNA expression. Confirming that ghrelin's orexigenic effect is mediated by specific modulation of AgRP/NPY neurons in the ARC, no change was demonstrated in the mRNA levels of the other feeding-promoting neuropeptides such as melanocyte stimulating hormone (MCH) and prepro-orexin (OX). Recent data indicate that the orexigenic effect of ghrelin is mediated by its modulation of hypothalamic adenosine monophosphate (AMP)-activated protein kinase (AMPK) enzyme activity.⁴⁰

The detection of ghrelin receptors on vagal afferent neurons in the rat suggests that ghrelin signals from the stomach are transmitted to the brain via the vagus nerve.³⁵ However, whether integrity of the vagus nerve is crucial for effects of ghrelin and whether vagotomy prevents its orexigenic effect in animal models and humans is not universally accepted, as cutting vagal afferents were not necessary for the orixigenic effect of the peripherally injected ghrelin in rats,⁴¹ and gastrectomy in humans accompanied by vagatomy did not prevent the orexigenic effects of ghrelin treatment, indicating an intact vagus is not required for its orexigenic effects.⁴²

Ghrelin and Glucose Homeostasis

Since 2000, numerous studies have suggested that ghrelin has an important role in regulating β -cell function of the pancreas and glucose homeostasis. Indeed, the weight of evidence could support even a more physiologically important function in the control of glucose homeostasis than appetite regulation.⁴³ The available data suggest a negative association between systemic ghrelin and insulin levels.⁴⁴ There

is controversy in the role of ghrelin in insulin secretion as ghrelin has been shown to inhibit insulin secretion in some experiments.⁴⁵⁻⁴⁷ and stimulate insulin release in others.^{48,49} These discrepancies may be due to differences in species and/or experimental design. Plasma ghrelin and insulin levels are affected by blood glucose level, as high glucose suppresses ghrelin secretion and stimulates insulin secretion. Ghrelin also inhibits insulin effects on glycogen synthesis and gluconeogenesis *in vitro*. Ghrelin may also inhibit secretion of the insulin-sensitising protein adiponectin from adipocytes and stimulate secretion of the counter-regulatory hormones, including GH, cortisol, epinephrine and (possibly) glucagon. More studies are needed to clarify the precise physiological role of ghrelin on the regulation of glucose homeostasis.⁵⁰ Pharmacological inhibition of GOAT⁵¹ and ghrelin ablation in ob/ob mice⁵² improves glucose tolerance and insulin sensitivity.

Ghrelin and Lipid Metabolism

Ghrelin is now thought to play a significant role in the regulation of lipid storage in white adipose tissue (WAT). Although acute ghrelin exposure also induces GH secretion, the net effect of prolonged ghrelin exposure is increased fat mass. Ghrelin has been reported to enhance adipogenesis, augment fat storage enzyme activity, elevate triglyceride content and reduce fat utilisation/lipolysis.⁵³⁻⁵⁵

Evidence has demonstrated that administration of peripheral ghrelin increases WAT mass in selective abdominal depots (retroperitoneal and inguinal) via a decrease in lipid export rather than a decrease in lipolysis per se. Thus, during periods of energy insufficiency, ghrelin may prevent lipid loss from responsive adipocytes thereby permitting depot-specific utilisation of energy reserves. It was also found that ghrelin-induced lipid accumulation is not specific to WAT, as exogenous ghrelin markedly increased the number of lipid droplets in the livers of treated rats and mice, an effect mediated by direct activation of its receptor on hepatocytes. Ghrelin receptor antagonism or gene deletion significantly decreased obesity-associated hepatic steatosis by suppression of *de novo* lipogenesis.^{55,56}

Is Ghrelin Essential for Life?

Total gastrectomy was shown to decrease the plasma concentrations of ghrelin to approximately 30–50 % of those of pregastrectomy when measured at 30 minutes after the operation. This concentration gradually increased to about 70 % of the level before the operation. These results indicate that decreased ghrelin production after gastrectomy is subject to compensatory production possibly by the intestines and pancreas.⁵⁷ The increased ghrelin levels after gastric bypass surgery were associated with altered ghrelin cell responsiveness to two major physiological modulators of ghrelin secretion – glucose and norepinephrine. This provides new insights into the regulation of ghrelin secretion and its relation to circulating ghrelin within the contexts of obesity and weight loss.⁵⁸

Although ghrelin is not essential for food intake in mice, it is required for certain food reward behaviours that occur in the setting of chronic calorie restriction. Ghrelin is also essential in mice to prevent hypoglycaemia and death when the animals are subjected to severe calorie restriction. The latter function of ghrelin became apparent in studies of genetically engineered mice that lack the gene encoding GOAT enzyme⁵⁹ and ghrelin-deficient mice.⁴⁰

Regulation of Ghrelin Secretion

The circulating level of ghrelin is determined by the balance among its secretion, degradation and clearance rates. Plasma esterases have

been reported to des-acylate acyl ghrelin, whereas plasma proteases account for the degradation of circulating ghrelin. Clearance of circulating ghrelin includes being captured by its receptor and excreted in urine. Besides, acyl ghrelin can transport across the blood–brain barrier bidirectionally through specific transport system in humans.⁶¹ Ghrelin secretion has been found to be modified under different conditions such as fasting, pathological conditions and surgery.⁶²

In contrast to other gut hormones, plasma ghrelin levels increase in response to fasting and decrease on refeeding.⁶³ Furthermore, plasma ghrelin levels are reduced by chronic intake of high-calorie diets and obesity in humans. In rodents, prolonged exposure to high-fat (HF) diets will result in a positive energy balance, obesity and a reduction of stomach production and secretion of ghrelin.⁶³⁻⁶⁵ However, an increase in the number of ghrelin secreting cells in response to the HF diet has been shown in a recent study.⁶⁶ The extent to which the increased adiposity exerts an inhibitory influence on stomach ghrelin production and secretion is not well known.⁶⁷ The mechanism of pre-prandial increase in ghrelin levels is evidenced to be noradrenergic mediated.⁶⁸ and the post-prandial decrease by increase in glucose and insulin. However, the regulating role of insulin on post-prandial ghrelin suppression is rather additive.^{68,69}

Low systemic ghrelin levels have been reported in obesity, untreated hyperthyroidism,⁷⁰ in male hypogonadism,⁷¹ in polycystic ovary syndrome⁷² and in the presence of *Helicobacter pylori*-induced gastritis.⁷³ Plasma ghrelin levels are high in anorexia nervosa patients and return to control levels after weight gain by renutrition, in lean people, Prader-Willi syndrome and after eradication of *H. pylori*.⁷³

Primary cell cultures of dispersed gastric mucosal cells from adult mice and new-born rats have been developed to investigate the mechanisms regulating ghrelin synthesis and secretion.^{74,75} Ghrelin-secreting immortalised cell lines developed from ghrelinomas in the stomachs and pancreatic islets of transgenic mice expressing SV40 large T-antigen under the control of preproghrelin promoter are now available models.^{64,76} Using these models, modulation of ghrelin release by different factors, such as peptide hormones, monoaminergic neurotransmitters, glucose, fatty acids, second messengers, potential downstream effector enzymes and channels, has now been investigated. Insulin, glucagon, oxytocin, somatostatin, dopamine, glucose and long-chain fatty acids have all been shown to regulate ghrelin secretion through their direct interaction with ghrelin cells.^{62,62,64,74-76}

Nutrients and Ghrelin Level

Isocaloric intestinal infusions of either glucose or amino acids have been found to suppress ghrelin levels more rapidly and effectively than lipid infusions. Theoretically, weak suppression of an orexigenic hormone by ingested lipids could be one of the mechanisms underlying HF dietinduced weight gain. The rate of nutrient absorption and the increase in osmolarity within the intestinal lumen may partly explain the difference in ghrelin suppression by different types of food. Glucose and amino acids, which are quickly absorbed from the gut, suppressed ghrelin rapidly and deeply. By contrast, lipids that require intestinal digestion before absorption lead to weak suppression of ghrelin levels.⁷⁷

The underlying mechanisms that mediate suppression of systemic ghrelin secretion by food are not well known. This may be due to direct nutrient sensing in ghrelin-producing cells and the gut peptides released in response to food intake as insulin, glucagon-like-peptide 1

(GLP-1), peptide YY (PYY) and cholecystokinin (CCK) as they increase rapidly after food intake and circulating ghrelin levels begin to fall simultaneously (reviewed in reference 78).

Hormones Regulating Ghrelin Expression and Secretion

Insulin

Several observations in humans and rats indicate that insulin may inhibit ghrelin secretion and decrease the total serum ghrelin level. GLP-1 has been reported to alleviate the pre-prandial rise of ghrelin in human beings because it is a potent stimulator for insulin secretion.79 This inhibitory effect of insulin on ghrelin level may underlie the suppression of glucose on ghrelin and the inverse relationship between bodyweight and ghrelin level. It may also explain the low ghrelin level in patients of type 2 diabetes mellitus.⁸⁰ The rise in ghrelin after insulin administration⁸¹ was explained by severe hypoglycaemia induced by rapid injection of high dose of insulin.82 Thus, the influence of insulin and glucose on ghrelin secretion is possibly contradictory and independent. Although some studies found a negative correlation between ghrelin level and insulin resistance,83,84 there is plenty of data suggesting the reverse, as ablation of ghrelin and ghrelin receptors improves insulin sensitivity.^{51,84,85} and ghrelin infusion in humans impairs glucose tolerance in hyperinsulinaemic euglycaemic clamp studies.⁸⁶

Glucagon

Glucagon may contribute to the pre-prandial surge of ghrelin as evidenced by the 1) glucagon receptor present in endocrine cells in gastric mucosa, 2) glucagon concentration increases during fasting, 3) plasma acyl ghrelin concentration rises transiently while des-acyl ghrelin increases persistently after administration of glucagon in rats, 4) ghrelin released from the rat stomach is augmented by glucagon perfusion, and 5) glucagon may directly stimulate the gene transcription of ghrelin. Ghrelin has recently been shown to be directly regulated by glucagon.³⁷

Leptin (Ghrelin–Leptin Tango)

Although some studies⁸⁸ found no correlation between ghrelin and leptin levels in obese children and adolescents, other studies suggest that there is a complex interaction between leptin and ghrelin.89 Immunoneutralisation of circulating plasma ghrelin with specific immunoglobulin (Ig)-G anti-ghrelin antibodies caused a marked increase in plasma leptin and decrease in food intake. By contrast, exogenous leptin, at the dose that raises plasma leptin to the level occurring postprandially, markedly reduced plasma levels of ghrelin and attenuated food intake. These effects are reversed by the administration of specific IgG anti-leptin antibodies.⁹⁰ It has been shown that leptin inhibits both the secretion of gastric ghrelin and the stimulation of feeding by ghrelin. This cross-talk between leptin and ghrelin has been termed as the 'ghrelin-leptin tango' in bodyweight regulation. This hypothesis clarifies that the weight-reducing effects of leptin are mediated not only by its direct central action on the hypothalamus but also through its peripheral inhibitory effect on the release and action of ghrelin.91

Growth Hormone/Insulin-like Growth Factor-1 Axis and Somatostatin

GH exerts a negative feedback action on ghrelin production and secretion so GH therapy in GH deficient patients significantly decreases the serum acyl ghrelin concentration.⁹² As insulin-like growth factor-1 (IGF-1) functions to inhibit GH secretion, it may induce ghrelin secretion either directly or indirectly.⁹³ Somatostatin possibly inhibits ghrelin synthesis directly. This inhibitory effect of somatostatin on ghrelin may

be considered as a negative feedback modulation as ghrelin increases the level of somatostatin in plasma.⁹⁴ Cortisol and fatty acids also exert a negative feedback on ghrelin secretion. The increase of ghrelin at and before midnight may be explained by low cortisol at that time.⁹⁵

Oestrogen

There is controversy in the effect of oestrogen on ghrelin levels. Many studies reported that oestrogen up-regulates the ghrelin level.⁹⁶ However, oestrogen-replacement therapy in post-menopausal women increases serum total and acyl ghrelin secretion only to an insignificant extent, or even decreases serum total ghrelin level.⁹⁷ It was also found that plasma acyl ghrelin concentration, ghrelin expressing cells and ghrelin mRNA levels in the stomach increase transiently after ovariectomy in female rats.⁹⁸ In addition, ghrelin and oestrogen receptor immunoreactivities were demonstrated in the same cells, suggesting that oestrogen may have a direct effect on ghrelin expression.⁹⁶

Autonomic Nervous System

The autonomic nervous system plays an important role in the regulation of ghrelin. Excitation of the vagus nerve can stimulate ghrelin secretion. In rats and humans, ghrelin levels rise after administration of muscarinic agonists and fall after administration of muscarinic antagonists.^{99,100} This stimulatory effect is probably a direct effect on ghrelin-producing cells that are governed by the enteric nervous system in stomach mucosa.⁶¹ Sympathetic nervous system is also involved in the regulation of ghrelin, as plasma acyl ghrelin concentration is induced by an α -adrenergic antagonist and a β -adrenergic agonist. The adrenergic agents act directly on β 1 receptors in ghrelin-secreting cells.⁶⁸ Vagotomy has been reported to inhibit the secretion of gastric ghrelin acutely, but activates its secretion in the long term, suggesting that ghrelin secretion is modulated by the balance between cholinergic and adrenergic tones that control the enteric nervous system.^{101,102}

Clinical Applications of Ghrelin and Ghrelin Antagonist

In the past decade, clinical applications of ghrelin have been attempted for various pathologies, based on its anabolic function, including applications for patients with cachexia, sarcopenia (muscle wasting due to ageing),¹⁰³ myopenia (muscle wasting due to chronic illness)¹⁰³ and frailty states.^{104,105} Among the first applications of ghrelin in human chronic illness were studies in congestive heart failure (CHF)¹⁰⁶ and chronic obstructive pulmonary disease (COPD).^{107,108}

In the field of surgery, ghrelin comprehensively improves the patients' general conditions and quality of life via its pleiotropic physiological functions. This characteristic is unique and different from the existing drugs. Therefore, ghrelin may be an indispensable supplement to prevent surgical stress and post-operative sequelae.¹⁰⁹

Several synthetic ghrelin mimetics are being pursued in clinical trials for diverse indications.⁴² Three compounds are currently in development. Macimorelin is in clinical trials for the diagnosis of GH deficiency.¹¹⁰ A second compound, anamorelin, is in clinical trials for the treatment of cancer cachexia.¹¹¹ A third compound, relamorelin (also known as RM-131) is currently in phase II clinical trials and is being developed for treatment of diabetic gastroparesis and other gastrointestinal (GI) disorders.¹¹²

The orexigenic and lipogenic effect of ghrelin provide a potential use of ghrelin antagonists or reverse agonists in the treatment of obesity.¹¹³ However, studies in this area show conflicting results as ghrelin

antagonist reduced bodyweight and food intake in some studies,114,115 while it increased weight gain and food intake in another studies.116,117 [D-Arg1, D-Phe5, D-Trp7,9, Leu11] substance P was identified as an inverse agonist on GHS-R1a,118 and several classes of ghrelin receptor antagonists have been developed (reviewed in reference 119).

Conclusion

Ghrelin exerts many physiological roles and is regulated by several factors. Understanding the mechanisms of ghrelin regulation by modifying its secretion, acylation and degradation will provide a better therapeutic benefit of ghrelin, ghrelin mimetics, inverse agonists and ghrelin antagonists.

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