

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.

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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*

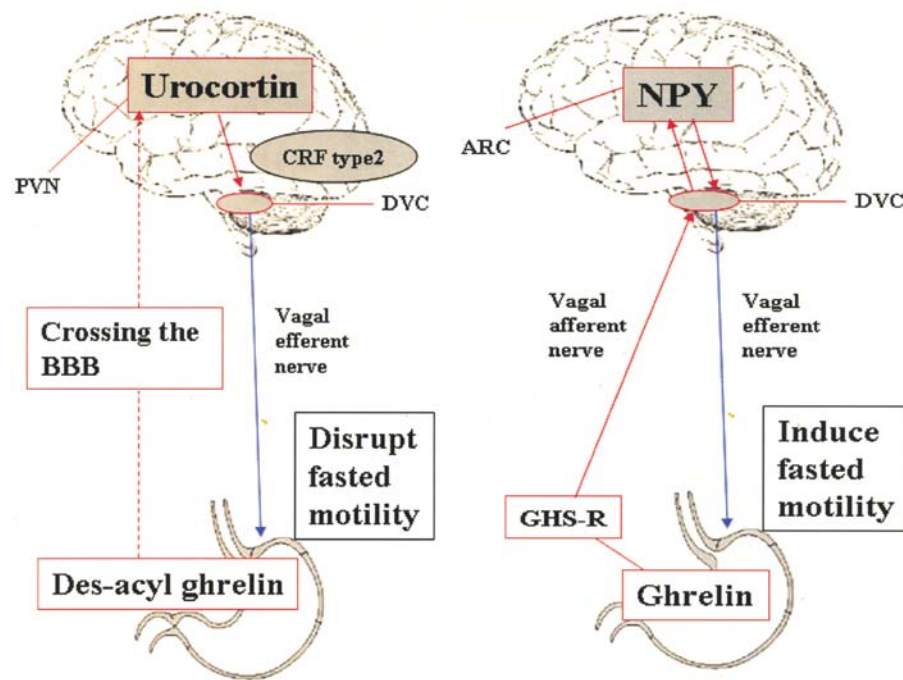


Figure 1. Summary diagram of ghrelin and des-acyl ghrelin on the gastroduodenal motility and brain mechanisms mediating the action of hypothalamic peptides.

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 glycogenolytic 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 1 β (IL-1 β), 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-1 β and IL-1 α 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 Intracerebro-ventricular 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



SPANDIDOS PUBLICATIONS 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	

^aConditions 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.

References

- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H and Kangawa K: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656-660, 1999.
- Hosoda H, Kojima M, Mizushima T, Shimizu S and Kangawa K: Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *J Biol Chem* 278: 64-70, 2003.
- Bowers CY, Momany F, Reynolds GA, Chang D, Hong A and Chang K: Structure-activity relationships of a synthetic pentapeptide that specifically releases growth hormone in vitro. *Endocrinology* 106: 663-667, 1980.
- Bowers CY, Momany FA, Reynolds GA and Hong A: On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* 114: 1537-1545, 1984.
- Bowers CY: Unnatural growth hormone-releasing peptide begets natural ghrelin. *J Clin Endocrinol Metab* 86: 1464-1469, 2001.
- Korbonits M, Goldstone AP, Gueorguiev M and Grossman AB: Ghrelin-A hormone with multiple functions. *Front Neuroendocrinol* 25: 27-68, 2004.
- Howard AD, Feighner SD, Cully DF, *et al.*: A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273: 974-977, 1996.
- Kojima M: The discovery of ghrelin - a personal memory. *Regul Pept* 145: 2-6, 2008.
- Tanaka M, Hayashida Y, Iguchi T, Nakao N, Nakai N and Nakashima K: Organization of the mouse ghrelin gene and promoter: occurrence of a short noncoding first exon. *Endocrinology* 142: 3697-3700, 2001.
- Hosoda H, Kojima M and Matsuo H: Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. *J Biol Chem* 275: 21995-22000, 2000.
- Bednarek MA, Feighner SD, Pong SS, *et al.*: Structure-function studies on the new growth hormone-releasing peptide, ghrelin: minimal sequence of ghrelin necessary for activation of growth hormone secretagogue receptor 1a. *J Med Chem* 43: 4370-4376, 2000.
- Date Y, Kojima M, Hosoda H, *et al.*: Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 141: 4255-4261, 2000.
- Kojima M, Hosoda H and Kangawa K: Purification and distribution of ghrelin: the natural endogenous ligand for the growth hormone secretagogue receptor. *Horm Res* 56: 93-97, 2001.
- Dass NB, Munonyara M, Bassil AK, *et al.*: Growth hormone secretagogue receptors in rat and human gastrointestinal tract and the effects of ghrelin. *Neuroscience* 120: 443-453, 2003.
- Andrews PL and Sanger GJ: Abdominal vagal afferent neurones: an important target for the treatment of gastrointestinal dysfunction. *Curr Opin Pharmacol* 2: 650-656, 2002.
- Bedendi I, Alloatti G, Marcantoni A, *et al.*: Cardiac effects of ghrelin and its endogenous derivatives des-octanoyl ghrelin and des-Gln14-ghrelin. *Eur J Pharmacol* 476: 87-95, 2003.
- Zhang JV, Ren PG, Avsian-Kretschmer O, Luo CW, Rauch R, Klein C and Hsueh AJ: Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 310: 996-999, 2005.
- Qi X, Li L, Yang G, *et al.*: Circulating obestatin levels in normal subjects and in patients with impaired glucose regulation and type 2 diabetes mellitus. *Clin Endocrinol* 66: 593-597, 2007.
- Nakahara T, Harada T, Yasuhara D, *et al.*: Plasma obestatin concentrations are negatively correlated with body mass index, insulin resistance index, and plasma leptin concentrations in obesity and anorexia nervosa. *Biol Psychiatry* 64: 252-255, 2008.
- Guan XM, Yu H, Palyha OC, *et al.*: Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* 48: 23-29, 1997.
- Willeesen MG, Kristensen P and Rømer J: Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 70: 306-316, 1999.
- Batterham RL, Cowley MA, Small CJ, *et al.*: Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 418: 650-654, 2002.
- Schwartz MW, Woods SC, Porte D Jr, Seeley RJ and Baskin DG: Central nervous system control of food intake. *Nature* 404: 661-671, 2000.
- Date Y, Murakami N, Toshinai K, *et al.*: The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 123: 1120-1128, 2002.
- Cummings DE and Foster KE: Ghrelin-leptin tango in body-weight regulation. *Gastroenterology* 124: 1532-1535, 2003.
- Fujimiya M, Asakawa A, Ataka K, Kato I and Inui A: Different effects of ghrelin, des-acyl ghrelin and obestatin on gastrointestinal motility in conscious rats. *World J Gastroenterol* 14: 6318-6326, 2008.
- Carreira MC, Camina JP, Smith RG and Casanueva FF: Agonist-specific coupling of growth hormone secretagogue receptor type 1a to different intracellular signaling systems. Role of adenosine. *Neuroendocrinology* 79: 13-25, 2004.
- Wren AM, Small CJ, Abbott CR, *et al.*: Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 50: 2540-2547, 2001.
- Tschöp M, Smiley DL and Heiman ML: Ghrelin induces adiposity in rodents. *Nature* 407: 908-913, 2000.
- Cummings DE, Foster-Schubert KE and Overduin J: Ghrelin and energy balance: focus on current controversies. *Curr Drug Targets* 6: 153-169, 2005.
- Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE and Weigle DS: A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50: 1714-1719, 2001.
- Natalucci G, Riedl S, Gleiss A, Zidek T and Frisch H: Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern. *Eur J Endocrinol* 152: 845-850, 2005.
- Cummings DE, Frayo RS, Marmonier C, Aubert R and Chapelot D: Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 287: 297-304, 2004.
- Pusztai P, Sarman B, Ruzicka E, Toke J, Racz K, Somogyi A and Tulassay Z: Ghrelin: a new peptide regulating the neurohormonal system, energy homeostasis and glucose metabolism. *Diabetes Metab Res Rev* 24: 343-352, 2008.

 SPANDIDOS³, Cuntz U, Fruehauf E, *et al*: Weight gain decreases plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* 145: 669-673, 2001.

36. Otto B, Tschöp M, Fruehauf E, Heldwein W, Fichter M, Otto C and Cuntz U: Postprandial ghrelin release in anorectic patients before and after weight gain. *Psychoneuroendocrinology* 30: 577-581, 2005.
37. Murata M, Okimura Y, Iida K, *et al*: Ghrelin modulates the downstream molecules of insulin-signaling in hepatoma cells. *J Biol Chem* 277: 5667-5674, 2002.
38. Broglio F, Arvat E, Benso A, *et al*: Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* 86: 5083-5086, 2001.
39. Svensson J, Lönn L, Jansson JO, *et al*: Two-month treatment of obese subjects with the oral growth hormone (GH) secretagogue MK-677 increases GH secretion, fat-free mass, and energy expenditure. *J Clin Endocrinol Metab* 83: 363-369, 1998.
40. Gauna C, Meyler FM, Janssen JA, *et al*: Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. *J Clin Endocrinol Metab* 89: 5035-5042, 2004.
41. Van der Lely AJ, Tschöp M, Heiman ML and Ghigo E: Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev* 25: 426-457, 2004.
42. Koo GC, Huang C, Camacho R, *et al*: Immune enhancing effect of a growth hormone secretagogue. *J Immunol* 166: 4195-4201, 2001.
43. Basa NR, Wang L, Arteaga JR, Heber D, Livingston EH and Tache Y: Bacterial lipopolysaccharide shifts fasted plasma ghrelin to postprandial levels in rats. *Neurosci Lett* 343: 25-28, 2003.
44. Wu R, Zhou M, Cui X, Simms HH and Wang P: Upregulation of cardiovascular ghrelin receptor occurs in the hyperdynamic phase of sepsis. *Am J Physiol Heart Circ Physiol* 287: 1296-1302, 2004.
45. Smith RG, Jiang H and Sun Y: Developments in ghrelin biology and potential clinical relevance. *Trends Endocrinol Metab* 16: 436-442, 2005.
46. Dixit VD, Schaffer EM, Pyle RS, *et al*: Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* 114: 57-66, 2004.
47. Masuda Y, Tanaka T, Inomata N, *et al*: Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 276: 905-908, 2000.
48. Sibilia V, Pagani F, Guidobono F, Locatelli V, Torsello A, Deghenghi R and Netti C: Evidence for a central inhibitory role of growth hormone secretagogues and ghrelin on gastric acid secretion in conscious rats. *Neuroendocrinology* 75: 92-97, 2002.
49. Brzozowski T, Konturek PC, Drozdowicz D, *et al*: Role of central and peripheral ghrelin in the mechanism of gastric mucosal defence. *Inflammopharmacology* 13: 45-62, 2005.
50. Zheng J, Ariga H, Taniguchi H, Ludwig K and Takahashi T: Ghrelin regulates gastric phase III-like contractions in freely moving conscious mice. *Neurogastroenterol Motil* 21: 78-84, 2008.
51. Fujino K, Inui A, Asakawa A, Kihara N, Fujimura M and Fujimura M: Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol* 550: 227-240, 2003.
52. Tack J, Depoortere I, Bisschops R, *et al*: Influence of ghrelin on interdigestive gastrointestinal motility in humans. *Gut* 55: 327-333, 2006.
53. Trudel L, Bouin M, Tomasetto C, *et al*: Two new peptides to improve post-operative gastric ileus in dog. *Peptides* 24: 531-534, 2003.
54. Trudel L, Tomasetto C, Rio MC, Bouin M, Plourde V, Eberling P and Poitras P: Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. *Am J Physiol Gastrointest Liver Physiol* 282: 948-952, 2002.
55. Venkova K and Greenwood-Van Meerveld B: Application of ghrelin to gastrointestinal diseases. *Curr Opin Investig Drugs* 9: 1103-1107, 2008.
56. Chen CY, Inui A, Asakawa A, *et al*: Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. *Gastroenterology* 129: 8-25, 2005.
57. Ataka K, Inui A, Asakawa A, Kato I and Fujimura M: Obestatin inhibits motor activity in the antrum and duodenum in the fed state of conscious rats. *Am J Physiol Gastrointest Liver Physiol* 294: 1210-1218, 2008.
58. De Smet B, Thijs T, Peeters TL and Depoortere I: Effect of peripheral obestatin on gastric emptying and intestinal contractility in rodents. *Neurogastroenterol Motil* 19: 211-217, 2007.
59. Gourcerol G, Coskun T, Craft LS, *et al*: Preproghrelin-derived peptide, obestatin, fails to influence food intake in lean or obese rodents. *Obesity* 15: 2643-2652, 2007.
60. Bassil AK, Häglund Y, Brown J, *et al*: Little or no ability of obestatin to interact with ghrelin or modify motility in the rat gastrointestinal tract. *Br J Pharmacol* 150: 58-64, 2007.
61. Volante M, Fulcheri E, Allia E, Cerrato M, Pucci A and Papotti M: Ghrelin expression in fetal, infant, and adult human lung. *J Histochem Cytochem* 50: 1013-1021, 2002.
62. Correia-Pinto J, Baptista MJ, Pedrosa C, Estevão-Costa J, Flake AW and Leite-Moreira AF: Fetal heart development in the nitrofen-induced CDH rat model: the role of mechanical and nonmechanical factors. *J Pediatr Surg* 38: 1444-1451, 2003.
63. Santos M, Bastos P, Gonzaga S, *et al*: Ghrelin expression in human and rat fetal lungs and the effect of ghrelin administration in nitrofen-induced congenital diaphragmatic hernia. *Pediatr Res* 59: 531-537, 2006.
64. Nagaya N, Kojima M, Uematsu M, *et al*: Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regul Integr Comp Physiol* 280: 1483-1487, 2001.
65. Baldanzi G, Filigheddu N, Cutrupi S, *et al*: Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. *J Cell Biol* 159: 1029-1037, 2002.
66. Dieguez C and Casanueva FF: Influence of metabolic substrates and obesity on growth hormone secretion. *Trends Endocrinol Metab* 6: 55-59, 1995.
67. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E and Heiman ML: Circulating ghrelin levels are decreased in human obesity. *Diabetes* 50: 707-709, 2001.
68. Ikezaki A, Hosoda H, Ito K, *et al*: Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. *Diabetes* 51: 3408-3411, 2002.
69. Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS and Jorgensen JO: Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol* 56: 203-206, 2002.
70. Levin BE and Keesey RE: Defense of differing body weight set points in diet-induced obese and resistant rats. *Am J Physiol* 274: 412-419, 1998.
71. Levin BE: Arcuate NPY neurons and energy homeostasis in diet-induced obese and resistant rats. *Am J Physiol* 276: 382-387, 1999.
72. English PJ, Ghatei MA, Malik IA, Bloom SR and Wilding JP: Food fails to suppress ghrelin levels in obese humans. *J Clin Endocrinol Metab* 87: 2984, 2002.
73. Inui A: Eating behavior in anorexia nervosa—an excess of both orexigenic and anorexigenic signaling? *Mol Psychiatry* 6: 620-624, 2001.
74. Shiya T, Nakazato M, Mizuta M, *et al*: Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 87: 240-244, 2002.
75. Aggarwal A: *Helicobacter pylori* infection: a cause of growth delay in children. *Indian Pediatr* 35: 191-192, 1998.
76. Rigamonti AE, Pincelli AI, Corrà B, *et al*: Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. *J Endocrinol* 175: 1-5, 2002.
77. Cuntz U, Fruehauf E, Wawarta R, *et al*: A role for the novel weight-regulating hormone ghrelin in anorexia nervosa. *Am Clin Lab* 21: 22-23, 2002.
78. Nedvídková J, Krykorková I, Barták V, Papezová H, Gold PW, Alesci S and Pacak K: Loss of meal-induced decrease in plasma ghrelin levels in patients with anorexia nervosa. *J Clin Endocrinol Metab* 88: 1678-1682, 2003.
79. Kaye WH, Berrettini W, Gwirtsman H and George DT: Altered cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. *Arch Gen Psychiatry* 47: 548-556, 1990.
80. Tanaka M, Naruo T, Muranaga T, *et al*: Increased fasting plasma ghrelin levels in patients with bulimia nervosa. *Eur J Endocrinol* 146: 1-3, 2001.
81. Colao A, Marzullo P, Di Somma C and Lombardi G: Growth hormone and the heart. *Clin Endocrinol* 54: 137-154, 2001.
82. Claycomb WC, Lanson NA Jr, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A and Izzo NJ Jr: HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proc Natl Acad Sci USA* 95: 2979-2984, 1998.

83. González-Juanatey JR, Piñeiro R, Iglesias MJ, Gualillo O, Kelly PA, Diéguez C and Lago F: GH prevents apoptosis in cardiomyocytes cultured in vitro through a calcineurin-dependent mechanism. *J Endocrinol* 180: 325-335, 2004.
84. Iglesias MJ, Piñeiro R, Blanco M, *et al*: Growth hormone releasing peptide (ghrelin) is synthesized and secreted by cardiomyocytes. *Cardiovasc Res* 62: 481-488, 2004.
85. Okumura H, Nagaya N, Enomoto M, Nakagawa E, Oya H and Kangawa K: Vasodilatory effect of ghrelin, an endogenous peptide from the stomach. *J Cardiovasc Pharmacol* 39: 779-783, 2002.
86. Wiley KE and Davenport AP: Comparison of the effects of atherosclerosis and nitrate therapy on responses to nitric oxide and endothelin-1 in human arteries in vitro. *Clin Sci* 48: 124-127, 2002.
87. Nagaya N, Uematsu M, Kojima M, *et al*: Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation* 104: 2034-2038, 2001.
88. Nagaya N, Uematsu M, Kojima M, *et al*: Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation* 104: 1430-1435, 2001.
89. Frascarelli S, Ghelardoni S, Ronca-Testoni S and Zucchi R: Effect of ghrelin and synthetic growth hormone secretagogues in normal and ischemic rat heart. *Basic Res Cardiol* 98: 401-405, 2003.
90. Matsumura K, Tsuchihashi T, Fujii K, Abe I and Iida M: Central ghrelin modulates sympathetic activity in conscious rabbits. *Hypertension* 40: 694-699, 2002.
91. Liu YL, Malik NM, Sanger GJ and Andrews PL: Ghrelin alleviates cancer chemotherapy-associated dyspepsia in rodents. *Cancer Chemother Pharmacol* 58: 326-333, 2006.
92. Suzuki H, Nishizawa T and Hibi T: Therapeutic strategies for functional dyspepsia and the introduction of the Rome III classification. *J Gastroenterol* 41: 513-523, 2006.
93. Tritos NA and Kokkotou EG: The physiology and potential clinical applications of ghrelin, a novel peptide hormone. *Mayo Clin Proc* 81: 653-660, 2006.
94. Inui A, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM and Fujimiya M: Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. *FASEB J* 18: 439-456, 2004.