

Ghrelin Gene Products and the Regulation of Food Intake and Gut Motility

CHIH-YEN CHEN, AKIHIRO ASAKAWA, MINEKO FUJIMIYA, SHOU-DONG LEE, AND AKIO INUI

Faculty of Medicine, National Yang-Ming University School of Medicine, Taipei (C.-Y.C, S.-D.L.), and Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan (C.-Y.C, S.-D.L.); Department of Anatomy, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan (M.F.); and Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan (A.A., A.I.)

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Address correspondence to: Dr. Akio Inui, Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima, 890-8520 Japan. E-mail: inui@m.kufm.kagoshima-u.ac.jp

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Abstract—A breakthrough using “reverse pharmacology” identified and characterized acyl ghrelin from the stomach as the endogenous cognate ligand for the growth hormone (GH) secretagogue receptor (GHS-R) 1a. The unique post-translational modification of *O*-*n*-octanoylation at serine 3 is the first in peptide discovery history and is essential for GH-releasing ability. Des-acyl ghrelin, lacking *O*-*n*-octanoylation at serine 3, is also produced in the stomach and remains the major molecular form secreted into the circulation. The third ghrelin gene product, obestatin, a novel 23-amino acid peptide identified from rat stomach, was found by comparative genomic analysis. Three ghrelin gene products actively participate in modulating appetite, adipogenesis, gut motility, glucose metabolism, cell proliferation, immune, sleep, memory, anxiety, cognition, and stress. Knockdown or knockout of acyl ghrelin and/or GHS-R1a, and overexpression of des-

acyl ghrelin show benefits in the therapy of obesity and metabolic syndrome. By contrast, agonism of acyl ghrelin and/or GHS-R1a could combat human anorexia-cachexia, including anorexia nervosa, chronic heart failure, chronic obstructive pulmonary disease, liver cirrhosis, chronic kidney disease, burn, and postsurgery recovery, as well as restore gut dysmotility, such as diabetic or neurogenic gastroparesis, and postoperative ileus. The ghrelin acyl-modifying enzyme, ghrelin *O*-Acyltransferase (GOAT), which attaches octanoate to serine-3 of ghrelin, has been identified and characterized also from the stomach. To date, ghrelin is the only protein to be octanoylated, and inhibition of GOAT may have effects only on the stomach and is unlikely to affect the synthesis of other proteins. GOAT may provide a critical molecular target in developing novel therapeutics for obesity and type 2 diabetes.

I. Introduction: Discovery of Growth Hormone Secretagogue Receptor, Ghrelin Gene Products (Acyl Ghrelin, Des-Acyl Ghrelin, and Obestatin), and Ghrelin *O*-acyltransferase

In 1996, a G protein-coupled receptor was successfully cloned from human and swine pituitary and hypothalamus and was identified as the target of growth hormone (GH¹) secretagogues (GHS), a class of peptide and non-

peptide compounds functioning in GH release from the anterior pituitary (Howard et al., 1996). Nucleotide sequence analysis indicated two subtypes of cDNAs derived from the same gene, located on chromosome 3q26.2 (McKee et al., 1997), which encodes two transcripts: Ia and Ib. An official International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification nomenclature designated growth hormone

¹ Abbreviations: 5-HT, 5-hydroxytryptamine; AgRP, agouti-related protein; AMPK, AMP-activated protein kinase; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid; CART, cocaine- and amphetamine-regulated transcript; CB₁-R, cannabinoid receptor subtype 1; CB₂-R, cannabinoid receptor subtype 2; CHF, chronic heart failure; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CRF₁-R, corticotropin-releasing factor receptor subtype 1; CRF₂-R, corticotropin-releasing factor receptor subtype 2; CRF, corticotropin-releasing factor; GH, growth hormone; GHRH, growth hormone-releasing hormone; GHS-R1a, growth hormone secretagogue receptor 1a; GOAT, ghrelin *O*-acyltransferase; GSK894281, *N*-(5-(*cis*-3,5-dimethyl-1-piperazinyl)-2-(methoxy) phenyl)-3-fluoro-4-(5-methyl-2-furanyl)benzenesulfonamide; IGF, insulin-

like growth factor; L-163,255, *N*-(1-(1,2-dihydro-1-methanesulfonylspiro(3*H*-indole-3,4'-piperidine-1'-yl)carbonyl)-4-phenylbutyl)-2-amino-2-methylpropanamide hydrochloride; MC₃-R, melanocortin receptor subtype 3; MC₄-R, melanocortin receptor subtype 4; MK-677, 2-dihydro-1-ethanesulfonylspiro-3*H*-indole-3,4'-piperidin-1'-yl]carbonyl-2-(phenylmethoxy)-ethyl-2-amino-2-methylpropanamide; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PYY, peptide YY; RT-PCR, reverse transcription-polymerase chain reaction; SHU9119, Ac-Nle(4)-c(Asp(5)-2'-Nal(7)-Lys(10)) α -MSH(4-10)-NH₂; SR141716, rimonabant; TM, trans-membrane domain; TNF, tumor necrosis factor; TZP-101, (4*R*,7*S*,10*R*,13*R*)-7-cyclopropyl-13-(4-fluorobenzyl)-3-oxa-6,9,12,15-tetraaza-4,9,10-trimethyl-4,5,6,7,10,12,13,15,16,17,18-undecahydro-1,2-benzocyclooctadecene-8,11,14-trione; UCP, uncoupling protein.

secretagogue receptor 1a (GHS-R1a) as the acyl ghrelin receptor, abbreviated when necessary to GRLN receptor (Davenport et al., 2005).

In 1999, acyl ghrelin was identified as the endogenous cognate ligand for the growth hormone secretagogue receptor, GHS-R1a (Kojima et al., 1999). The discovery of acyl ghrelin, a 28-amino acid peptide, as the first endogenous ligand for a GHS-R (previously known as an orphan receptor) isolated from the stomach, involves the idea and technique of “reverse pharmacology” (Kojima et al., 1999). Acyl ghrelin acts on GHS-R1a to stimulate GH release, which is distinct from its stimulation by hypothalamic GH-releasing hormone (GHRH). Acyl ghrelin features a unique post-translational modification of *O*-*n*-octanoylation at serine 3, and the post-translational modification of octanoylation is the first in peptide discovery history (Kojima et al., 1999; Kojima, 2008). The second endogenous cognate ligand for GHS-R, des-Gln¹⁴-ghrelin, another novel 27-amino acid peptide, is created by alternative splicing of the ghrelin gene, and constitutes one fifth of ghrelin immunoreactivity of the rat stomach. It is as potent as ghrelin for stimulation of GH secretion (Hosoda et al., 2000a). In addition to stimulating GH secretion (Kojima et al., 1999; Seoane et al., 2000; Wren et al., 2000; Broglio et al., 2001), a unique post-translational modification of *O*-*n*-octanoylation at serine 3 of ghrelin is also indispensable for acyl ghrelin’s ability to enhance food intake (Wren et al., 2000, 2001a,b; Druce et al., 2005; Druce et al., 2006) and adiposity (Tschöp et al., 2000; Wren et al., 2001b) in humans and rats. To date, acyl ghrelin is the only peripheral hormone that increases meal size (Woods, 2004; Chen et al., 2008c). Finally, acyl ghrelin and GHRH are both endogenous GH-releasing peptides. GHRH acts on the GHRH receptor, distinct from GHS-R1a, to activate adenylate cyclase and increase intracellular cAMP, which serves as a second messenger to activate protein kinase A (Kojima and Kangawa, 2005).

In contrast, des-acyl ghrelin, lacking *O*-*n*-octanoylation at serine 3, is also produced in the stomach and remains the major molecular form secreted into the circulation. Plasma des-acyl ghrelin/acyl ghrelin ratio ranges from 2.5:1 (Broglio et al., 2004c), 3:1 (Garcia et al., 2005), to 9:1 (Hosoda et al., 2000b; Yoshimoto et al., 2002) from different studies. At first, des-acyl ghrelin was regarded as a nonfunctional ghrelin ligand. Des-acyl ghrelin has been shown to actively participate in food intake (Asakawa et al., 2005; Chen et al., 2005a,b; Matsuda et al., 2006; Toshinai et al., 2006; Muscaritoli et al., 2007; Inhoff et al., 2008; Stengel et al., 2008), gut motility (Asakawa et al., 2005; Chen et al., 2005a,b), body size development (Ariyasu et al., 2005; Asakawa et al., 2005), adipogenesis (Thompson et al., 2004), insulin secretion and resistance (Broglio et al., 2004b; Gauna et al., 2004, 2005, 2007; Iwakura et al., 2005; Qader et al., 2008; Zhang et al., 2008b; Kiewiet et al., 2009), to in-

crease tension of guinea pig papillary muscle ex vivo (Bedendi et al., 2003), and cell proliferation and survival in vitro (Baldanzi et al., 2002; Cassoni et al., 2004, 2006; Granata et al., 2006, 2007; Filigheddu et al., 2007). A more recent study revealed that des-acyl ghrelin was secreted in a highly regulated manner in response to food deprivation in mice (Kirchner et al., 2009). Hence, des-acyl ghrelin should not only be an “innocent bystander,” especially after the discovery of ghrelin *O*-acyltransferase (GOAT). Des-acyl ghrelin is speculated to bind to an additional as-yet unidentified receptor different from GHS-1a. However, it is ambiguous whether the effects of des-acyl ghrelin are direct or not.

The third ghrelin gene product, obestatin, a novel 23-amino acid peptide identified from rat stomach, was derived from the mammalian prepro-ghrelin gene, which also encodes ghrelin, by comparative genomic analyses (Zhang et al., 2005). It was originally projected that obestatin binds to an orphan G protein-coupled receptor, termed GPR39, to inhibit food intake (Zhang et al., 2005). However, the effects of obestatin on food intake remain debatable. On the other side, obestatin manifested various biological functions, such as improving memory performance, causing anxiolytic effects (Carlini et al., 2007b), inhibiting thirst in rats (Samson et al., 2007), activating cortical neurons (Dun et al., 2006), stimulating proliferation of retinal pigment epithelial cells in vitro (Camiña et al., 2007), and profoundly influencing sleep (Szentirmai and Krueger, 2006b; Szentirmai et al., 2009).

Serine-3 of ghrelin, which is acylated with an eight-carbon fatty acid, octanoate, is inextricably required for the multifaceted endocrine functions of acyl ghrelin. Despite the crucial role for octanoylation in the physiology of ghrelin, the lipid transferase that mediates this novel modification had remained unknown until 2008. In 2008, ghrelin *O*-acyltransferase (GOAT), attaching octanoate to serine-3 of ghrelin, was identified and characterized by two independent research groups (Yang et al., 2008a; Gutierrez et al., 2008). Ghrelin seems the sole substrate for GOAT (Yang et al., 2008a). GOAT is located in the endoplasmic reticulum, and the presumed donor for octanoylation is octanoyl-CoA (Yang et al., 2008a). Expression of GOAT was demonstrated to be limited to the gastrointestinal tract, intestine, testis (Yang et al., 2008a), and the pancreas (Gutierrez et al., 2008), the major ghrelin-secreting tissues. The discovery of GOAT, an enzyme specific for octanoylation of ghrelin, identified the mechanism for this unique derivatization of ghrelin and also holds the promise of answering some major questions about the unanswered but important physiological roles of ghrelin (Gardiner and Bloom, 2008). The sequential steps of the production of three ghrelin gene products and their relationships with GOAT are illustrated in Fig. 1.

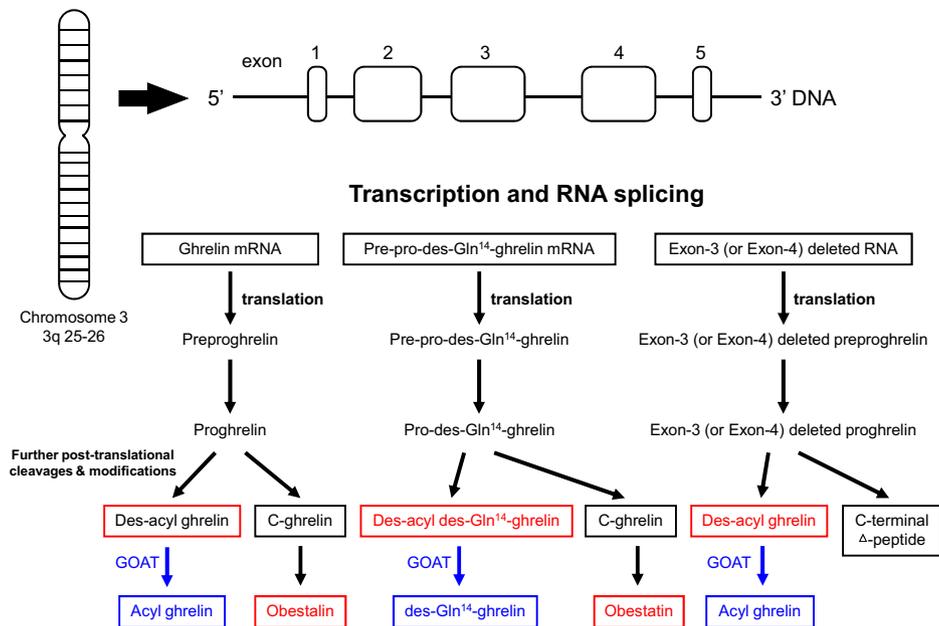


FIG. 1. Diagram illustrates sequential steps of the production of three ghrelin gene products and their relationship with GOAT.

II. Normal Physiology of Acyl Ghrelin and Regulation of Its Expression and Interactions

Acyl ghrelin is recognized to stimulate GH release. When administered intravenously, acyl ghrelin elicits a marked stimulatory effect on GH secretion (Seoane et al., 2000). Studies from humans and in vitro organ culture systems showed that GH, somatostatin, and cortistatin, but not GHRH or IGF-1, directly inhibit acyl ghrelin secretion (Kojima et al., 1999; Broglio et al., 2002b; Seoane et al., 2007a). These results illustrate the stimulatory effect of acyl ghrelin on GH release and negative feedback control of GH and somatostatin on gastric acyl ghrelin secretion. In addition to affecting on GH release, intravenous administration of acyl ghrelin induced elevation of circulating somatostatin and pancreatic polypeptide concentrations, whereas suppressed plasma insulin levels accompanied hyperglycemia in humans (Arosio et al., 2003). Ex vivo measurement of the release of acyl ghrelin and des-acyl ghrelin in vascularly perfused rat stomach showed that the release of des-acyl ghrelin from the perfused stomach was greater at pH 2 than at pH 4, whereas the release of acyl ghrelin was not affected by intragastric pH (Mizutani et al., 2009).

Acyl ghrelin was detected from the stomach to the colon, the hypothalamic regions (including arcuate nucleus and pituitary), pancreas, adipose tissue, heart, and lung (Kojima et al., 1999; Date et al., 2000), the highest concentrations being from the neck to the base of X/A cells of gastric oxyntic gland (Kojima et al., 1999; Date et al., 2000), as well as in the pituitary and small intestine (Ueberberg et al., 2009). The distribution of ghrelin-immunoreactive cells in human gastric mucosa followed that of gastric parietal cells (Tanaka-Shintani and Watanabe, 2005). In situ hybridization revealed that ghre-

lin cells were found to be localized in the mucous membrane of the stomach, duodenum, ileum, cecum, and colon, but not in myenteric plexus, and they can be classified into open- and closed-type cells (Sakata et al., 2002). The apical cytoplasmic process of the opened-type cells contacted the gastric lumen, thought to be functionally regulated by receiving luminal information such as nutrients and pH, whereas closed-type cells are modulated by hormones, neuronal stimulation, or mechanical distension (Sakata et al., 2002). The opened-type cells gradually increased in the direction from stomach to the lower gastrointestinal tract (Sakata et al., 2002). These results suggest that the two types of ghrelin cells may be distinctly regulated and play different physiological roles in various regions of the gastrointestinal tract. The fact that plasma ghrelin level decreased immediately 1 h after gastric resection in patients with early gastric cancer confirmed 70% circulating ghrelin produced from the stomach (Jeon et al., 2004).

A. Regulation of Food Intake by Acyl Ghrelin

Acyl ghrelin acts as a signal for mealtime hunger and meal initiation; to date, it is the only known gastrointestinal peptide to stimulate appetite and increases meal size, among dozens of enzymes, hormones, and other factors secreted by the gastrointestinal tract in response to food in the lumen (Woods, 2004; Chen et al., 2008c). In fact, fasting induced the elevation of acyl or total ghrelin in mice (Kirchner et al., 2009) and rats (Guo et al., 2008), whereas refeeding restored it. In humans, preprandial rise (Cummings et al., 2001; Natalucci et al., 2005; Liu et al., 2008) and postprandial fall (Tschöp et al., 2001a; Natalucci et al., 2005; Liu et al., 2008) in plasma ghrelin levels support the concept that

ghrelin plays a physiological role in meal initiation. It is noteworthy that short- and long-term exogenous injection of central and peripheral acyl ghrelin induced hyperphagia in rats (Wren et al., 2001a,b), and short-term exogenous administration of acyl ghrelin accelerated gastric emptying and elicited hunger in normal-weight humans (Levin et al., 2006), as well as stimulating energy intake in lean human volunteers (Druce et al., 2005; Druce et al., 2006). In addition to increasing caloric intake, central administration of acyl ghrelin preferentially enhanced fat ingestion in rats (Shimbara et al., 2004). However, we need to be cautious upon data interpretation because of the possibility of leakage into periphery during central injection at higher dosage. Finally, long-term or repeated injection of acyl ghrelin enhances food intake in rats and humans under pathological conditions: counteracting body weight loss in rats implanted with malignant tumor (Hanada et al., 2003), improving appetite and food intake in patients with cancer (Neary et al., 2004), stimulating appetite in humans with chronic wasting diseases, such as chronic obstructive pulmonary disease (Nagaya et al., 2005) and chronic kidney disease (Ashby et al., 2009), and increasing energy intake in patients receiving dialysis (Ashby et al., 2009).

In vivo and ex vivo animal studies in rats revealed that "anticipation of eating," as well as fasting/feeding status, influenced pre- and postprandial plasma ghrelin levels in rats (Drazen et al., 2006; Seoane et al., 2007b). A novel organ-culture model of gastric tissue explants from rat donors demonstrated that fasting induced gastric ghrelin release, whereas refeeding fully reversed it (Seoane et al., 2007b). The ex vivo experiments also showed that when animals were allowed 15 min before explant extraction to see or smell, but not eat, the food, total ghrelin secretion was suppressed just like in gastric explants from refed rats (Seoane et al., 2007b), whereas this effect was blocked by surgical vagotomy or atropine sulfate. These results indicate that sensorial stimuli related to food, but without food ingestion (tease feeding), are able to modulate gastric ghrelin secretion and circulating ghrelin levels. The food-related stimuli are vagal cholinergic pathway-dependent and mediated by a medium-term memory mechanism from the system of sensory neurons integrated in the enteric nervous system (Seoane et al., 2007b). These findings suggest a role for total ghrelin in the regulation of anticipatory processes involved in food intake and nutrient disposition, independent of nutrient status. However, a human study observed preprandial increases and postprandial suppression of plasma total ghrelin levels among human subjects initiating meals voluntarily, in the absence of time- and food-related cues, as well as a close overlap between the temporal profiles of total ghrelin levels and hunger scores, consistent with the hypothesis that ghrelin functions as an important physiological meal initi-

ator (Cummings et al., 2004). The discrepancies may result from species differences.

B. Regulation of Whole-Body Energy Homeostasis by Acyl Ghrelin

Besides its influence on appetite, acyl ghrelin affects body weight and adiposity. Unlike other gut-derived satiation factors, such as cholecystokinin (which, when administered continuously, changed only meal patterns but not body weight), long-term or repeated injection of acyl ghrelin increased body weight in rats (Wren et al., 2001b) and in humans with chronic wasting conditions, including chronic heart failure (Nagaya et al., 2004) and chronic obstructive pulmonary disease (Nagaya et al., 2005). Central and peripheral administration of acyl ghrelin also decrease energy expenditure in mice and rats, either via increasing respiratory quotient (Tschöp et al., 2000), decreasing oxygen consumption (Asakawa et al., 2001b), suppressing thermogenesis in brown adipose tissue through directly inhibiting brown adipose tissue sympathetic nerve activity (Yasuda et al., 2003), and up-regulating mRNA expression of uncoupling protein 1 (UCP1) in brown fat tissue and uncoupling protein 2 (UCP2) in white adipose tissue (Tsubone et al., 2005), resulting in increase in fat mass. Peripheral exogenous injection with small dose of acyl ghrelin, which did not stimulate food intake, did increase body weight gain and enhance adiposity in mice and rats (Tschöp et al., 2000; Tsubone et al., 2005), indicating that the adiposity-inducing effect of acyl ghrelin is independent of its appetite-stimulating actions. A recent study revealed that during periods of energy insufficiency, exposure to acyl ghrelin may limit energy utilization in specific white adipose tissue depots by GHS-R1a-dependent lipid retention (Davies et al., 2009). Central administration of acyl ghrelin preferentially enhanced fat ingestion in rats (Shimbara et al., 2004), which predisposes the development of obesity.

C. Regulation of Mucosa Protection by Acyl Ghrelin

Acyl ghrelin could be a new gastroprotective factor in gastric mucosa. Increases in the plasma total and acyl ghrelin levels preceded the formation of duodenal ulcers in rats treated with cysteamine, which inhibited the release of somatostatin and induces the formation of duodenal ulcers (Fukuhara et al., 2005). Intracerebroventricular acyl ghrelin dose dependently decreased ethanol-induced gastric ulcers, and the gastroprotective effect of central acyl ghrelin was mediated by endogenous NO release and required the integrity of sensory nerve fibers (Sibilia et al., 2003). Pretreatment with intraperitoneal acyl ghrelin prevented either intragastric ethanol-induced, water immersion and restraint stress-induced, or ischemia-reperfusion-induced gastric mucosal lesions (Brzozowski et al., 2004, 2006; Konturek et al., 2004, 2006), and gastric mucosal expression of tumor necrosis factor α (TNF- α) mRNA (Konturek et al., 2004,

2006; Brzozowski et al., 2006) and nuclear factor κ B-p65 protein (Konturek et al., 2006) in rats. The protective effect of acyl ghrelin on either intragastric ethanol-induced, water immersion and restraint stress-induced, or ischemia-reperfusion-induced gastric mucosa damage was accompanied by rise in gastric mucosa blood flow and generation of mucosal prostaglandin E₂ (Brzozowski et al., 2004, 2006; Konturek et al., 2004), and sensory nerves dependent (Brzozowski et al., 2004, 2006; Konturek et al., 2006).

D. Regulation of Water Intake by Acyl Ghrelin

Centrally and peripherally administered acyl ghrelin potently inhibits water intake in rats (Hashimoto et al., 2007), whereas intracerebroventricular acyl ghrelin attenuated water intake stimulated under dipsogenic conditions, such as short-term injection of angiotensin II or hypertonic saline (Mietlicki et al., 2009), indicating that the actions of acyl ghrelin are not limited to food intake but can also include alterations in water intake. Intracerebroventricular acyl ghrelin also inhibited water intake in chicks under ad libitum and 17-h water-deprived conditions, suggesting that acyl ghrelin acts as an antidipsogenic peptide across species (Tachibana et al., 2006). Hence, acyl ghrelin participates in the regulation of both energy intake (stimulation) and water intake (inhibition), which gives it a full role in ingestive behavior.

E. Regulation of Gastrointestinal Motility and Gastric Acid Secretion by Acyl Ghrelin

Electrophysiologic recordings indicated that two distinct population of gastric distension-sensitive neurons existed in the dorsal vagal complex in rats: gastric distension-excited neurons were activated by ghrelin, whereas gastric distension-inhibited neurons were suppressed by acyl ghrelin (Wang et al., 2008). Acyl ghrelin is closely involved in the regulation of gastrointestinal motility and secretion of gastric acid and pancreatic enzymes, coupled with ingestive behavior, to facilitate digestive process and energy absorption. Pathophysiology of ghrelin gene products in feeding-associated gastrointestinal motility will be fully elucidated and discussed in section VIII. Regulation of gastric acid secretion by ghrelin is reviewed by Chen et al. (2008c). Intravenous boluses of exogenous fragments of acyl ghrelin, Gly-Ser-Ser(*n*-octanoyl)-Phe, reduced the volume of pancreatic-biliary juice, protein, and trypsin outputs under both basal and cholecystokinin-8-stimulated conditions in a dose-dependent manner in anesthetized rats (Kapica et al., 2006), whereas exogenous fragments of acyl ghrelin failed to affect the pancreatic secretion in rats subjected to vagotomy, capsaicin deactivation of afferents, or pretreatment with tarazepide, suggesting that acyl ghrelin might control exocrine pancreas secretion by affecting duodenal neurohormonal mechanism(s) involving cholecystokinin and vagal nerves in rats.

F. Regulation of Acyl Ghrelin Secretion

Preprandial acyl ghrelin surges are probably triggered by sympathetic nervous system. Plasma acyl ghrelin levels in rats were increased by a muscarinic agonist, an α -adrenergic antagonist, and a β -adrenergic agonist, whereas the levels were decreased by a muscarinic antagonist and an α -adrenergic agonist (Hosoda and Kangawa, 2008). Furthermore, vagotomy inhibited acyl ghrelin secretion in the short term but enhanced it over the long term in rats (Hosoda and Kangawa, 2008). In addition, vagotomy affected neither baseline total ghrelin levels nor the suppression of total ghrelin by a nutrient load in rats (Williams et al., 2003b). The food deprivation-induced elevation of plasma total ghrelin levels was completely prevented by subdiaphragmatic vagotomy, whereas, in a separate experiment, the deprivation-related rise in plasma total ghrelin was substantially reduced by atropine methyl nitrate treatment, indicating that the response to fasting is driven by increased vagal efferent tone (Williams et al., 2003b) and elucidating the dissociation between nutrient load- and deprivation-related ghrelin responses. The results, in turn, indicated that the regulation of circulating total ghrelin levels involved separate mechanisms operating through anatomically distinct pathways. Collectively, acyl ghrelin is modulated by both the cholinergic and adrenergic pathways from the autonomic nervous system. The dissociation between the short- and long-term effects of vagotomy on plasma acyl ghrelin levels implies an additional neural control mechanism involved in the regulation of acyl ghrelin release (Hosoda and Kangawa, 2008). Another study demonstrated that the neural, instead of the neurohumoral, branch of the sympathetic nervous system could directly stimulate total ghrelin secretion in rats (Mundinger et al., 2006). On the other hand, intravenous injection of acyl ghrelin was shown to act on the central nervous system to decrease arterial pressure and renal sympathetic nerve activity in rabbits (Matsumura et al., 2002), demonstrating the participation of central acyl ghrelin in modulating the sympathetic nerve activity to the kidney and the baroreceptor reflex.

Postprandial suppression of plasma ghrelin is not mediated by nutrients in the stomach or duodenum, where most total ghrelin is synthesized and secreted, because either jejunal, gastric, or duodenal nutrient infusions suppressed ghrelin levels (Overduin et al., 2005). The meal-related total ghrelin suppression requires postgastric (pre- or postabsorptive) feedback, because intragastric infusion of glucose did not reduce plasma ghrelin level in rats with occluded pylorus (Williams et al., 2003a). Postprandial suppression of plasma ghrelin is regulated by postmeal increases in lower intestinal osmolarity, circulating insulin surges, and probably blood glucose (Overduin et al., 2005). Furthermore, the depth of postprandial ghrelin suppression was proportional to

ingested caloric load, but the recovery of plasma ghrelin was not a critical determinant of intermeal interval (Callahan et al., 2004). Moreover, macronutrient type also influences total ghrelin regulation. Glucose and amino acids suppressed ghrelin more rapidly and strongly than did lipids and fructose in rats and women (Teff et al., 2004; Overduin et al., 2005). Electrophysiological recordings revealed that the activity of the glucose-sensing neurons in the nucleus of the solitary tract could be modulated by the acyl ghrelin, and the primary effect of acyl ghrelin on glucose-inhibited and glucose-excited neurons was inhibitory (Wang et al., 2008). The results provide us the mechanisms by which acyl ghrelin diversely influences cellular excitability and neuronal circuitry in the hindbrain, contributing its biological actions to food intake. The suppressive effect on plasma total ghrelin levels by free fatty acids is independent of ambient GH and insulin levels in healthy men (Gormsen et al., 2006). The relatively weak suppression of total ghrelin by lipids and fructose compared with glucose and amino acids could lead to increased caloric intake and contribute to weight gain and obesity during long-term consumption of diets high in lipids and fructose. Two human studies using advanced technique measuring acyl ghrelin confirmed the above findings (Tannous dit El Khoury et al., 2006; Foster-Schubert et al., 2008). It is noteworthy that they found biphasic suppression of plasma ghrelin: a new but intriguing phenomenon featuring an initial decrease in acyl and total ghrelin and a marked rebound above preprandial values of acyl and total ghrelin after carbohydrate ingestion alone (Foster-

Schubert et al., 2008), suggesting the mechanisms contributing to the actions of high-protein/low-carbohydrate diets to promote weight loss and high-fat diets to enhance weight gain. The authors theorized that partial substitution of dietary protein for carbohydrate or fat might promote longer-term postprandial acyl ghrelin suppression and satiation (Tannous dit El Khoury et al., 2006). Another study also showed that consumption of a “higher protein-acute meal” led to lower respiratory exchange ratio, lower carbohydrate oxidation, and higher fat oxidation compared with a “normal protein-acute meal,” whereas the “higher protein-acute meal” also led to reduced self-reported postprandial hunger and desire to eat and lower postprandial plasma total ghrelin compared with the normal protein-acute meal in women (Leidy et al., 2007).

G. Interactions between Acyl Ghrelin and Other Neuropeptides.

The hypothalamic arcuate nuclei coexpressing neuropeptide Y (NPY) and agouti-related protein (AgRP), both prototypic anabolic neuropeptides to promote positive energy homeostasis (Schwartz et al., 2000; Seoane et al., 2003; Andrews et al., 2008), are the most well proven central targets for the biological actions of acyl ghrelin. Almost all NPY/AgRP neurons in the arcuate nuclei express GHS-R1a (Willesen et al., 1999; Mondal et al., 2005) (Fig. 2). Short- and long-term injection of acyl ghrelin increased hypothalamic NPY and AgRP mRNA levels with either stimulation of food intake or gain of body weight (Kamegai et al., 2000, 2001). Intra-

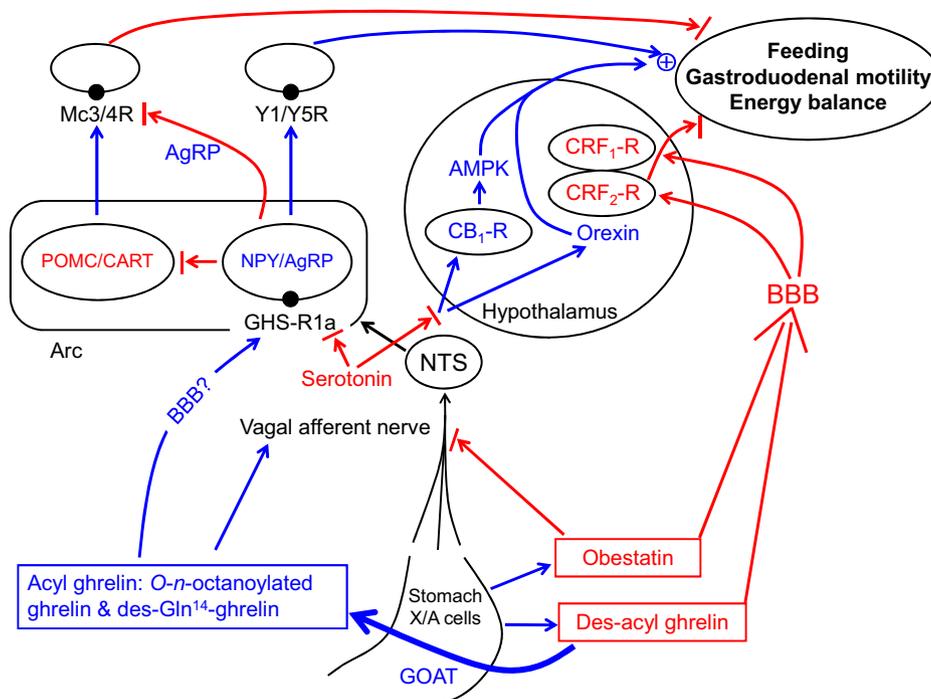


FIG. 2. Schematic diagram depicts the activation of distinct neuroendocrine signaling pathways by acyl ghrelin, des-acyl ghrelin, and obestatin from the stomach and the effects of their biological activities on feeding, gastroduodenal motility, and energy balance. Arc, the arcuate nucleus; BBB, blood-brain barrier; NTS, nucleus of the solitary tract.

cerebroventricular injection with small doses of acyl ghrelin enhanced food intake without altering the episodic GH release in conscious rats (Kamegai et al., 2000), demonstrating that the feeding-inducing action of acyl ghrelin is independent of its GH-stimulating effect. Acyl ghrelin definitely activates NPY/AgRP neurons in the arcuate nuclei, as shown by the increase of intracellular Ca^{2+} concentration, which was via an AMPK-mediated, protein kinase A- and N-type channel-dependent signaling mechanism (Kohno et al., 2003, 2008) and *c-fos* immunoreactivity (Hewson and Dickson, 2000; Kamegai et al., 2000, 2001; Shintani et al., 2001; Cowley et al., 2003; van den Top et al., 2004; Chen et al., 2005b; Date et al., 2005; Kobelt et al., 2005; Inhoff et al., 2008) in the arcuate nuclei. Chemical (Tamura et al., 2002), vector-mediated (Bewick et al., 2005), pharmacological (Nakazato et al., 2001; Asakawa et al., 2001b; Keen-Rhinehart and Bartness, 2007), or genetic blockade (Chen et al., 2004; Shaw et al., 2005) of NPY and/or AgRP signaling cascade completely abolished or attenuated the orexigenic effects of acyl ghrelin. Among them, central Y_1 receptor (Asakawa et al., 2001b; Shintani et al., 2001) as well as melanocortin receptor subtype 3 and 4 (MC_3 -R and MC_4 -R) (Shaw et al., 2005; Keen-Rhinehart and Bartness, 2007) are responsible for acyl ghrelin-induced hyperphagic effects.

In addition to NPY/AgRP neurons in the arcuate nuclei, orexin, serotonin, and cannabinoid signaling pathways are actively participating in acyl ghrelin-stimulatory effects (Fig. 2). First, ghrelin-immunoreactive axonal terminals made direct synaptic contacts with orexin-producing neurons that made orexin very possible in the involvement of acyl ghrelin-induced feeding. Intracerebroventricular administration of acyl ghrelin induced *c-fos* expression, a marker of neuronal activation, in orexin-producing neurons in the lateral hypothalamus, whereas acyl ghrelin remained competent to induce *c-fos* expression in orexin-producing neurons even after pretreatment with anti-NPY immunoglobulin G (Toshinai et al., 2003). Pretreatment with anti-orexin-A and anti-orexin-B immunoglobulin G attenuated acyl ghrelin-induced feeding. In addition, acyl ghrelin-induced feeding was also suppressed in orexin knockout mice (Toshinai et al., 2003). These data reveal a novel hypothalamic pathway that links acyl ghrelin and orexin in the regulation of feeding behavior and energy homeostasis. Second, brain serotonin (5-hydroxytryptamine; 5-HT) systems also contribute to regulation of eating behavior and energy homeostasis. A selective serotonin-reuptake inhibitor decreased the effects of acyl ghrelin on food intake at the level of hippocampus (Carlini et al., 2007a), showing that the effects of acyl ghrelin on feeding in the hippocampus could depend on the availability of serotonin. $5-HT_{2C}$ receptors and $5-HT_{1B}$ receptors have been shown to mediate anorectic effects of 5-HT drugs. Twenty-four hours fasting increased the expression of hypothalamic *5-HT_{2C} receptor* and *5-HT_{1B} receptor* genes in association with increases in plasma acyl ghrelin levels

compared with fed state in mice (Nonogaki et al., 2006). Treatment with serotonin reuptake inhibitor or $5-HT_{2C}$ receptor agonist significantly inhibited the increases in plasma active ghrelin levels without altering the expression of hypothalamic *NPY*, *AgRP*, and *ghrelin* genes, suggesting that there is a negative feedback system between brain 5-HT systems and plasma acyl ghrelin levels in energy homeostasis in mice (Nonogaki et al., 2006). Furthermore, fenfluramine, which stimulates the release of serotonin by disrupting vesicular storage of the neurotransmitter and reversing the serotonin transporter, inhibited marked food intake, which could be only partially reversed by intravenous acyl ghrelin or oral TJ-43, a spray-dried powder from herbal extracts of rikkunshito (Fujitaka et al., 2009), implying that the serotonin system acted on upstream regulation of ghrelin system on food intake. Third, acyl ghrelin was found to increase the endocannabinoid content of the hypothalamus in wild-type mice, and this effect was abolished by pretreatment with rimobant, a cannabinoid receptor subtype 1 (CB_1 -R) antagonist, whereas no effect was observed in CB_1 -R-knockout animals (Kola et al., 2008). Intraperitoneal injection of acyl ghrelin did not induce an orexigenic effect in CB_1 -R-knockout mice, whereas both the genetic lack of CB_1 -R and the pharmacological blockade of CB_1 -R inhibited the effect of peripheral acyl ghrelin on AMPK activity. Electrophysiological studies showed that acyl ghrelin can inhibit the excitatory inputs on the parvocellular neurons of the paraventricular nucleus and that this effect was abolished by administration of a CB_1 -R antagonist, an inhibitor of the diacylglycerol lipase (the enzyme responsible for 2-arachidinoylethanolamide synthesis), or the presence of BAPTA (an intracellular calcium chelator) (Kola et al., 2008). The evidence makes it clear that an intact cannabinoid signaling pathway is necessary for the stimulatory effects of acyl ghrelin on AMPK activity and food intake and for the inhibitory effect of acyl ghrelin on paraventricular neurons. However, early tolerance to the hypophagic effect of SR141716 (i.e., rimonabant) was reported to develop within 5 days; in contrast, however, its body weight-reducing action remained in rats, pointing out the important role of cannabinoid system in the regulation of body weight (Rigamonti et al., 2006). A study indicated that both cannabinoids and acyl ghrelin stimulated AMPK activity in the hypothalamus and the heart while inhibiting AMPK in liver and adipose tissue, demonstrating that AMPK is linked not only to the orexigenic effects of endocannabinoids and acyl ghrelin in the hypothalamus but also to their effects on the metabolism of peripheral tissues (Kola et al., 2005). To date, however, no information is available for interactions between CB_2 -R and acyl ghrelin on food intake and body weight from PubMed search. Hence, studies using CB_2 -R antagonist, such as AM-630, to investigate its actions with acyl ghrelin on food intake and body weight are demanded in the future.

III. Normal Physiology of Des-Acyl Ghrelin and Regulation of Its Expression and Interactions

Fasting induced the elevation of des-acyl ghrelin, the second ghrelin gene product, in mice (Kirchner et al., 2009), rats (Seoane et al., 2007a), and humans (Liu et al., 2008; Mondal et al., 2008), whereas feeding suppressed it (Seoane et al., 2007a; Liu et al., 2008; Mondal et al., 2008; Kirchner et al., 2009). Des-acyl ghrelin has been demonstrated to decrease food intake in mice (Asakawa et al., 2005) and rats (Chen et al., 2005a,b) centrally and peripherally in the fasted state. The action of des-acyl ghrelin was characterized by rapid onset (1 h) and short duration (2 h), but the suppressive effect was not durable (Chen et al., 2005b). In addition, the anorexigenic effects induced by des-acyl ghrelin in food-deprived rodents are mediated through distinct brain nuclei (compared with those by acyl ghrelin) by real-time RT-PCR and immunohistochemical studies. Intraperitoneal injection of des-acyl ghrelin induced mRNA expression of cocaine- and amphetamine-regulated transcript (CART) and urocortin 1, which are both anorexigenic neuropeptides to reduce food intake, in the mouse hypothalamus (Asakawa et al., 2005). Furthermore, intraperitoneal administration of des-acyl ghrelin elicited the amount of *c-fos* expression in the rat paraventricular nucleus neurons, presumably corticotropin-releasing factor (CRF) neurons (Chen et al., 2005b), without activating the nucleus of the solitary tract in the brainstem (Asakawa et al., 2005; Chen et al., 2005b) (Fig. 2). However, another two studies show discrepant results. Administration of des-acyl ghrelin did not affect feeding in fasted and freely fed mice (Neary et al., 2006; Toshinai et al., 2006), whereas central administration of des-acyl ghrelin increased light phase food intake in freely fed rats and mice by activating orexin-expressing neurons in the lateral hypothalamic area (Toshinai et al., 2006). It is very difficult to detect the effect of a satiation peptide during light phase under ad libitum feeding, most likely because of the satiation signals and the lack of the ability to further reduce the very low food intake during this phase. Des-acyl ghrelin has been reported to suppress dark-phase cumulative food intake and body weight gain in freely fed obese Zucker rats (Stengel et al., 2008) and to oppose acyl ghrelin-induced hyperphagic effects in freely fed lean rats (Inhoff et al., 2008), most likely to be mediated via central pathways. Moreover, repeated injection of des-acyl ghrelin for a period of 8 days induced a significant reduction of body weight gain that was not compensated or recovered in the 4 days after treatment (Stengel et al., 2008). A more recent study revealed that des-acyl ghrelin was secreted in a highly regulated manner in response to food deprivation in mice (Kirchner et al., 2009), supporting the idea that des-acyl ghrelin should play an as-yet undefined physiological role in feeding behavior rather than being an "innocent bystander." These authors also sug-

gested that des-acyl ghrelin might bind to an additional, unknown ghrelin receptor (Kirchner et al., 2009). Both peripheral and central injection of des-acyl ghrelin before acyl ghrelin consistently blocked the stimulatory effects on food intake induced by acyl ghrelin alone in the goldfish (*Carassius auratus*) (Matsuda et al., 2006), indicating that the interactions between acyl ghrelin and des-acyl ghrelin could be observed across species. Promisingly, des-acyl ghrelin was demonstrated to be involved in the regulation of human appetite (Muscarioti et al., 2007). Moreover, a decrease in body weight and fat pad mass weight accompanied moderately decreased linear growth in des-acyl ghrelin-overexpressing mice has been shown by two independent studies (Ariyasu et al., 2005; Asakawa et al., 2005), implying that, in contrast to acyl ghrelin, des-acyl ghrelin generates a negative energy balance (Asakawa et al., 2005). Taken together, the evidence indicates that des-acyl ghrelin is closely linked with ingestive behavior, metabolism, and body weight regulation, probably via counteracting the elevated level of endogenous acyl ghrelin under certain physiological situations and then dampening its biological effects, such as feeding and energy balance. Moreover, the anorexigenic effect of des-acyl ghrelin even appeared in rats without intact leptin signaling (Stengel et al., 2008). Therefore, des-acyl ghrelin alone or combined with leptin might offer new options of pharmacotherapy for the treatment of human obesity and metabolic syndrome. However, long-term impacts of des-acyl ghrelin on animal and human body weight regulation need to be established before the induction of pharmacologic interventions. Finally, des-acyl ghrelin did not affect water intake in chicks (Tachibana et al., 2006)

IV. Normal Physiology of Obestatin and Regulation of Its Expression and Interactions

The third ghrelin gene product, obestatin, a 23-amino acid peptide, was recently identified from rat stomach (Zhang et al., 2005). High levels of GPR39 mRNA were found abundantly in the amygdala, the hippocampus, and the auditory cortex but not in the hypothalamus (McKee et al., 1997; Jackson et al., 2006). Plasma obestatin levels were lower in obese subjects and those who had undergone gastrectomy (Huda et al., 2008). However, recent studies indicate that obestatin is not the endogenous cognate ligand for GPR39 (Lauwers et al., 2006; Holst et al., 2007; Chartrel et al., 2007), whereas a more recent study demonstrated that obestatin was a metabolic hormone capable of binding to GPR39 and, in turn, of regulating the diverse biological functions of gastrointestinal and adipose tissues (Zhang et al., 2008a). The plasma obestatin concentration did not change after a 450-kcal (Mondal et al., 2008) or even a 1550-kcal (Huda et al., 2008) meal in humans, identifying that obestatin secretion is not influenced by dietary nutrients. The effects of obestatin on food intake

remain controversial (Chen et al., 2009). Obestatin has been demonstrated to inhibit food intake in rodents (Zhang et al., 2005; Bresciani et al., 2006; Sibilias et al., 2006; Chartrel et al., 2007; Zizzari et al., 2007; Carlini et al., 2007; Green et al., 2007), whereas several research groups fail to show this anorexigenic effect (Seoane et al., 2006; Gourcerol et al., 2007; Nogueiras et al., 2007; Yamamoto et al., 2007; De Smet et al., 2007; Annemie et al., 2009; Kobelt et al., 2008; Mondal et al., 2008; Unniappan et al., 2008). Obestatin had no effect on body weight (Sibilias et al., 2006; Unniappan et al., 2008). However, the N-terminal obestatin (residues 1–13) was reported to reduce food intake and body weight, whereas the middle fragment (residues 6–18) did not affect food intake and body weight in mice (Nagaraj et al., 2008). Obestatin boluses increased the protein output and trypsin activity of pancreatic-biliary juice in anesthetized rats, whereas vagotomy abolished the effects of exogenous obestatin administration (Kapica et al., 2007). The data support that obestatin stimulates the secretion of pancreatic juice enzymes through a vagal pathway. Fasting induced elevation of plasma obestatin, whereas refeeding suppressed it, which adds another clue to the physiological functions of obestatin in regulating metabolism and energy homeostasis (Guo et al., 2008). However, peripheral obestatin failed to affect food intake in *ghrelin*-knockout mice (Depoortere et al., 2008). Acclimation of experimental animals, skills during experiments, and numbers of each group being inadequate to reach statistical power could all account for these discrepant results of obestatin on food intake. The cutting-edge knowledge of obestatin on food intake is reviewed by Chen et al. (2009). On the other hand, intracerebroventricular injection of obestatin inhibited water drinking in ad libitum-fed and -watered rats and in food- and water-deprived rats and inhibited angiotensin II-induced water drinking in rats provided free access to water and food (Samson et al., 2007), and the authors claimed that the effect of obestatin on food intake might be secondary to an initial action in inhibiting thirst phenomenon referred as “dehydration anorexia.” Dehydration anorexia could be one of the mechanisms through which to explain the discrepant results of obestatin on food intake. In an extended study, during a hypovolemic challenge, intracerebroventricular administration of obestatin significantly inhibited drinking of water but not saline (0.3 M NaCl) (Samson et al., 2008). Central obestatin also inhibited, in a dose-related fashion, dehydration-induced vasopressin secretion without affecting plasma oxytocin levels and vasopressin release induced by central angiotensin II (Samson et al., 2008), indicating that obestatin might be an important contributor to the physiologic regulation of fluid and electrolyte homeostasis by inhibiting the vasopressin system (Samson et al., 2007, 2008). However, another recent study failed to show an inhibitory effect of intracerebroventricular obestatin on water intake in mice (Annemie et al.,

2009). Three recent studies also remind us to take major instability and impurity of obestatin peptides under biomedical investigations into account during interpretation for those discrepant data obtained in feeding behavior (Pan et al., 2006; De Spiegeleer et al., 2008; Vergote et al., 2008). Hence, in-depth exploration of obestatin on mammalian appetitive behavior, including microstructures of feeding and correction of situational physiological bias, should be further conducted (Chen et al., 2009).

V. Normal Physiology, Expression, and Regulation of Growth Hormone Secretagogue Receptor

The human full-length GHS-R1a cDNA encodes the predicted polypeptide of 366 amino acids with seven-transmembrane domains (TM), whereas GHS-R1b is predicted to encode a truncated polypeptide of 289 amino acids with only five TM. The *GHS-R* gene consists of two exons: the first exon encodes TM-1 to TM-5, and the second exon encodes TM-6 to TM-7. GHS-R1b is derived from the only first exon, encodes only five of the seven predicted TMs, and is thus a COOH-terminally truncated form of GHS-R1a and is pharmacologically inactive (Howard et al., 1996; Kojima and Kangawa, 2005). Acyl ghrelin acts on GHS-R1a, which belongs to family A, and, in turn, signals via a $G_{q/11}\alpha$ -subunit that results in the release of inositol trisphosphate and Ca^{2+} (Howard et al., 1996; Guan et al., 1997; Wettschureck et al., 2005). GHS-R1b interacts with GHS-R1a and neurotensin receptor 1 to form heterodimer (Takahashi et al., 2006b). Emerging evidence suggests that the cloned GHS-R1a alone cannot be the responsible for all acyl ghrelin and GHS-mediated influence on food intake, gut motility, sleep, memory and behavior, glucose and lipid metabolism, cardiovascular performance, cell proliferation, immunological response, and reproduction. The cloned GHS-R1b isoform is apparently nonresponsive to ghrelin/GHS, despite demonstration of expression in neoplastic tissues responsive to ghrelin not expressing GHS-R1a; GHS-R1a homologs sensitive to acyl ghrelin are capable of interaction with GHS-R1b, forming heterodimeric species. [Heterogeneity, molecular identity, and transduction of the multiple levels of information, including the conservation across species for signaling molecules and receptors, of GHS-R1a and GHS-R1b are reviewed by Muccioli et al. (2007) (see Table 1).]

The GHS-R1a is expressed very selectively in the brain centers related to regulation of energy homeostasis, such as hypothalamic nuclei, three components of the dorsal vagal complex (area postrema, nucleus of the solitary tract, and the dorsal motor nucleus of the vagus), the hippocampus, dopaminergic neurons in the ventral tegmental area and substantia nigra, parasympathetic preganglionic neurons, and in the dorsal and medial raphe nuclei, the pituitary, and the dentate gyrus (Gnanapavan et al., 2002; Kojima and Kangawa,

TABLE 1

Summary of ghrelin gene products associated receptor types, their corresponding cognate ligand, and intracellular signaling mechanisms

Receptor type	Cognate Ligands	Signaling	Antagonists
GHS-R1a	Acyl ghrelin, short acyl ghrelin fragments, synthetic peptidyl GHRP-6, partial peptidyl and non-peptidyl GHS	G α_q : PLC, IP3, DAG, \uparrow Ca $^{2+}$, PKC \uparrow ERK1/2, CREB and SRE	[D-Lys 3]GHRP-6
GHS-R1b	NTS-R1/GHS-R1b heterodimer	Heterodimer formation with GHS-R1a and NTS-R1	Unknown
GHS-Rx (uncloned)	Des-acyl ghrelin	N.A.	N.A.
Some unknown receptor should be other than GPR39	Obestatin	N.A.	N.A.

GHRP-6, growth hormone-releasing peptide 6; PLC, phospholipase C; IP3, inositol trisphosphate 3; DAG, diacylglycerol; PKC, protein kinase C; ERK1/2, extracellular signal-regulated kinase 1 and 2; CREB, cAMP-responsive element-binding protein; SRE, serum-responsive element; NTS-R1, neurotensin receptor 1; N.A., not applicable; GPR39, G-protein coupled receptor 39.

2005). RT-PCR analysis revealed that GHS-R1a mRNA expression was detected in many human organs, including heart, lung, liver, kidney, pancreas, stomach, small and large intestine, adipose tissue, and immune cells (Gnanapavan et al., 2002; Sun et al., 2007), the highest levels occurring in pituitary, adrenal gland, and spinal cord (Sun et al., 2007; Ueberberg et al., 2009). These findings ascertain that acyl ghrelin has multiple functions in these central and peripheral tissues. By contrast, expression of GHS-R1b was found to be more widespread than that of GHS-R1a in all tissues investigated (Gnanapavan et al., 2002), raising the possibility that acyl ghrelin might function in a widespread fashion independent of GHS-R1a. The hypothalamic and hind-brain targets for acyl ghrelin are not well protected by the blood-brain barrier, and site-specific saturable transport of acyl ghrelin across the blood-brain barrier may provide additional access to GHS-R1a and deeper targets within the brain (Banks et al., 2002; Pan et al., 2006). GH-stimulating effect by peripheral acyl ghrelin was reported through direct central mechanisms in humans (le Roux et al., 2005a). On the other hand, the feeding-stimulating effects by peripheral acyl ghrelin could occur via indirect mechanisms; this idea is supported by the fact that peripheral acyl ghrelin had no effect on feeding in patients who had undergone vagotomy (le Roux et al., 2005a) and in rodents with surgical or chemical vagotomy (Asakawa et al., 2001b; Date et al., 2002; Chen et al., 2005b) or lesions in caudal brain stem (Date et al., 2002; Gilg and Lutz, 2006). However, contradictory opinion exists (Arnold et al., 2006). In addition, the hypothalamus produced small quantities of acyl and des-acyl ghrelin by itself (Cowley et al., 2003; Mondal et al., 2005; Sato et al., 2005), which would act locally as neuropeptides modulating neurons for energy balance.

Consistent with the notion that acyl ghrelin expression is closely modulated by food intake, GHS-R1a is regulated according to feeding status. Acyl ghrelin mRNA levels in the stomach, and GHS-R1a mRNA levels in the hypothalamus and pituitary gland, increased after 48-h fasting in rats, whereas refeeding decreased them (Kim et al., 2003b). Body weight loss by sleeve gastrectomy in obese rats induced by high-fat diet was

accompanied decrease of plasma ghrelin concentration and increased expression of GHS-R1a in the hypothalamus compared with sham controls (Wang and Liu, 2009). The increased GHS-R1a in the hypothalamus and/or pituitary gland could contribute to augment the actions of acyl ghrelin in these organs and may be considered a physiologic mechanism to increase appetite and adiposity in energy-deficient state (Kim et al., 2003b; Wang and Liu, 2009). Intracerebroventricular leptin decreased GHS-R1a mRNA in the arcuate nucleus accompanied inhibition of food intake and body weight gain, whereas intracerebroventricular acyl ghrelin increased GHS-R1a mRNA in the arcuate nucleus in rats (Nogueiras et al., 2004). During fasting (with high levels of plasma acyl ghrelin and low levels of plasma leptin) and in obese Zucker rats (insensitive to leptin), increased GHS-R1a mRNA was detected in the arcuate nucleus (Nogueiras et al., 2004), indicating that satiation and hunger signals are both involved in the regulation of GHS-R1a expression in the brain in addition to feeding. Direct morphological evidence identified that GHS-R1a was produced in the stomach-projected afferent neurons of the nodose ganglion in rats, suggesting that ghrelin signals from the stomach are transmitted to the brain via vagal afferent nerves (Sakata et al., 2003). Obesity-prone rats fed a high-fat diet had increased body weight, increased adiposity, and increased mRNA expression of GHS-R1a and CB $_1$ -R in the nodose ganglion compared with low-fat diet-fed control rats or high-fat diet-induced obesity-resistant rats (Paulino et al., 2009), demonstrating that shifts in the balance between orexigenic and anorexigenic signals within the vagal afferent pathway might influence food intake and body weight gain induced by high-fat diet.

VI. Normal Physiology, Expression, and Regulation of Ghrelin O-acyltransferase

The acylated modification of ghrelin with an eight-carbon fatty acid, octanoate, at serine-3 is crucial for the physiological effects of acyl ghrelin, such as GH release, food eating, adiposity, and insulin secretion. GOAT, a polytopic membrane-bound enzyme that attaches octanoate to serine-3 of ghrelin, was recently identified and character-

ized (Yang et al., 2008a; Gutierrez et al., 2008). Analysis of the mouse genome revealed that GOAT belongs to a family of 16 hydrophobic membrane-bound acyltransferases that includes Porcupine, which attaches long-chain fatty acids to Wnt proteins (Yang et al., 2008a). GOAT is a conserved orphan membrane-bound *O*-acyl transferase (MBOAT) that specifically octanoylates serine-3 of the ghrelin peptide. GOAT is the only member of this family that octanoylates ghrelin when coexpressed in cultured endocrine cell lines with prepro-ghrelin (Yang et al., 2008a). GOAT activity requires catalytic asparagine and histidine residues that are conserved in this family. GOAT is the only member of the membrane-bound *O*-acyl transferase family, the expression of which is highly enriched within gastric ghrelin cells and the whole body distribution of which colocalizes with that of ghrelin (Sakata et al., 2009). The occurrence of ghrelin and GOAT in stomach and pancreas tissues demonstrates the relevance of GOAT in the acylation of ghrelin and further implicates acyl ghrelin in pancreatic function in addition to its gastric function (Gutierrez et al., 2008).

GOAT was subjected to end-product inhibition, and this inhibition was better achieved with substrates having the octanoyl group attached through an amide linkage rather than the corresponding ester (Yang et al., 2008b). Unexpectedly, although the main active form of ghrelin is modified by *n*-octanoic acid, a recent enzymological analysis showed that GOAT had a strong preference for *n*-hexanoyl-CoA over *n*-octanoyl-CoA as an acyl donor (Ohgusu et al., 2009). However, the concentration of *n*-hexanoyl ghrelin in the mouse stomach is very low compared with that of *n*-octanoyl ghrelin (Ohgusu et al., 2009), raising the presumption that different concentrations between *n*-hexanoyl-CoA and *n*-octanoyl-CoA in the stomach may affect the production and concentration of various acyl-modified ghrilins. From PubMed search, there is no information regarding the biological actions of hexanoyl-ghrelin. These insights may facilitate the future design of useful inhibitors of GOAT.

Because nonacylated peptide does not stimulate appetite, it is intriguing to determine whether GOAT activity modulates in feeding regulation. Chronic undernutrition markedly increased the expression of GOAT mRNA levels in stomach mucosae, offer a mechanistic explanation of the increased acyl ghrelin levels observed in patients with severe malnutrition (González et al., 2008). Using absorbable medium-chain fatty acid, GOAT-ghrelin system could act as a nutrient sensor to tell the brain that high caloric food is available, leading to fat storage, nutrient partitioning, and growth signals (Kirchner et al., 2009). A recent study indicated that meals inhibited secretion of acyl and des-acyl ghrelin, yet long-term fasting inhibited acylation but not total secretion, suggesting that acylation might be regulated independently of secretion by nutrient availability in the gut or by esterases that cleave the acyl group (Liu et al., 2008).

VII. Pathophysiology of Ghrelin Gene Products in Feeding and Body Weight Regulation

A. Relationships between Plasma Levels of Ghrelin Gene Products and Body Weight Homeostasis

Circulating ghrelin concentrations significantly correlate with human body mass index and body fatness and are low in obese persons. The negative association between ghrelin secretion and body weight is emphasized by evidence that weight decrease augments circulating ghrelin levels in anorexia and weight increase reduces circulating ghrelin levels in obesity. Plasma ghrelin levels increase in energy deficient conditions (see section XI). Moreover, the increase in ghrelin level is closely coupled with markedly elevated expression of GHS-R1a in the hypothalamus in fasting or long-term food restriction (Kim et al., 2003b; Nogueiras et al., 2004; Kurose et al., 2005), reflecting an enhanced feeding feedback for ghrelin-induced stimulation of appetite during weight loss. Conversely, circulating ghrelin levels decrease in energy excessive conditions (also see Section XI). Prader-Willi syndrome, a “hereditary hyperghrelinemia,” is an exception to have increased body weight and plasma ghrelin concentrations. Therefore, ghrelin/GHS-R1a system contributes to the adaptive metabolic mechanisms to such alterations and feedback signaling cascade among human nutrient intake and the central nervous system, and actively participates in the regulation of short- and long-term mammalian energy balance and body weight.

B. Acyl Ghrelin in Feeding and Body Weight Regulation

Long-term central administration of rat acyl ghrelin significantly increased food intake and body weight (Kamegai et al., 2001). Furthermore, the feeding-eliciting action of exogenous acyl ghrelin was not diminished by a long-term hyperghrelinemia in transgenic mice overexpressing human *ghrelin* gene, indicating that the food ingestive pathway of the GHS-R1a was not susceptible to “desensitization” (Wei et al., 2006). In contrast, the epididymal fat pad growth and the GH responses to exogenous acyl ghrelin were blunted in *ghrelin* transgenic mice with chronic hyperghrelinemia (Wei et al., 2006). Short-term exogenous administration of high-dose acyl ghrelin stimulated energy intake in obese and in lean subjects, whereas low-dose acyl ghrelin increased food intake only in obese people (Druce et al., 2005). Administration of acyl ghrelin provoked palatability only in the obese group, but not in the lean group, whereas the GH-releasing effect was attenuated in obese subjects compared with lean subjects (Druce et al., 2005). Collectively, the evidence revealed that the feeding response to acyl ghrelin was maintained in obesity. Obese people were sensitive to appetite-stimulating effects of acyl ghrelin, and the orexigenic ability was independent of GH release in obesity. This highlights the idea that inhibition of circulating acyl ghrelin may be a

useful therapeutic target in the treatment of obesity. However, an animal study showed that an increased fat mass per se did not exert an inhibitory effect on acyl ghrelin homeostasis during ingestion of a 5-week high-fat diet in rats (Qi et al., 2008), presuming that endogenous signal for activation of ingestive behavior could still remain intact despite excess stored calories in high fat-fed rats. In addition, rats fed with 5 weeks on diets rich in sucrose and/or fat showed elevated expression of leptin mRNA in the stomach and serum leptin concentration in response to sucrose-rich rather than fat-rich diets, linking leptin with sucrose metabolism and suppressed expression of ghrelin in the stomach and serum total ghrelin level by all palatable diets, including sucrose and fat (Lindqvist et al., 2005). Rats fed on palatable diets overeat; increased body weight and adiposity despite raising satiation peptide (leptin) and lowering hunger peptide (ghrelin). These results imply that leptin/ghrelin systems were blunted, and only the above endogenously responsive hormones are insufficient to control appetite, body weight gain, and adiposity in palatable food diet.

C. Interactions between Ghrelin Gene Products and the Blood-Brain Barrier

The blood-brain barrier prevents the unrestricted exchange of substances between the central nervous system and the blood and also conveys information between the central nervous system and the gastrointestinal tract through several mechanisms. Saturable systems transported human acyl ghrelin from brain to blood and from blood to brain, whereas mouse acyl ghrelin was a substrate for the brain-to-blood transporter but not for the blood-to-brain transporter (Banks et al., 2002). The second ghrelin gene product, des-acyl ghrelin, entered

the brain by nonsaturable transmembrane diffusion and was sequestered once within the central nervous system (Banks et al., 2002). In contrast, the third ghrelin gene product, obestatin, lacked specific binding and endocytosis, and did not cross the blood-brain barrier. Small amounts of internalized obestatin showed rapid intracellular degradation before the radioactivity was released by exocytosis (Pan et al., 2006). These pharmacokinetic experiments illustrated distinctive physiological interactions of three ghrelin gene products with the central nervous system (Fig. 3). Moreover, serum factors and physiological states affect the ghrelin gene products across the blood-brain barrier. First, obesity and old age lost the ability to transport intravenous human acyl ghrelin across the blood-brain barrier, resulting in an inverse relationship between body weight and acyl ghrelin blood-brain barrier permeability (Banks et al., 2008). Second, serum triglycerides promoted transport of intravenous acyl ghrelin across the blood-brain barrier (Banks et al., 2008). Third, fasting tended to enhance acyl ghrelin transport across the blood-brain barrier (Banks et al., 2008). Collectively, high fat diet and starvation would augment orexigenic effects of acyl ghrelin on the central nervous system by increasing its crossing the blood-brain barrier, whereas obesity and old age would ameliorate the actions of acyl ghrelin on the brain by decreasing its crossing the blood-brain barrier (Fig. 3). Emerging evidence reveals that the orexigenic effects by peripheral acyl ghrelin could occur via indirect mechanisms and be dependent on the vagal afferent pathway (Asakawa et al., 2001b; Date et al., 2002; Chen et al., 2005b; le Roux et al., 2005a; Gilg and Lutz, 2006), whereas GH release induced by peripheral acyl ghrelin could occur via direct mechanism (le Roux et al., 2005a).

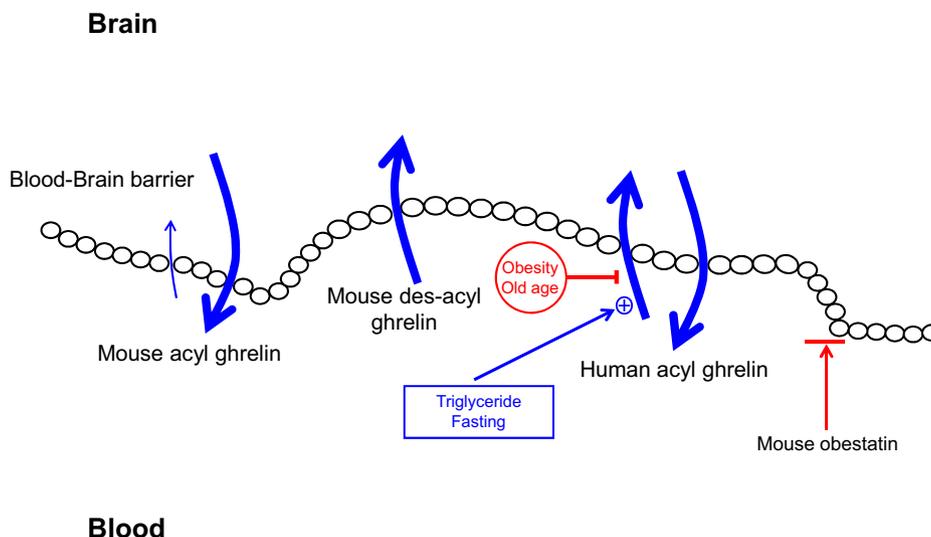


FIG. 3. Differential transportation of mouse acyl ghrelin, mouse des-acyl ghrelin, human acyl ghrelin, and mouse obestatin across the blood-brain barrier, which regulates communications between the gut and the brain. Obesity, old age, serum triglyceride, and fasting affect acyl ghrelin across the blood-brain barrier.

D. Interactions between Acyl Ghrelin and Helicobacter pylori on Feeding

Chronic noninvasive bacterial infection causes altered feeding behavior. Chronic *Helicobacter pylori* infection in mice caused increased mononuclear cell infiltration in gastric corpus, larger gastric area, delayed gastric emptying, elevated plasma acyl ghrelin and cholecystokinin levels, and lowered pro-opiomelanocortin (POMC) mRNA expression in the arcuate nucleus; all returned to control values after eradication of *H. pylori* (Bercik et al., 2009). However, altered feeding behavior, such as decreased amount of food per bout and increased frequency of bouts, persisted after eradication of *H. pylori*, which was paralleled by persistently elevated gastric CD3⁺ T cells in the stomach and increased expression of TNF- α mRNA in the brain (Bercik et al., 2009), implying that central nervous system changes might be slow to resolve after chronic infection and persist after eradication of the triggering gastrointestinal infection, supporting a role for altered gut-brain pathways in persistent abnormal feeding behavior after postinfection gut dysfunction.

E. Total Acyl Ghrelin and Burn Injury

Burn injury is a common clinical problem and induces persistent hypermetabolism and muscle wasting. Total plasma ghrelin was reduced 1 day after burn in rats (Balasubramaniam et al., 2006). Intraperitoneal injection of acyl ghrelin greatly stimulated 2-h food intake in rats on five separate days after burn, whereas on day 15 after the burn, plasma growth hormone levels were significantly lower than in control rats, and this was restored to normal levels by exogenous acyl ghrelin supplement (Balasubramaniam et al., 2006). These observations suggest that ghrelin retains its ability to favorably modulate both the peripheral anabolic and central orexigenic signals, even after thermal injury, despite ongoing changes from prolonged and profound hypermetabolism (Balasubramaniam et al., 2006). Long-term treatment with acyl ghrelin may attenuate endocrine- and feeding-related dysfunctions in patients with burn injury in the future.

F. Growth Hormone Secretagogue Receptor Agonist and Postoperative Anorexia-Cachexia

Postoperative ileus is an impairment of coordinated gastrointestinal motility that develops as a consequence of abdominal surgery. The abnormal gastrointestinal motility often leads to abdominal bloating, vomiting, lack of defecation, and poor appetite after surgery. Repetitive intravenous administrations of ipamorelin, a novel synthetic GHS-R1a agonist, significantly increased laparotomy and intestinal manipulation-induced reduction of food intake and body weight in rats, in addition to its effect on cumulative fecal pellet output (Venkova et al., 2009), implying that postsurgical mul-

tipale intravenous bolus infusion of ipamorelin might ameliorate the symptoms of anorexia-cachexia in patients with postoperative ileus.

G. Total Acyl Ghrelin and Functional Dyspepsia

Functional dyspepsia is a common gastrointestinal disorder that is defined by the Rome III criteria. Plasma total ghrelin in patients with functional dyspepsia could be lower (Takamori et al., 2007; Lee et al., 2009), similar (Shinomiya et al., 2005; Pilichiewicz et al., 2008), or higher (Nishizawa et al., 2006) compared with healthy subjects. Plasma acyl ghrelin levels correlated with "subjective symptom score" in female patients with functional dyspepsia (Shinomiya et al., 2005), whereas plasma acyl and total ghrelin levels correlated with "ingestive score" in another group of patients with functional dyspepsia (Nishizawa et al., 2006). Repeated intravenous administration of acyl ghrelin tended to increase daily food intake and significantly induced hunger sensation in patients with functional dyspepsia (Akamizu et al., 2008).

H. Acyl Ghrelin, Reward Feeding, and Related Body Weight Regulation

In addition to expression in the hypothalamus, GHS-R1a is expressed in brain sites other than the hypothalamus, such as the ventral tegmental area, a primary node of the mesolimbic reward neuropathway, which sends dopaminergic projections to the nucleus accumbens (Kelley and Berridge, 2002), suggesting central effects of ghrelin on energy balance and other functions, which may recruit extrahypothalamic circuits. Emerging evidence reveals that besides acting through visceral vagal afferent associated gut-brain axis and blood-mediated signals directly on the level of the hypothalamus (i.e., an energy homeostatic process), ghrelin gene products are also deeply involved in modulating reward and motivation in enhancing the hedonic and incentive responding to food-related cues (Wellman et al., 2005; Malik et al., 2008a) or context (Smith et al., 2010). For instance, acyl ghrelin was reported to alter the appetitive and consummatory response to learned cues associated with food in mice and rats in nonhomeostatic brain feeding nuclei (Johnson et al., 2009). Microinjection of acyl ghrelin into the ventral tegmental area, the key structures of the mesolimbic dopamine system, elicited robust feeding behavior, whereas its microinjection into the nucleus accumbens induced a moderate eating pattern in rats (Naleid et al., 2005; Abizaid et al., 2006). Intraperitoneal administration of acyl ghrelin induced locomotor stimulation, conditioned place preference, and accumbal dopamine overflow in mice (Jerlhag, 2008). Phasic dopamine release is crucial in reward behavior (Hajnal et al., 2009). A microdialysis study showed that subcutaneous administration of acyl ghrelin in rats increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens, the main

projecting structure of the mesolimbic dopamine system (Quarta et al., 2009). These findings suggest that systemic acyl ghrelin may regulate the valence of reinforcers such as food and drugs of abuse by interfering with mesolimbic dopamine activity. Blockade of dopamine β -hydroxylase in the hindbrain abolished acyl ghrelin-induced feeding (Date et al., 2006), which implies that the action of acyl ghrelin action could be noradrenergic neuron-dependent and could be involved in rewarding mechanisms. Using functional magnetic resonance imaging techniques, the neuronal response to food pictures increased in the amygdala, orbitofrontal cortex, anterior insula, and striatum regions of the brain after intravenous administration of acyl ghrelin in healthy human volunteers, implicating acyl ghrelin in encoding the incentive value of food cues (Malik et al., 2008b). In addition, the actions of acyl ghrelin in the amygdala and orbitofrontal cortex were correlated with self-rated hunger gradings (Malik et al., 2008b). A recent study demonstrated that alcohol-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference were abolished in models of suppressed central acyl ghrelin signaling: GHS-R1a was either knocked out or knocked down, pointing to a requirement for central acyl ghrelin signaling for alcohol reward (Jerlhag et al., 2009). The preprandial rise in plasma acyl ghrelin may increase the incentive value for motivated behaviors such as food seeking, and ghrelin signaling constitutes a potential target for the treatment of compulsive overeating or alcohol addiction (Jerlhag, 2008; Jerlhag et al., 2009). Moreover, fasting plasma acyl ghrelin levels were higher in persons with chronic alcoholism during a period of abstinence than in control subjects (Kim et al., 2005a). Besides, a positive correlation was observed between acyl ghrelin levels and the duration of abstinence, whereas daily alcohol intake before abstinence was inversely related to acyl ghrelin levels. Hence, these findings suggest that acyl ghrelin plays a role in the pathogenesis of "alcohol dependence," particularly during the abstinence period, in persons with chronic alcoholism (Kim et al., 2005a). Emerging evidence from rodents to humans indicates the inextricably biological roles of acyl ghrelin in metabolism and reward linked to regulation of eating behavior. The rapid growing obesity epidemic may be partially mediated by an increase in the expo-

sure to cues for food in our modern society. Biologic pathways regulating appetite and craving are often overlapped and interacted (Kalra and Kalra, 2004). Appetitive behavior is influenced by both strong regulatory drives and taste-hedonics, whereas cognitive-rewarding-social-emotional mechanisms can often override homeostatic control systems by various palatable food stimuli; therefore, targeting ghrelin gene products, via either responsible homeostatic or reward (nonhomeostatic) neuronal circuits, or both, holds the promise of the induction of satiation, control of food intake, and, eventually, diminished body energy stores, normalization of body weight regulation under obesogenic environments, and combat against alcohol or drug craving during withdrawal in humans in this modern society.

VIII. Normal Physiology and Pathophysiology of Ghrelin Gene Products in Feeding-Associated Gastrointestinal Motility

Ghrelin gene products actively participate in the regulation of feeding-associated gastrointestinal motility. Results from electrophysiologic studies provide us the mechanisms by which acyl ghrelin diversely influences cellular excitability and neuronal circuitry in the hindbrain, contributing its biological actions on feeding and gastric motility (Wang et al., 2008). Circulating acyl ghrelin levels were fluctuating and their peak was highly associated with gastric migrating motor complex cycle (Ariga et al., 2007). Endogenous acyl ghrelin released from gastric X/A cells mediated gastric phase III-like contractions via vagal-dependent pathways in mice (Zheng et al., 2009a) and in rats (Ariga et al., 2007; Taniguchi et al., 2008), whereas serotonin released from enterochromaffin cells of duodenal mucosa mediated intestinal phase III-like contractions via 5-HT₄ receptors located on intrinsic primary afferent neurons in rats (Taniguchi et al., 2008). Intravenous exogenous acyl ghrelin increased motility index in the antrum in dogs (Yin and Chen, 2006). Intracerebroventricular and intravenous exogenous acyl ghrelin increased motility index in the antrum and induced in the duodenum a motor activity pattern resembling that after fasting in conscious fed rats (Fujino et al., 2003) (see Table 2). The effects of intracerebroventricular and intravenous injected acyl

TABLE 2
Differential regulation of ghrelin gene products on gastroduodenal motility in freely moving conscious rats

Drugs	Drug Administration	Status	MI		Frequency of Phase III-Like Contractions	
			Antrum	Duodenum	Antrum	Duodenum
Rat acyl ghrelin	i.v. and i.c.v.	Acutely fed	↑	↑		
		Fasted	N.A.	N.A.	↑	↑
Rat des-acyl ghrelin	i.v. and i.c.v.	Acutely fed			N.A.	N.A.
		Fasted	N.A.	N.A.	↓	
Rat obestatin	i.v.	Acutely fed	↓		↓	
		Fasted	N.A.	N.A.		↓

i.c.v., intracerebroventricular; i.v., intravenous; MI, motility index; N.A., not applicable.

ghrelin were blocked by GHS-R antagonist given via the same route and also were blocked by immunoneutralization of NPY in the brain. Intravenous infusion of acyl ghrelin induced in the antrum and duodenum in vagotomized rats a motor pattern similar to that found after fasting, whereas intracerebroventricular injection of acyl ghrelin failed to affect motor activity, suggesting that once the brain mechanism was eliminated by truncal vagotomy, acyl ghrelin might primarily regulate fasting motor activity through GHS-R1a on the stomach and duodenum (Fujino et al., 2003). Furthermore, intraperitoneal acyl ghrelin injection elicited *c-fos* immunoreactivity in the nucleus of the solitary tract and arcuate nucleus (Chen et al., 2005b), revealing that brain NPY neurons (Fujino et al., 2003), probably via Y₂ or Y₄ receptors (Fujimiya et al., 2008), and vagal afferent pathways, were involved in acyl ghrelin-induced stimulation of a fasting gastrointestinal motor activity pattern (see Table 3). Acyl ghrelin dose-dependently shortened the migrating motor complex cycle length in the duodenum and jejunum through intrinsic cholinergic neurons in awake rats (Edholm et al., 2004). In addition, intraperitoneal exogenous acyl ghrelin administration enhanced antropyloric coordination, which could contribute to accelerated solid gastric emptying induced by acyl ghrelin in rats (Ariga et al., 2008c). Acyl ghrelin-induced gastric phase III-like contractions were also found via vagal cholinergic pathways in mice (Zheng et al., 2009a). A recent study demonstrated that fenfluramine decreased plasma acyl ghrelin concentration and suppressed gastric and duodenal phase III-like contractions and that this could be reversed by intravenous acyl ghrelin or oral TJ-43 (Fujitsuka et al., 2009), also implying that the ghrelin system acted at the down-stream regulation of serotonin system on gastroduodenal motility. Intravenous infusion of acyl ghrelin provoked a premature gastric phase III migrating motor complex in healthy human volunteers that was not mediated through release of motilin (Tack et al., 2006), revealing that the migrating motor complex-inducing ability by acyl ghrelin is present across species. The stimulation of acyl ghrelin on human gastric phase III migrating motor complex was accompanied by increased plasma levels of pancreatic polypeptide and prolonged increased tone of the proximal stomach (Tack et al., 2006).

Exogenous intravenous acyl ghrelin administration stimulated gastric motility (Masuda et al., 2000), gastric myoelectrical activity (in terms of increasing dominant frequency and normal slow wave, and decreasing domi-

nant power), and enhanced liquid gastric emptying (Tümer et al., 2008) in rats. The stimulatory effects on gastric myoelectrical activity and liquid gastric emptying were not fully blocked by subcutaneous pretreatment with atropine sulfate, suggesting that the gastroprokinetic actions of acyl ghrelin are mediated not only by the vagal cholinergic muscarinic pathway but also by intrinsic local pathways via action on as-yet undefined receptors (speculated) and signaling noncholinergic excitatory neurotransmitters in the enteric nervous system in gastric smooth muscle (Tümer et al., 2008). However, intravenous injection of canine acyl ghrelin stimulated GH release in a dose-dependent manner, but did not stimulate the motor activity of the digestive tract in either the fasted or the fed state in dogs (Ohno et al., 2006). Moreover, intravenous high-dose canine acyl ghrelin significantly reduced the motility index in the gastric body in the fasted state. Acyl ghrelin did not accelerate gastric emptying in dogs, either (Ohno et al., 2006). These results differ from previous reports dealing with rodents and remind us to be cautious during interpretation of experimental results obtained in research with larger animals such as dogs.

Central and peripheral application of acyl ghrelin enhanced nutrient solid and non-nutrient semiliquid gastric emptying in mice (Asakawa et al., 2001b; Dornonville de la Cour et al., 2004; Kitazawa et al., 2005; De Winter et al., 2004) and in rats (Trudel et al., 2002; Fukuda et al., 2004; Chen et al., 2005a, 2008b; Depoortere et al., 2005; Levin et al., 2005). Central and peripheral administered acyl ghrelin also stimulated small intestinal transit in rats (Trudel et al., 2002; Fukuda et al., 2004; Chen et al., 2005, 2008b). The ghrelin analog RC-1139 (Poitras et al., 2005) promoted non-nutrient semiliquid gastric emptying in mice (Dornonville de la Cour et al., 2004). Intravenous or subcutaneous administration of acyl ghrelin elicited hunger (in terms of appetite score), accelerated solid meal gastric emptying in normal-weight healthy volunteers and normal-weight patients with GH deficiency (Levin et al., 2006), and increased energy intake and enhanced the perceived palatability of the food offered (pleasantness, in terms of palatability score) in leaner human volunteers and obese subjects (Druce et al., 2005, 2006). The authors also showed that the gastric emptying-stimulating ability of intravenous acyl ghrelin was not mediated via GH and motilin (Levin et al., 2006). The effects of acyl ghrelin on gastric emptying and small intestinal transit are reviewed by Chen et al. (2008c). Taken together, these

TABLE 3
Differential activation of corresponding hypothalamic neurons, brain receptors, and neuroendocrine pathways induced by peripheral effects of ghrelin gene products

Drugs	Hypothalamic Neuron Activated	Brain Receptors Mediated	Vagal Afferent Dependence
Rat acyl ghrelin	NPY	Y ₂ and Y ₄ receptors	Yes
Rat des-acyl ghrelin	CRF	CRF ₂ -R	No
Rat obestatin	CRF and urocortin 2	CRF ₁ -R and CRF ₂ -R	Partially

couple acyl ghrelin with orexigenic, gastroprokinetic, and hedonic-rewarding effects. Solid meal gastric emptying in children with Prader-Willi syndrome was delayed (Choe et al., 2005) and non-nutrient liquid gastric emptying in adults with Prader-Willi syndrome was normal (Hoybye et al., 2007), despite higher acyl ghrelin levels, suggesting that the voracious appetite associated with Prader-Willi syndrome could be something other than the gastric emptying mechanism.

Regarding colonic motility, intracerebroventricular and intraperitoneal injection of acyl ghrelin decreased colonic transit time in conscious fed rats, which was blocked by pretreatment with intracerebroventricular Y_1 receptor antagonist, but not Y_2 receptor antagonist (Tebbe et al., 2005b). Furthermore, acyl ghrelin bilaterally microinjected into the paraventricular nucleus induced a significant stimulation of colonic propulsion, resulting in the shortening of colonic transit time. The enhanced colonic motility induced by microinjection of acyl ghrelin into the paraventricular nucleus was abolished by pretreatment with the nonselective CRF-R antagonist and Y_1 receptor antagonist inside the paraventricular nucleus, but not by the selective CRF₂-R antagonist (Tebbe et al., 2005a). Collectively, acyl ghrelin could act in the central nervous system, including inside the paraventricular nucleus, to modulate colonic motor function using Y_1 receptor and CRF₁-R dependent mechanisms (Fig. 4). In addition, intrathecal application of CP-464709-18 (Carpino et al., 2002; Atcha et al., 2009), a synthetic GHS-R1a agonist, at L6-S1, but not application at pontomedullary levels or to the thoracic spinal cord, elicited propulsive contractions. The stimulation evoked by intravenous CP-464709-18 was prevented if the pelvic nerve outflows were severed, but not if the spinal cord was cut rostral to the defecation center at L6-S3, and was also blocked by hexamethonium (Shimizu et al., 2006). The findings also suggested that

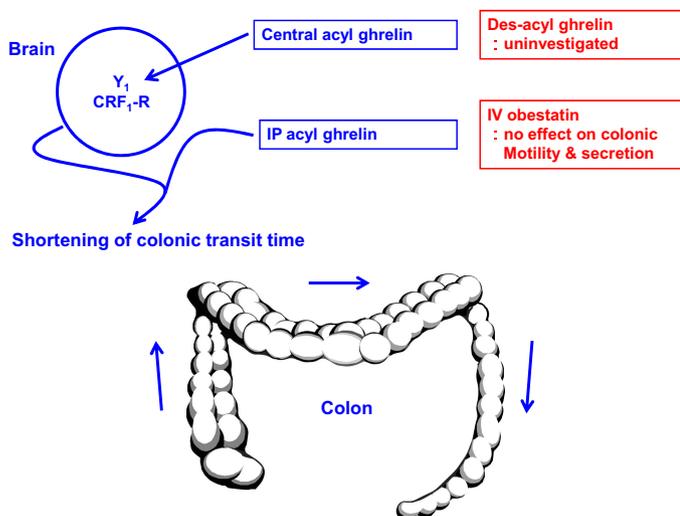


FIG. 4. Differential regulation of acyl ghrelin, des-acyl ghrelin, and obestatin on mammalian colonic motility and secretion. IP, intraperitoneal; IV, intravenous.

activation of GHS-R1a in the lumbosacral spinal cord, via the pelvic nerves, triggered coordinated propulsive contractions that emptied the colorectum. Oral administration of a centrally acting GHS-R1a agonist, GSK894281, to conscious rats also triggered defecation (Shafton et al., 2009). Therefore, oral administration of GHS-R1a agonists that enter the central nervous system could possibly be used to relieve acute cases of constipation or to clear the bowel before colonoscopy.

Conversely, intracerebroventricular and intravenous injection of des-acyl ghrelin decreased gastric phase III-like contractions and disrupted motor activity pattern after fasting in the antrum in conscious rats (Chen et al., 2005b). Intracerebroventricular and intravenous effects of des-acyl ghrelin on stomach motility after fasting were blocked by truncal vagotomy, whereas disruptive effects by intravenous des-acyl ghrelin on stomach motility after fasting were not altered by capsaicin treatment. These results, together with elicitation of *c-fos* immunoreactivity in the arcuate nucleus and paraventricular nucleus (Asakawa et al., 2005; Chen et al., 2005b) without activation of that in nucleus of the solitary tract (Chen et al., 2005b) by intraperitoneal des-acyl ghrelin administration, suggest that the actions of peripheral des-acyl ghrelin could cross the blood-brain barrier and might occur through vagal efferent signaling but independent of vagal afferent pathways. Pretreatment with nonselective CRF-R antagonist and the selective CRF₂-R antagonist, but not CRF₁-R antagonist, blocked the disruptive effects of des-acyl ghrelin on motor activity after fasting in the antrum, indicating that CRF₂-R in the brain mediated this action (Chen et al., 2005b). Intracerebroventricular and intravenous effects of des-acyl ghrelin consistently suppressed solid gastric emptying in mice (Asakawa et al., 2005), whereas intracisternal injection of des-acyl ghrelin inhibited semiliquid gastric emptying in rats (Chen et al., 2005a). The disruptive effects by des-acyl ghrelin on the gastric motility after fasting in the antrum through activation of CART and urocortin 1, then subsequently signaling via CRF₂-R in the brain, could contribute to the delayed semiliquid gastric emptying induced by des-acyl ghrelin in rodents. However, subcutaneous injection of des-acyl ghrelin did not antagonize cholecystinin-induced inhibition of non-nutrient semiliquid gastric emptying in conscious mice (Dornonville de la Cour et al., 2004).

Similar to ingestive behavior, the effects of obestatin on the upper gastrointestinal motility are still debatable. Using a freely moving conscious rat model, intravenous obestatin decreased motility index in the antrum and inhibited phase III-like contractions in the duodenum (Ataka et al., 2008). Immunohistochemical analysis revealed that intravenous obestatin elicited *c-fos* immunoreactivity in the arcuate nucleus, paraventricular nucleus, and nucleus of the solitary tract and activated CRF- and urocortin 2-containing neurons in the brain (Ataka et al., 2008). The effects of peripheral obestatin

on the duodenum, but not on the antrum, were abolished by perivagal capsaicin treatment, whereas intracerebroventricular injection of the selective CRF₁-R and CRF₂-R antagonists blocked the suppressive effects of intravenous obestatin on the antrum and duodenum. Collectively, these results imply that the inhibitory actions of peripheral obestatin on fed gastroduodenal motility required brain CRF₁-R and CRF₂-R, and vagal afferent pathways might be partially involved (Ataka et al., 2008; Fujimiya et al., 2008) (see Tables 2 and 3). In contrast, intraperitoneal obestatin did not affect gastric emptying parameters and did not inhibit the prokinetic effects of acyl ghrelin (De Smet et al., 2007). Mouse and rat intestinal and fundic smooth muscle strips did not respond to obestatin either in the absence or in the presence of electrical field stimulation (De Smet et al., 2007). Lower dose of obestatin (0.1–1 nM) reduced the ability of acyl ghrelin to facilitate electrical field stimulation-evoked contractions of the stomach, but higher concentrations (10–1000 nM) only tended to reduce the response to acyl ghrelin but changes were not statistically significant (Bassil et al., 2007a). Intravenous acyl ghrelin increased liquid gastric emptying and reduced migrating motor complex cycle time, whereas intravenous infusion of obestatin had no effects (Bassil et al., 2007a). Intravenous obestatin failed to prevent intravenous acyl ghrelin-induced shortening of cycle time in terms of duodenal phase III-like contractions (Ataka et al., 2008) and small intestinal migrating motor complex (Bassil et al., 2007a), as well as the increase of motility index in the antrum (Ataka et al., 2008) in rats. In addition, peripheral obestatin failed to affect gastric emptying in *ghrelin*-knockout mice (Depoortere et al., 2008). A novel *in vivo* animal model simultaneously measuring colonic motility and secretion showed that intravenous obestatin administration has no effect on colonic motor and secretory functions in rats, whereas human/rat CRF had stimulatory effects in conscious fed rats (Chen et al., 2008a) (Fig. 4). Taking these data together, obestatin has little ability to modulate gastric emptying, and seems not to be a physiological opponent to counteract acyl ghrelin to modify rodent gastrointestinal motility.

A novel relationship of ingestive behavior and gastric motor function was recently proposed. Fixed feeding increased plasma acyl ghrelin levels, potentiated interdigestive gastric contractions, and accelerated solid gastric emptying (Ariga et al., 2008a), implying that irregular eating habits might impair gastric migrating motor complex and induce postprandial symptoms in humans.

Mice chronically infected with *H. pylori* manifested with higher plasma acyl ghrelin level and delayed gastric emptying rate, whereas eradication of *H. pylori* restored these two abnormalities but not feeding behavior, implying that central nervous system changes might be slow to recover and persist after eradication of the triggering gut infection after chronic infection.

Delayed gastrointestinal transit is a common problem in patients with severe burn. Severe cutaneous burn injury significantly delayed non-nutrient liquid gastric emptying, intestinal transit, and colonic transit compared with sham injury, whereas intraperitoneal application of acyl ghrelin normalized both non-nutrient liquid gastric emptying and intestinal transit, but not the colonic transit in rats (Sallam et al., 2007). Subcutaneous atropine blocked the prokinetic effects of acyl ghrelin on gastric emptying and intestinal transit, suggesting that the prokinetic effects of acyl ghrelin are exerted via the cholinergic pathway (Sallam et al., 2007). Therefore, acyl ghrelin may have a therapeutic potential for clinical burn patients with delayed upper gastrointestinal transit.

Postoperative ileus develops as a consequence of abdominal surgery or other major surgical procedures and is a generalized event involving the entire gastrointestinal tract and is not restricted to the region directly subjected to manipulation. Proposed mechanisms of postoperative ileus include the stimulation of neuronal responses, such as excitation of afferent neurons and activation of noradrenergic and noncholinergic neuronal pathways, and the induction of an intestinal inflammatory response (Greenwood-Van Meerveld, 2007). However, the treatment of postoperative ileus is very limited. To date, GHS-R1a agonists (i.e., acyl ghrelin, acyl ghrelin analog, and acyl ghrelin mimetic) are the only effective drugs for treating postoperative ileus. Intravenous acyl ghrelin significantly reversed postoperative gastric and small intestinal ileus but not colonic ileus in rats (Trudel et al., 2002), whereas high doses of motilin or erythromycin were ineffective, and postoperative gastric ileus was only partially improved by the calcitonin gene-related peptide receptor antagonist CGRP_{8–37}. Subsequent experiments showed that CGRP_{8–37} and acyl ghrelin were potent prokinetics, improving postoperative gastric ileus in dogs (Trudel et al., 2003). Intravenous administration of acyl ghrelin analog RC-1139 reversed not only postoperative gastric ileus but also morphine-induced gastric ileus in rats (Poitras et al., 2005). Single or triple intravenous boluses of TZP-101, a small-molecule GHS-R1a agonist with bioavailability superior to that of acyl ghrelin, were equally effective to abolish abdominal surgery-induced ileus, morphine-induced gastric ileus, small intestinal ileus (Venkova et al., 2007), and colonic ileus (Fraser et al., 2009) in rats. The prokinetic action of TZP-101 was more profound in the stomach than in the small intestine and colon. TZP-101 administration shortened the time to the first bowel movement in rats with postoperative ileus, and restored postsurgery morphine-induced decrease of cumulative fecal pellet output, as measured by the number of fecal pellets and the stool weight, but had no effect on postoperative morphine-induced anorexia and reduced body weight (Fraser et al., 2009). Therefore, TZP-101 may represent a new therapeutic approach for the treatment

of gastric, small intestinal, and colonic dysmotility in patients with postoperative ileus. Repetitive intravenous administration of ipamorelin, a novel synthetic GHS-R1a agonist, significantly increased laparotomy and intestinal manipulation-induced reduction of cumulative fecal pellet output, food intake and body weight in rats (Venkova et al., 2009), implying that postsurgical multiple intravenous bolus infusion of ipamorelin might ameliorate the symptoms in patients with postoperative ileus.

Meal gastric emptying was delayed in patients with functional dyspepsia (Takamori et al., 2007; Lee et al., 2009). However, plasma ghrelin levels showed no significant correlation with delayed gastric emptying (Takamori et al., 2007; Lee et al., 2009) or psychological disorders in these patients. Investigation with more subjects is necessary to determine the true roles of acyl and des-acyl ghrelin in functional dyspepsia.

IX. Access of Ghrelin Gene Products to the Central Nervous System: Direct and Indirect Neuroendocrine Pathways

Blood-born acyl ghrelin, secreted by gastric X/A cells, was originally thought to stimulate food intake by acting in the hypothalamic arcuate nucleus, which is devoid of blood-brain barrier, based on the presence of GHS-R1a there and the fact that acyl ghrelin, injected directly into the arcuate nucleus, induced eating (Wren et al., 2001b; Bagnasco et al., 2003; Currie et al., 2005). Rats with lesions in the area postrema, a caudal brain stem center devoid of blood-brain barrier, did not respond to peripheral acyl ghrelin-induced stimulation of food intake (Gilig and Lutz, 2006). A recent patch-clamp electrophysiological study showed that acyl ghrelin exerted a direct effect on electrical activity of neurons in the area postrema, another brain region lacking the blood-brain barrier, supporting the notion that acyl ghrelin could act via the area postrema to regulate feeding and energy homeostasis (Fry and Ferguson, 2009). In addition, electrophysiological recordings found that acyl ghrelin stimulated the activity of arcuate NPY neurons and mimicked the effect of NPY in the paraventricular nucleus of the hypothalamus, revealing that release of acyl ghrelin at the arcuate nucleus and hypothalamic paraventricular nucleus might stimulate the release of orexigenic peptides and neurotransmitters, thus representing a regulatory circuit controlling energy homeostasis (Cowley et al., 2003). Administration of acyl ghrelin into the three other brain sites, including the hypothalamic paraventricular nucleus (Wren et al., 2001b; Olszewski et al., 2003a; Currie et al., 2005), the ventral tegmental area (Naleid et al., 2005; Abizaid et al., 2006; Jerlhag et al., 2007), and the dorsal vagal complex (Faulconbridge et al., 2003), also enhanced feeding. Furthermore, GHS-R1a mRNA is present in numerous other brain regions in which the effects of acyl ghrelin have not been ex-

plored (Zigman et al., 2006). Central acyl ghrelin injection, including ventricular, lateral hypothalamus, and paraventricular nucleus, recruiting oxytocin and orexin neurons (Olszewski et al., 2003b, 2007), induced feeding driven by energy needs not reward, as evidenced by increased intake of chow but not sucrose in rats treated with central acyl ghrelin (Bomberg et al., 2007). Pharmacokinetic studies also identified the saturable systems transported human acyl ghrelin from brain to blood and from blood to brain, whereas mouse acyl ghrelin was a substrate for the brain-to-blood transporter but not for the blood-to-brain transporter (Banks et al., 2002) (also see Fig. 3).

Several studies suggested that peripheral acyl ghrelin stimulates feeding via a vagally mediated abdominal action. First, electrophysiologic evidence demonstrated that acyl ghrelin directly suppressed firing of gastric vagal nerves (Asakawa et al., 2001b; Date et al., 2002) and inhibited visceral afferent activation of cholecystokinin-sensitive catecholamine neurons in the solitary tract nucleus (Cui and Appleyard, 2009). Second, either total subdiaphragmatic vagotomy, selective gastric vagotomy, or perivagal capsaicin application blocked the feeding-stimulatory effect of intravenous or intraperitoneal injection of acyl ghrelin in rats (Date et al., 2002; Chen et al., 2005b) and mice (Asakawa et al., 2001b). Third, intravenous administration of acyl ghrelin stimulated GH secretion, but failed to enhance energy intake in patients who had undergone vagotomy as a result of lower esophageal or gastric surgery (le Roux et al., 2005a). Finally, peripheral acyl ghrelin elicited *c-fos* immunoreactivity in the arcuate nucleus (Date et al., 2002, 2005; Chen et al., 2005b; Kobelt et al., 2005; Inhoff et al., 2008), paraventricular nucleus (Chen et al., 2005b), and solitary tract nucleus (Date et al., 2005), whereas capsaicin treatment or coadministration of cholecystokinin abolished acyl ghrelin-induced neuronal activity in either the arcuate nucleus (Date et al., 2002; Kobelt et al., 2005) or solitary tract nucleus (Date et al., 2005). Collectively, these results support the important roles of peripheral acyl ghrelin in the activation of vagal afferent-solitary tract nucleus-arcuate nucleus/paraventricular nucleus pathways in stimulating food intake. However, another two studies showed that vagal afferents were not necessary for eating-stimulatory effect of intraperitoneal injected acyl ghrelin (Arnold et al., 2006), and hindbrain catecholamine neurons only contributed to the GH but not the feeding response to peripheral acyl ghrelin (Emanuel et al., 2009) in rats. Hence, more evidence is required to validate the role of vagal afferent nerves in mediating GH-releasing and appetite-stimulating effects.

Hypophysectomy prevented acyl ghrelin-induced adiposity and increased gastric ghrelin secretion in rats, suggesting a negative gastro-hypophyseal regulatory feedback loop involving the stomach and the pituitary to regulate gastric ghrelin secretion (Tschöp et al., 2002).

Arcuate nucleus neurons, with projections to the median eminence, are well known to be responsive to a wide array of hormones and nutrients, including leptin, insulin, gonadal steroids, and glucose and to act as conduits or conditional pacemakers for diverse signals relevant to feeding and energy homeostasis (Cone et al., 2001; van den Top et al., 2004). Hence, reciprocal afferent humoral signals, composed of anorexigenic leptin from white adipose tissue and orexigenic acyl ghrelin from stomach, to the arcuate nuclei integrate the moment-to-moment regulation of energy homeostasis (Kalra and Kalra, 2004). Whole-cell patch-clamp recordings from rat arcuate nucleus neurons in hypothalamic slice preparations revealed widespread expression of functional ATP-sensitive potassium channels within the nucleus (van den Top et al., 2007). ATP-sensitive potassium channels were expressed in orexigenic NPY/AgRP and ghrelin-sensitive neurons and in anorexigenic CART neurons, indicating a crucial role for these ion channels in central sensing of metabolic and energy status (van den Top et al., 2007). Several *in vivo* lines of evidence, using either chemical ablation (Tamura et al., 2002) or vector-mediated ablation (Bewick et al., 2005) of NPY/AgRP neurons, or *NPY(-/-)/AgRP(-/-)* double knockout techniques (Chen et al., 2004), suggest that the orexigenic action of acyl ghrelin is mediated through NPY/AgRP neurons in the arcuate nucleus, because these NPY/AgRP neuron-manipulated animals, which were often lean and of a hypophagic phenotype, failed to respond to the feeding-stimulating effects of peripheral acyl ghrelin. However, NPY/AgRP neurons are not essential for feeding responses to glucoprivation (Luquet et al., 2007). Central Y_1 , Y_5 , and melanocortin receptors are responsible for central acyl ghrelin-induced hyperphagic effect in rats (Nakazato et al., 2001), whereas this orexigenic action is mediated via central Y_1 but not Y_5 receptors in mice (Asakawa et al., 2001b). MC_3 -R and MC_4 -R are involved in acyl ghrelin-induced feeding behavior, based on the fact that intracerebroventricular melanotan II, a synthetic $MC_{3/4}$ -R agonist, completely blocked food deprivation- and peripheral acyl ghrelin-induced increase in food intake and attenuated food deprivation- and peripheral acyl ghrelin-induced increase in food hoarding in Siberian hamsters (Keen-Rhinehart and Bartness, 2007); in addition, the effects of intraperitoneal acyl ghrelin on food intake were reduced in MC_3 -R and MC_4 -R double-knockout mice, and female MC_3 -R and MC_4 -R double-knockout mice exhibited a diminished responsiveness to the GH-releasing effects of intraperitoneal acyl ghrelin (Shaw et al., 2005). In addition, incubation of the hypothalamic explants with acyl ghrelin significantly increased NPY and AgRP mRNA expression in the presence, but not absence, of dexamethasone *in vitro* (Goto et al., 2006). The stimulatory effects of acyl ghrelin on NPY gene expression were abolished in the presence of cycloheximide, which blocked translation, suggesting that *de novo* protein synthesis is required for

ghrelin action (Goto et al., 2006). Furthermore, short- and long-term central infusion of acyl ghrelin increased NPY, AgRP, and somatostatin mRNA expression in the arcuate nucleus (Kamegai et al., 2001; Seoane et al., 2003). Mice with an AgRP neuron-specific deletion of vesicular GABA transporter were lean, resistant to obesity, and had an attenuated hyperphagic response to acyl ghrelin, elucidating that GABA release from AgRP neurons is important in regulating acyl ghrelin-mediated food intake and energy balance (Tong et al., 2008). Acyl ghrelin is known to activate hypothalamic AMPK, a crucial metabolic sensor controlling energy balance. A recent study showed that CaMKK2 mediated this effect by forming a unique complex of AMPK α/β with acetyl-CoA carboxylase in a pathway distinct from the more established AMP/LKB1 pathway (Anderson et al., 2008). Short-term pharmacologic inhibition of CaMKK2 in wild-type mice, but not *CaMKK2*-null mice, inhibited appetite and promoted weight loss consistent with decreased NPY and AgRP mRNAs (Anderson et al., 2008). In mice, acyl ghrelin was found to initiate robust changes in hypothalamic mitochondrial respiration that were dependent on UCP2, whereas activation of this mitochondrial mechanism was critical for acyl ghrelin-induced mitochondrial proliferation and electric activation of NPY/AgRP neurons, for acyl ghrelin-triggered synaptic plasticity of POMC-expressing neurons, and for acyl ghrelin-induced food intake (Andrews et al., 2008). The UCP2-dependent action of acyl ghrelin on NPY/AgRP neurons was driven by a hypothalamic fatty acid oxidation pathway involving AMPK, carnitine palmitoyltransferase 1, and free radicals that were scavenged by UCP2, revealing a signaling modality connecting mitochondria-mediated effects of GPR on neuronal function and associated ingestive behavior (Andrews et al., 2008). Taken together, these results extend our understanding of the molecular basis and intracellular mechanisms, certainly including the imperative roles of NPY/AgRP neurons in the arcuate nucleus and hypothalamic AMPK/UCP2 system involved in the effect of acyl ghrelin on food intake and GH secretion.

Des-acyl ghrelin does not bind to GHS-R1a, but it crosses the blood-brain barrier to the brain by a nonsaturable transmembrane diffusion manner (Banks et al., 2002). Several results support the idea that peripheral des-acyl ghrelin, also secreted by gastric X/A cells, acts directly in the brain to inhibit feeding. First, intraperitoneal administration of des-acyl ghrelin suppressed food intake in lean mice and rats (Asakawa et al., 2005; Chen et al., 2005b), and food intake and body weight gain in obese Zucker rats (Stengel et al., 2008). Second, perivagal capsaicin application did not abolish the food-inhibitory effect of intraperitoneal administration of des-acyl ghrelin in rats (Chen et al., 2005b). Third, intraperitoneal injection of des-acyl ghrelin induced mRNA expression of CART and urocortin 1 in mice (Asakawa et al., 2005), which are both anorectic neu-

ropeptides. Fourth, peripheral des-acyl ghrelin elicited *c-fos* immunoreactivity in the arcuate nucleus (Asakawa et al., 2005; Chen et al., 2005b; Stengel et al., 2008) and paraventricular nucleus (Asakawa et al., 2005; Chen et al., 2005b; Stengel et al., 2008) without change in the solitary tract nucleus (Chen et al., 2005b), indicating that the activation of des-acyl ghrelin on brain neurons was devoid of a vagal afferent nerve-solitary tract nucleus pathway. Fifth, intraperitoneal des-acyl ghrelin attenuated acyl ghrelin-induced *c-fos* immunoreactivity in the arcuate nucleus in lean rats (Inhoff et al., 2008). Sixth, pharmacological blockage revealed CRF₂-R to be responsible for the activation of des-acyl ghrelin actions on the brain. Seventh, immunofluorescence double staining confirmed colocalization of *c-fos* and CRF in the paraventricular nucleus in the hypothalamus by intraperitoneal injection of des-acyl ghrelin in rats (Chen et al., 2005b). Finally, pharmacokinetic experiments detected that des-acyl ghrelin entered the brain by nonsaturable transmembrane diffusion and was sequestered once within the central nervous system to mediate its actions there (Banks et al., 2002).

The biological actions and signaling pathway of obestatin are still debatable. Intravenous obestatin elicited *c-fos* immunoreactivity in the arcuate nucleus, paraventricular nucleus, and solitary tract nucleus (Ataka et al., 2008), implying that the vagal afferent pathway was involved, at least partially, in actions of peripheral obestatin activating the brain neurons. Pharmacological blockage revealed both CRF₁-R and CRF₂-R responsible for the activation of obestatin actions on the brain. Immunohistochemical analysis demonstrated that CRF- and urocortin-2-containing neurons in the paraventricular nucleus in the hypothalamus were activated by intravenous injection of obestatin in rats (Ataka et al., 2008; Fujimiya et al., 2008). However, another study claimed that intraperitoneal administration of obestatin did not elicit *c-fos* immunoreactivity in ingestive behavior-relevant brain nuclei in rodents (Kobelt et al., 2008). The rapid intracellular degradation and major instability of obestatin in the plasma should be taken into account during data interpretation (Pan et al., 2006; Vergote et al., 2008).

X. Direct Effects of Ghrelin Gene Products on Other Tissues

A. Glucose Metabolism

A previous study indicated that acyl ghrelin possessed insulin-like action stimulating the receptor substrate 1–growth factor receptor-bound protein 2–mitogen-activated protein kinase pathway and anti-insulin action suppressing Akt activity and up-regulation of gluconeogenesis in hepatoma cell lines (Murata et al., 2002). This raises the possibility of acyl ghrelin's involvement in the regulation of glucose homeostasis and insulin secretion. Acyl ghrelin induced several genes, including IA-2 β , a

pancreas β -cell autoantigen for type 1 diabetes (Doi et al., 2006). Administration of acyl ghrelin or overexpression of IA-2 β inhibited glucose-stimulated insulin secretion in MIN6 insulinoma cells. Furthermore, inhibition of IA-2 β expression by the RNA interference technique ameliorated acyl ghrelin-related inhibitory effects on glucose-stimulated insulin secretion (Doi et al., 2006), implying the link among acyl ghrelin, IA-2 β , and glucose-stimulated insulin secretion. In vitro, acyl ghrelin elicited glucose output whereas des-acyl ghrelin suppressed glucose release by primary hepatocytes (Gauna et al., 2005). In addition, des-acyl ghrelin was able to antagonize acyl ghrelin-induced glucose output through a GHS-R1a independent pathway (Gauna et al., 2005), suggesting that acyl and des-acyl ghrelin could be separate hormones able to modulate hepatic glucose metabolism by acting directly on the liver.

Exogenous injection of acyl ghrelin at specific doses inhibited intravenous glucose-stimulated insulin secretion in mice by a direct action on the islets, most likely by a distal action on the β -cell signaling machinery (Reimer et al., 2003). GHS-R1a antagonist, [D-Lys³]GHRP-6, and immunoneutralization of endogenous acyl ghrelin enhanced glucose-induced insulin release from perfused pancreas, whereas exogenous acyl ghrelin suppressed it ex vivo (Dezaki et al., 2006). In addition, acyl ghrelin inhibited insulin release from islets isolated from mice and rat pancreas, whereas des-acyl ghrelin blunted acyl ghrelin's effect at a 10-fold higher concentration ex vivo (Qader et al., 2008). Acyl ghrelin has been reported to activate GHS-R1a of pancreatic β -cells, which is coupled with pertussis toxin-sensitive heterotrimeric G-protein G α_{i2} , which in turn attenuates membrane excitability via activation of voltage-dependent K⁺ channels (Kv2.1) and eventually suppresses Ca²⁺ influx and insulin secretion ex vivo (Dezaki et al., 2004, 2007) (Fig. 5).

Exogenous acyl ghrelin administration produced higher blood glucose and marked lower insulin levels after intraperitoneal glucose injection in mice, implying that short-term administration of acyl ghrelin might suppress insulin release in vivo (Sun et al., 2006). Acyl ghrelin infused into the portal vein inhibited glucose-stimulated insulin secretion in anesthetized rats, whereas the effect was attenuated by either hepatic vagotomy or coinfusion with atropine methyl bromide, supporting the inhibitory effect of exogenous acyl ghrelin on glucose-induced insulin release via the vagus nerve (Cui et al., 2008). The decrease in acyl ghrelin level after a meal might be important for the occurrence of the incretin effect in rats (Cui et al., 2008). Subsequent human studies demonstrated that exogenous acyl ghrelin exerted effects at a similar level to decrease plasma insulin and increase plasma glucose levels in young and elderly normal volunteers (Broglia et al., 2001, 2003a); administration of acyl ghrelin was followed by transient insulin release during oral free fatty acid loading, whereas acyl ghrelin blunted the insulin

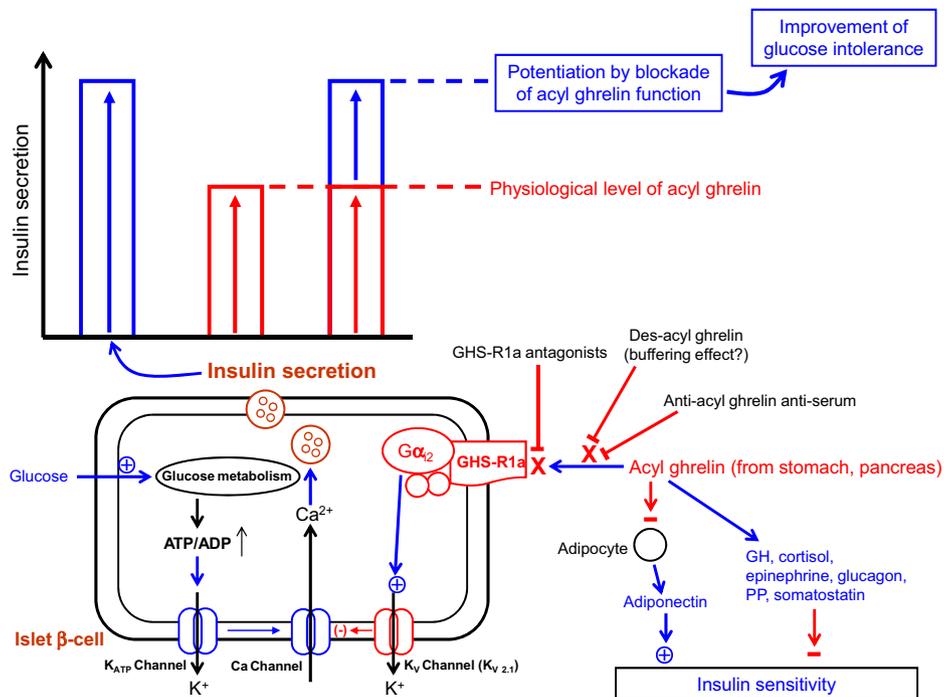


FIG. 5. Acyl ghrelin signal pathway in pancreatic β -cell and insulinostatic function of endogenous acyl ghrelin on islets. Acyl ghrelin of pancreas origin interacts with GHS-R1a in β -cell in a paracrine/autocrine manner. Acyl ghrelin attenuates glucose-induced insulin release, and thus determines the physiological level of insulin secretion. Acyl ghrelin also interacts with GH, cortisol, epinephrine, and possibly glucagon, pancreatic polypeptide (PP), and somatostatin, as well as adiponectin from adipocytes.

response to arginine and enhanced arginine-induced increase in glucose level in healthy humans (Broglia et al., 2003b). Intravenous infusion of acyl ghrelin suppressed plasma insulin concentration with hyperglycemia in normal weight healthy volunteers (Arosio et al., 2003). Supraphysiological acyl ghrelin levels impaired human insulin sensitivity (Vestergaard et al., 2007b). The above four human studies support the existence of a functional link between acyl ghrelin and the endocrine pancreas. By contrast, des-acyl ghrelin administration potently and dose-dependently increased insulin concentration induced by intravenous glucose tolerance test in the portal, and, to a lesser extent, the systemic circulation in anesthetized fasted rats (Gauna et al., 2007a). The des-acyl ghrelin-induced stimulation of insulin secretion was completely blocked by coadministration of exogenous acyl ghrelin at equimolar concentrations. Likewise, [D-Lys³]GHRP-6, alone or in combination with acyl ghrelin and des-acyl ghrelin, enhanced the portal insulin response to intravenous glucose tolerance test, whereas exogenous acyl ghrelin alone exert no further effect in vivo (Gauna et al., 2007a). In patients with GH deficiency, intravenous administration of acyl ghrelin reduced insulin sensitivity, whereas combination of acyl plus des-acyl ghrelin strongly improved insulin sensitivity (Gauna et al., 2004). Furthermore, intravenous acyl ghrelin increased plasma glucose and decrease plasma insulin levels, whereas des-acyl ghrelin coadministered with acyl ghrelin abolished these effects in healthy volunteers (Broglia et al., 2004b), suggesting that des-acyl

ghrelin counteracts the metabolic response to acyl ghrelin in humans. Most recently, continuous exposure to acyl ghrelin in humans enhanced somatotroph secretion but also worsened glucose metabolism, although it inhibited lipolysis (Broglia et al., 2008). Coadministration of acyl ghrelin and des-acyl ghrelin as a single intravenous bolus injection caused a significant decrease in insulin concentration in nondiabetic subjects suffering from morbid obesity, a condition characterized by insulin resistance and low GH levels, whereas glucose concentration did not change in the first hour after combination administration of acyl ghrelin and des-acyl ghrelin (Kiewiet et al., 2009), suggesting a strong improvement in insulin sensitivity. Further human studies in which des-acyl ghrelin with or without acyl ghrelin administered for a longer time are warranted. Hence, des-acyl ghrelin, or more likely the acyl versus des-acyl ghrelin ratio, may be implicated in the regulation of insulin release. Des-acyl ghrelin may act as a secretagogue of insulin by removing the suppressive tone of acyl ghrelin on pancreatic β -cells and subsequently stimulate insulin secretion from pancreatic islets. The other speculation is that the relative increase of des-acyl ghrelin fraction in the peripheral circulation reflects the buffering of acyl ghrelin metabolic actions, thus improving peripheral insulin sensitivity (Gauna et al., 2007b). Therefore, des-acyl ghrelin might be of therapeutic value to treat insulin resistance and impaired insulin release in patients with type 2 diabetes.

On the other hand, short-term nutritional changes, such as short-term enteral or parenteral administration, may differentially regulate acyl and des-acyl ghrelin concentrations. For example, a short-term intravenous glucose load in anesthetized fasted rats inhibited des-acyl ghrelin in portal and systemic circulation, whereas it blunted acyl ghrelin concentration in only prehepatic but not systemic circulation (Gauna et al., 2007b). Secretion of total ghrelin was inhibited by glucose load, via either oral (Nakagawa et al., 2002; Broglio et al., 2004b) or intravenous (Nakagawa et al., 2002) route, but was unaffected by intravenous glucagons and arginine in humans.

The data concerning the influence of obestatin on glucose metabolism are controversial. Exogenous obestatin acted as a potent inhibitor of insulin secretion (similar to acyl ghrelin) under glucose-stimulated conditions in anesthetized fasted rats in vivo and in cultured islets in vitro (Ren et al., 2008). Exogenous obestatin was shown to inhibit insulin release from mouse and rat pancreas isolated islets ex vivo (Qader et al., 2008). Intraperitoneal administration of obestatin inhibited feeding-induced elevation of plasma glucose and insulin levels in mice but exhibited no direct actions on glucose homeostasis or insulin secretion (Green et al., 2007). However, another study indicated that intravenous obestatin affected neither basal nor intravenous glucose-stimulated insulin secretion and glucose metabolism in the systemic and portal circulation of fasted rats (Kiewiet et al., 2008). Furthermore, incubation with obestatin did not alter insulin release from pancreatic islets and modify blood glucose (Unniappan et al., 2008), which did not support the notion that obestatin was a hormone with metabolic actions. On the contrary, obestatin increased insulin secretion in both absence and presence of glucose, stimulated the expression of main regulatory β -cell genes, and promoted β -cell and human islet cell survival in vitro (Granata et al., 2008). A recent study demonstrated that obestatin exerted a dual effect on glucose-induced insulin secretion in a perfused rat pancreas model: obestatin potentiated the insulin response to glucose at a low concentration, whereas it inhibited the insulin release evoked by glucose stimulus (Egido et al., 2009). The discrepancy regarding obestatin modulating glucose metabolism and insulin action needs further investigation.

Genetic studies also support the involvement of acyl ghrelin, des-acyl ghrelin, and GHS-R, in the regulation of glucose metabolism. First, *acyl ghrelin*-knockout mice displayed enhanced glucose-induced insulin release from isolated pancreatic islets ex vivo, whereas islet density, size, insulin content, and insulin mRNA levels were unaltered (Dezaki et al., 2006). Second, *acyl ghrelin* knockout mice had higher plasma insulin and lower blood glucose levels after intraperitoneal glucose injection and possessed a higher glucose disposal rate in hyperinsulinemic-euglycemic clamp studies compared

with wild-type mice (Sun et al., 2006). Third, insulin-induced blood-glucose-lowering effect was greatly enhanced in mice lacking acyl ghrelin compared with wild-type control mice (Sun et al., 2006). Fourth, deletion of *acyl ghrelin* exhibited lower fasting blood glucose, higher fasting plasma C-peptide, and higher postprandial plasma insulin level in *ob/ob* mice (Sun et al., 2006). Fifth, double knockout of *acyl ghrelin* and *leptin* manifested higher plasma insulin secretion and prevented hyperglycemia induced by intraperitoneal glucose injection, compared with *ob/ob* mice (Sun et al., 2006). The authors suggested that ablation of acyl ghrelin rescued diabetic phenotype of *ob/ob* mice through augmenting glucose-dependent insulin secretion from the pancreas β -cells by reducing pancreatic *Ucp2* expression and improving peripheral insulin sensitivity (Sun et al., 2006). In another two studies, absence of acyl ghrelin protected against hyperinsulinemia and hyperglycemia induced by a high-fat diet (Wortley et al., 2005; Dezaki et al., 2006). In addition, mice lacking GHS-R demonstrated lower blood glucose and serum insulin compared with wild-type control mice (Zigman et al., 2005; Longo et al., 2008; Sun et al., 2008) and greater "metabolic flexibility" under diet-induced metabolic stress in terms of utilization of energy (Longo et al., 2008). Furthermore, blood glucose levels fell in wild-type mice and in both *acyl ghrelin*-null and *GHS-R*-null genotypes, but the drop in blood glucose was significantly greater in *acyl ghrelin* knockout and *GHS-R* knockout mice than in their wild-type littermates during negative energy balance by 50% caloric restriction (Sun et al., 2008). On the other hand, age-related glucose intolerance was observed in a gain-of-function genetic model of increased circulating acyl ghrelin in transgenic mice (Reed et al., 2008). More recently, another study using transgenic mice indicated that overexpression of plasma acyl ghrelin was associated with hyperphagia, increased energy expenditure, glucose intolerance, decreased glucose-stimulated insulin secretion, and reduced leptin sensitivity (Bewick et al., 2009). Collectively, acyl ghrelin does play a fundamental role in regulating pancreatic β -cell function by inhibiting glucose-stimulated insulin release. The results imply that strategies designed to antagonize acyl ghrelin action may reduce appetite and improve glucose homeostasis (Bewick et al., 2009). By contrast, transgenic mice overexpressing des-acyl ghrelin exhibited lower blood glucose (Zhang et al., 2008b) and lower plasma insulin (Iwakura et al., 2005) levels after intraperitoneal glucose injection and had a greater hypoglycemic response to insulin administration (Zhang et al., 2008b) than control animals. However, other studies indicated that transgenic mice overexpressing des-acyl ghrelin showed small phenotype (Ariyasu et al., 2005; Asakawa et al., 2005), and tendencies with lower blood glucose and plasma insulin levels. It is conceivable that these genetic data imply the differential roles of acyl versus des-acyl ghrelin: acyl ghrelin may inhibit insulin

release, decrease insulin sensitivity, and predispose to hyperglycemia, whereas des-acyl ghrelin may enhance insulin sensitivity and maintain a euglycemic condition.

In humans, acyl ghrelin peaks anticipated food episodes and were coupled with the lowest insulin levels, whereas food intake was followed by a prompt decrease in ghrelin coupled with an increase in insulin levels (Cummings et al., 2001), supporting a negative functional relationship between insulin and ghrelin secretion under normal physiologic conditions (Purnell et al., 2003; Cummings et al., 2004). An *ex vivo* study from a novel organ-culture model of gastric tissue explants from rat donors also confirmed this negative association: fasting induced elevation of plasma ghrelin level and suppression of plasma insulin concentration, whereas refeeding or “tease feeding” reversed them (Seoane et al., 2007b). A human study revealed that acyl ghrelin was negatively correlated with circulating insulin levels across all meals, and they suggested that insulin was a key determinant of meal-induced acyl ghrelin suppression (Tannous et al., 2006). However, plasma acyl and total ghrelin levels were enhanced in rats with streptozotocin-induced diabetes, suggesting adaptation to the negative energy balance condition (Masaoka et al., 2003). Likewise, plasma fasting acyl ghrelin level was increased, whereas des-acyl ghrelin level was decreased in patients with obesity-related type 2 diabetes compared with lean subjects (Rodríguez et al., 2009). Moreover, plasma total ghrelin level negatively correlated with hemoglobin A_{1c} in patients with diabetes, suggesting that long-term poor glycemic control might impair ghrelin secretion (Ueno et al., 2007). A recent study indicated that metformin therapy prolonged the postprandial fall of plasma total ghrelin concentration and thus had concomitant effects on appetite in type 2 diabetes, contributing to its actions in promoting weight loss and attenuating weight gain in these patients (English et al., 2007).

Overall, the ghrelin system plays a pivotal role in the entero-insular axis. Acyl ghrelin influences glucose metabolism not only from its endocrine effect, but also from its direct effects on hepatocytes by stimulating hepatic glucose production (Murata et al., 2002; Gauna et al., 2005). In addition, acyl ghrelin inhibited secretion of the insulin-sensitizing protein adiponectin from adipocytes (Ott et al., 2002) and stimulated release of the counter-regulatory hormones, including GH (Broglia et al., 2001, 2003a, 2004c), cortisol, epinephrine (Broglia et al., 2003a, 2004c), and possibly glucagon, pancreatic polypeptide, and somatostatin (Qader et al., 2008) (Fig. 5). The effects of acyl ghrelin on glucose metabolism and insulin secretion should be mediated by GHS-R1a, whereas those of des-acyl ghrelin might be mediated through a receptor other than GHS-R1a (Soares and Leite-Moreira, 2008). In clinical practice, bariatric surgery is a promising mode to resolve human type 2 diabetes mellitus (Vetter et al., 2009). Diabetes resolves 84

to 98% after bypass procedure and 48 to 68% for restrictive procedure. Bariatric surgery alters secretion of gut hormones, resulting in enhanced insulin secretion and improved glycemic control in humans (Vetter et al., 2009). However, effects of gastric bypass on ghrelin levels are inconsistent (Vetter et al., 2009). Two recent studies pointed out that measurement of total ghrelin did not adequately reflect acyl ghrelin and des-acyl ghrelin concentrations, which highlights the importance of evaluation of acyl ghrelin and des-acyl ghrelin using specific two-site assays (Mackelvie et al., 2007; Liu et al., 2008). Hence, further studies, particularly using state-of-art technique to separately measure acyl ghrelin, des-acyl ghrelin, and obestatin, are necessary to clarify this controversial issue, and facilitate refinement of surgical procedures and development of drugs with the same effects but without using a “knife.”

B. Lipid Metabolism

Des-acyl ghrelin, acyl ghrelin, short acyl ghrelin fragments, and synthetic GHS were shown to act directly as antilipolytic factors on the adipose tissue *in vitro* through binding to a specific receptor that is distinct from GHS-R1a (Muccioli et al., 2004). Acyl and des-acyl ghrelin were expressed in human abdominal-subcutaneous adipose tissue (Kos et al., 2009). Both acyl and des-acyl ghrelin mediated fat deposition in part via Y₁ receptor, whereas des-acyl ghrelin affected lipolysis, lipogenesis, and leptin secretion (Kos et al., 2009). The results suggest the potential importance of the gut-fat-brain axis in determining ghrelin's effects on lipid metabolism. In differentiating omental adipocytes, incubation with both acyl and des-acyl ghrelin significantly increased peroxisome proliferator-activated receptor γ and sterol-regulatory element binding protein-1 mRNA levels, as well as fat storage-related proteins, including acetyl-CoA carboxylase, fatty acid synthase, lipoprotein lipase, and perilipin (Rodríguez et al., 2009). Consequently, both acyl and des-acyl ghrelin stimulated intracytoplasmic lipid accumulation (Rodríguez et al., 2009). A study indicated that both cannabinoids and acyl ghrelin stimulated AMPK activity in the hypothalamus and the heart but inhibited AMPK in liver and adipose tissue (Kola et al., 2005). The activation of AMPK through phosphorylation resulted in decreased hypothalamic levels of malonyl-CoA and increased carnitine palmitoyltransferase 1 (López et al., 2008). These novel effects of cannabinoids and acyl ghrelin on AMPK provide a mechanism for an increase in adipose tissue, in addition to the well known stimulation of appetite. The effects of ghrelin on lipogenesis (lipogenic) and carbohydrate metabolism (diabetogenic) can also be explained by its ability to activate AMPK.

Subcutaneous injection of acyl ghrelin in rats induced tissue-specific changes in mitochondrial and lipid metabolism genes favoring triglyceride deposition in liver over skeletal muscle (Barazzoni et al., 2005). Long-term

intravenous infusion of acyl ghrelin, but not des-acyl ghrelin, induced a depot-specific increase in white adipose tissue in retroperitoneal and inguinal regions and promoted hepatic steatosis in rats by a GHS-R1a-dependent lipid retention mechanism (Davies et al., 2009). Direct injection of acyl and des-acyl ghrelin, but not GHS-R1a agonist (L-163,255), into the right tibial bone marrow cavity promoted bone marrow adipogenesis in rats (Thompson et al., 2004). The *in vivo* experiment demonstrated that the direct adipogenic effects of acyl and des-acyl ghrelin were mediated by a receptor other than GHS-R1a and were independent of GH secretion. Central acyl ghrelin is of physiological relevance in the control of cell metabolism in adipose tissue, because it induces adiposity (Tschöp et al., 2000) and stimulates lipid storage (Theander-Carrillo et al., 2006) independently from acyl ghrelin-induced hyperphagia and seems to be mediated by the sympathetic nervous system (Theander-Carrillo et al., 2006). Most recently, central acyl ghrelin was demonstrated to regulate peripheral lipid metabolism in a GH-independent fashion (Sangiao-Alvarellos et al., 2009). These results imply a new central nervous system-based neuroendocrine circuit regulating metabolic homeostasis of adipose tissue.

On the other way, free fatty acids in physiological range had an independent suppressive effect on plasma total ghrelin level in humans, serving as a negative feedback control, whereas this effect was evident with intra-arterial free fatty acid concentration between 0 and 1 nM (Gormsen et al., 2006). Moreover, neither GH nor GHS-R blockade (using pegvisomant) had any effect on circulating total ghrelin concentrations (Gormsen et al., 2007).

Regarding obestatin, the N-terminal (residues 1–13) reduced cholesterol and triglyceride levels and perirenal and epididymal fat, whereas the middle fragment (residues 6–18) reduced triglyceride levels and epididymal fat in mice (Nagaraj et al., 2008).

C. Cardiovascular System

Widespread expression of ghrelin and GHS-R1a was shown in the human cardiovascular system (Kleinz et al., 2006). Therefore, it is intriguing to speculate about the link among ghrelin, obesity, and major cardiovascular comorbidities, such as hypertension, atherosclerosis, and heart disease. Acyl ghrelin has been demonstrated to exhibit direct vasodilatory effects in humans. Short-term intra-arterial infusion of human acyl ghrelin increased forearm blood flow in healthy volunteers in a dose-dependent manner (Okumura et al., 2002). Short-term intravenous infusion of acyl ghrelin decreased mean arterial pressure, increased cardiac index and stroke volume index, and elicited GH, prolactin, adrenocorticotropin, cortisol, and epinephrine secretion, but with no alteration in heart rate in healthy subjects (Nagaya et al., 2001a). These direct vasodilatory effects were through endothelium-independent, antagonizing

in vitro vasoconstrictory action of endothelin-1 (Wiley and Davenport, 2002) and *in vivo* GH/IGF-1/nitric oxide-independent (Okumura et al., 2002) mechanisms. Micro-injection of both acyl and des-acyl ghrelin in the nucleus tractus solitarius decreases blood pressure in rats (Lin et al., 2004; Tsubota et al., 2005). Subcutaneous injection of acyl ghrelin attenuated the progression of long-term hypoxia-induced pulmonary hypertension in rats involving down-regulation of endothelin-1 and prevention of impaired NO-mediated vasodilation and thickened wall-to-lumen ratio in peripheral pulmonary arteries (Schwenke et al., 2008b). These results indicate that acyl ghrelin may be an effective prophylactic therapy for attenuating the adverse responses to chronic hypoxia.

Two endogenous ligands of GHS-R, acyl ghrelin and des-Gln¹⁴-ghrelin, as well as des-acyl ghrelin, increase tension of guinea pig papillary muscle *ex vivo* (Bedendi et al., 2003). Ghrelin unexpectedly increased coronary perfusion pressure and constricted isolated coronary arterioles in rats (Pemberton et al., 2004), whereas intracoronary acyl ghrelin infusion unexpectedly decreased coronary blood flow in anesthetized pigs (Grossini et al., 2007). The mechanisms were shown to involve L-type Ca²⁺ channel and protein kinase C activation (Pemberton et al., 2004) and the inhibition of a tonic coronary β_2 -adrenergic receptor-mediated vasodilatory effect related to the release of nitric oxide (Grossini et al., 2007). By contrast, subcutaneous injection of the GHS-R1a antagonist [D-Lys-3]GHRP-6 increased arterial pressure and heart rate dose-dependently via modulation of sympathetic activity in rats (Vlasova et al., 2009). Therefore, this finding raises the concern that the use of GHS-R1a antagonists as therapeutic targets for reduction in food intake might lead to serious side effects, such as elevated blood pressure in humans, most of whom already have elevated blood pressure as part of their metabolic syndrome (Vlasova et al., 2009). On the other hand, bolus intravenous injection of obestatin does not change blood pressure level of spontaneously hypertensive rat (Li et al., 2009).

Exogenous ghrelin inhibited vascular oxidative stress in spontaneously hypertensive rats as a result of inhibition of vascular NADPH oxidases (Kawczynska-Drozd et al., 2006). The finding may indicate the potential and important antiatherosclerotic effect of ghrelin. Acyl ghrelin inhibited angiotensin II-induced proliferation and contraction in a dose-response manner via the cAMP/protein kinase A pathway (Rossi et al., 2009). Repeated administration of acyl ghrelin improved endothelial dysfunction and increased endothelial nitric-oxide synthase expression in GH-deficient rats, elucidating GH-independent mechanisms (Shimizu et al., 2003b). These data regarding the effects of acyl ghrelin on rodent and human aortic smooth muscle cell functions open the way to considering ghrelin as a potential therapeutic target in vascular damage and remodeling. The beneficial action of acyl ghrelin on human vascular endothelium has been

shown (Tesauro et al., 2005). However, total ghrelin concentrations and carotid artery atherosclerosis were found to be positively correlated in men after adjustment for the commonly recognized risk factors of atherosclerosis (Pöykkö et al., 2006). Therefore, experimental and prospective studies are warranted to clarify the roles of ghrelin in atherosclerosis. Novel vascular actions of acyl ghrelin were observed to directly stimulate production of NO from vascular endothelial cells using phosphoinositide-3-kinase-dependent signaling pathways that mimic those of insulin (Iantorno et al., 2007), implying that ghrelin could be beneficial for metabolic and cardiovascular diseases characterized by reciprocal relationships between insulin and endothelial dysfunction.

Exogenous administration of acyl ghrelin after rat myocardial infarction prevented an increase in cardiac sympathetic tone (Schwenke et al., 2008a; Soeki et al., 2008), attenuated early cardiac remodeling (Soeki et al., 2008), improved left ventricular function (Soeki et al., 2008), and reduced mortality (Schwenke et al., 2008a), implying that early acyl ghrelin treatment within the first several hours after myocardial infarction may improve early survival prognosis and provide clinicians with critical time for implementing supplementary therapeutic measures. In addition, ghrelin and GHS-R1a in the myocardium were up-regulated during isoproterenol-induced myocardial injury (Li et al., 2006). The protective effect of acyl ghrelin against isoproterenol-induced myocardial injury and fibrosis was more potent than that of des-acyl ghrelin, which suggests that acyl and des-acyl ghrelin could be an endogenous cardioprotective factor in ischemic heart disease (Chang et al., 2004; Li et al., 2006), and these effects were mediated via GHS-R1a-dependent and -independent pathways (Li et al., 2006). Subcutaneous or intravenous infusion of acyl ghrelin stimulated left ventricular function but not modified endothelium-dependent vasodilation, with (Enomoto et al., 2003) or without (Vestergaard et al., 2007a) altering ejection fraction in men. The beneficial effects of ghrelin on heart function, including reduction of myocyte apoptosis, can also be explained by its ability to activate AMPK (Kola et al., 2005).

D. Endocrine System

1. Hypothalamic-Pituitary-Adrenal Axis. Acyl ghrelin strongly stimulates GH secretion and facilitates secretion of GHRH. On the other hand, acyl ghrelin affects somatotrophic and hypothalamic-pituitary-adrenal axis. In fact, acyl ghrelin induces secretion of adrenocorticotropin and cortisol/prolactin (or corticosterone) in humans (Takaya et al., 2000; Coiro et al., 2005) and rats (Asakawa et al., 2001a). Acyl ghrelin was shown to increase release of CRF in rats (Mozid et al., 2003) and arginine-vasopressin in humans (Coiro et al., 2005) and in rats (Ishizaki et al., 2002; Mozid et al., 2003). The mechanisms of GH and adrenocorticotropin regulation

by acyl ghrelin may include hypothalamic release of GHRH, CRF, arginine vasopressin, and NPY (Wren et al., 2002).

Expression of *prepro-ghrelin*, *GHS-R1a*, and *GOAT* genes was notably higher in the cortex than in medulla, whereas high expression of *GOAT* gene was found in the zona glomerulosa. Direct stimulating effect of acyl ghrelin on corticosterone output by cultured rat adrenocortical cells was also demonstrated (Rucinski et al., 2009).

2. Hypothalamic-Pituitary-Thyroid Axis. Hypothyroidism resulted in an increase in gastric ghrelin mRNA and circulating plasma total ghrelin levels, both being decreased in hyperthyroid rats (Camino et al., 2002). Reduced serum acyl ghrelin levels were observed in patients with hyperthyroidism (Riis et al., 2003; Röjdmarm et al., 2005; Altinova et al., 2006a, 2006b; Braclik et al., 2008) but not in subclinical hyperthyroidism (Tanda et al., 2009). Ghrelin levels were decreased in hyperthyroidism and increased when euthyroidism was achieved after treatment with antithyroid drugs (Röjdmarm et al., 2005; Tanda et al., 2009; Theodoropoulou et al., 2009). Body mass index and insulin were the main factors influencing ghrelin concentration in hyperthyroidism, whereas T3 and T4 levels had no effect on ghrelin levels (Theodoropoulou et al., 2009). GH release after acyl ghrelin, GHRP-6, and GHRH administration was decreased in thyrotoxicosis, whereas acyl ghrelin's ability to increase glucose levels was not altered in thyrotoxicosis (Nascif et al., 2007). Acyl ghrelin-mediated pathways of adrenocorticotropin release might be activated by thyroid hormone excess, but adrenocortical reserve was maintained during thyrotoxicosis (Nascif et al., 2009). These observations suggest that thyroid hormone excess interferes with GH-releasing and affects adrenocorticotropin-releasing pathways activated by acyl ghrelin. Circulating total ghrelin in thyroid dysfunction was related to insulin resistance and not to hunger, food intake, or anthropometric changes (Giménez-Palop et al., 2005). Thyroid hormone stimulates food intake in humans and rodents. The hypothalamic neuropeptide Y/Y₁ receptor pathway, activated by a reduction in circulating leptin but not by an increase in circulating ghrelin, contributed to hyperphagia associated with triiodothyronine-induced thyrotoxicosis (Ishii et al., 2003).

Hypothyroidism is not accompanied by significant changes in circulating ghrelin (Tanda et al., 2009). Serum ghrelin levels are increased in patients who are hypothyroid (Braclik et al., 2008; Gjedde et al., 2008) and become normalized by L-thyroxine treatment (Gjedde et al., 2008).

E. Cell Proliferation

Acyl and des-acyl ghrelin stimulated the proliferation of a somatotroph pituitary tumor cell line via the mitogen-activated protein kinase pathway (Nanzer et al., 2004). Because ghrelin has been shown to be expressed in both normal and adenomatous pituitary

tissue, locally produced acyl and des-acyl ghrelin may play a role in pituitary tumorigenesis via an autocrine/paracrine pathway. [³H]Thymidine incorporation with both hexarelin and acyl ghrelin was shown to be stimulated rat hippocampal progenitor cells (Johansson et al., 2008), suggesting a novel cell protective and proliferative role for GHS in the central nervous system. The third ghrelin gene, obestatin, was reported to mediate proliferation of human retinal pigment epithelial cells (Camiña et al., 2007).

Natural (acyl ghrelin) and synthetic (hexarelin) GH secretagogues both were demonstrated to stimulate H9c2 cardiomyocyte cell proliferation, supporting the potential peripheral effects of GHS on the cardiovascular system independent of increased GH secretion (Pettersson et al., 2002). Both acyl ghrelin and des-acyl ghrelin have been reported to inhibit cell death in primary adult and H9c2 cardiomyocytes and endothelial cells through extracellular signal-regulated kinase 1/2 and phosphoinositide-3-kinase/AKT pathways (Baldanzi et al., 2002). Furthermore, acyl ghrelin and des-acyl ghrelin recognize common high-affinity binding sites on H9c2 cardiomyocytes, which do not express GHS-R (Baldanzi et al., 2002). The third ghrelin gene product, obestatin, was not a relevant metabolic or viability modifier on cardiomyocyte (Iglesias et al., 2007).

Both acyl ghrelin and des-acyl ghrelin were reported to stimulate proliferating C2C12 skeletal myoblasts, which did not express GHS-R1a, to differentiate and to fuse into multinucleated myotubes in vitro through activation of p38 (Filigheddu et al., 2007). The results provide the evidence that the described activities on C2C12 are probably mediated by this novel, as-yet unidentified receptor for both ghrelin forms.

Ghrelin and GHS-R production were demonstrated to be present in pancreatic β -cells and related endocrine tumors, indicating that autocrine/paracrine circuits of ghrelin/GHS-R may be active in neoplastic conditions (Volante et al., 2002). Des-acyl ghrelin and acyl ghrelin were also shown to prevent cell death and apoptosis of pancreatic β -cells, implying that an as-yet unknown receptor other than GHS-R1a was likely to be involved in these survival mechanisms (Granata et al., 2006). A subsequent study further identified that acyl ghrelin and des-acyl ghrelin promoted survival of both β -cells and human islets, whereas these effects were independent of GHS-R1a and were probably mediated by acyl ghrelin/des-acyl ghrelin binding sites and involved cAMP/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphoinositide-3-kinase/Akt signaling pathways (Granata et al., 2007). The third ghrelin gene product, obestatin, has been proven to promote β -cell and human islet cell survival and to stimulate the expression of main regulatory β -cell genes (Granata et al., 2008). Taken together, new roles for all three ghrelin gene products were identified within the endocrine pancreas.

Finally, the ghrelin system was shown to affect various cancer cell lines. For example, acyl ghrelin and des-acyl ghrelin induced in vitro a dose-dependent inhibition of cell proliferation and increased apoptosis of the H345 small cell carcinoma cell line, despite the absence of GHS-R1a (Cassoni et al., 2006), whereas acyl ghrelin and des-acyl ghrelin inhibited DU-145 cell proliferation, and displayed a biphasic effect in PC-3 cells in human prostate neoplasm-related cell lines via specific GHS binding sites other than GHS-R1a and -1b (Cassoni et al., 2004).

F. Immune Function

In fact, various studies reveal that acyl ghrelin regulate immune cell proliferation and activation and secretion of proinflammatory cytokines. Ghrelin stimulated phagocytosis through a GHS-R1a dependent pathway in fish, the most primitive vertebrate (Yada et al., 2006). Expressions of GHS-R1a and acyl ghrelin were detectable in all immune cells regardless of the maturity and cell types, including T cells, B cells, monocytes, and neutrophils (Hattori et al., 2001; Gnanapavan et al., 2002; Dixit et al., 2004), where acyl ghrelin acts via GHS-R1a to specifically inhibit the expression of proinflammatory anorectic cytokines such as interleukin-1 β , interleukin-6, and TNF- α (Dixit et al., 2004). Acyl ghrelin was expressed in human T cells and preferentially segregated within the lipid raft domains upon T-cell antigen receptor ligation (Dixit et al., 2009). Genetic ablation of both *acyl ghrelin* and *GHS-R1a* led to loss of thymic epithelial cells and an increase in adipogenic fibroblasts in the thymus (Youm et al., 2009). Acyl ghrelin and GHS-R1a expression within the thymus diminished with progressive aging (Dixit et al., 2007), whereas infusion of acyl ghrelin into 14-month-old mice significantly improved the age-associated changes in thymic architecture and thymocyte numbers. In addition, acyl ghrelin expression also declined with increasing age in spleen and T cells, whereas exogenous acyl ghrelin administration in old mice reduced proinflammatory cytokines, showing that ghrelin functions in an autocrine and paracrine capacity to regulate proinflammatory cytokine expression in human and murine T cells and may contribute in regulating so called "inflammaging" (Dixit et al., 2009). Acyl ghrelin-induced thymopoiesis during aging was associated with enhanced early thymocyte progenitors and bone marrow-derived Lin(-)Sca1(+)cKit(+) cells, whereas *acyl ghrelin*- and *GHS-R*-deficient mice displayed enhanced age-associated thymic involution (Dixit et al., 2007). Functional loss of *acylghrelin/GHS-R1a* interactions induced thymic adipogenesis with age, suggesting that acyl ghrelin may preserve the thymic stromal cell microenvironment by controlling age-related adipocyte development within the thymus (Youm et al., 2009). An age-related increase in thymic adiposity is associated with reduced thymopoiesis and compromised immune surveil-

lance in the elderly, which is a very clinically relevant issue.

In contrast, des-acyl ghrelin is not involved in immunomodulation (Li et al., 2004; Dixit et al., 2007). On the other hand, obestatin increases binding of oxidized low-density lipoprotein to thioglycollate-elicited mouse peritoneal macrophages, and when administered together with acyl ghrelin, decreased vascular cell adhesion molecule-1 expression on endothelial cells (Kellokoski et al., 2009).

G. Bone Metabolism

In healthy children, acyl ghrelin was a significant negative predictor of whole-body bone mineral density and bone mineral content, whereas in obese children, des-acyl ghrelin was found to be associated with whole-body bone mineral density, whole-body bone mineral content, and height (Pacifico et al., 2009). However, testosterone was reported to stimulate markers of bone formation (procollagen type N-terminal propeptide) and bone resorption (type I carboxyl-terminal telopeptide) in early puberty, whereas total ghrelin level had no direct influence on bone turnover markers in boys at different stages of puberty (Jürimäe et al., 2009b). In addition, ghrelin had no direct influence on bone mineral density in young male competitive swimmers (Jürimäe et al., 2009a). In elderly Italian men, serum ghrelin was significantly associated with femoral neck bone mineral density (Gonnelli et al., 2008). Exogenous infusion of acyl ghrelin had no immediate effects on bone resorption (as measured by type 1 collagen β C-telopeptide) and bone formation (as measured by procollagen type 1 amino-terminal propeptide) in healthy volunteers and subjects who had undergone gastrectomy (Huda et al., 2007). However, there was an inverse relationship between baseline total ghrelin and bone resorption (Huda et al., 2007). GOAT mRNA was expressed in murine cartilage explants and in the cultured murine chondrogenic ATDC-5 cell line. Likewise, GOAT was also expressed in human immortalized chondrocyte cell lines and in human cultured primary chondrocytes, demonstrating that chondrocytes were equipped with biochemical machinery for the synthesis of acylated ghrelin (Gómez et al., 2009). Moreover, ghrelin was synthesized and secreted by chondrocytes (Camino et al., 2005). These data suggest a novel role of the ghrelin axis in prehypertrophic and hypertrophic chondrocyte differentiation during endochondral ossification.

Bone weight, bone mineral density and bone mineral content of the lumbar vertebrae and humerus were decreased in the fundectomized pigs compared with sham-operated pigs, in parallel with lower serum GH, ghrelin, and IGF-1 concentrations (Tatara et al., 2007). These findings established the role of the gastric-hypothalamic-pituitary axis in osteopenia. Subcutaneous injection of acyl ghrelin partially reversed total gastrectomy-induced reduction of body weight, lean body mass, and

body fat but not bone mass in mice (Dornonville de la Cour et al., 2005). Ghrelin and GHS-R1a were identified in osteoblast-like cells (Fukushima et al., 2005). Acyl ghrelin stimulated proliferation of human osteoblastic TE85 cells via NO/cGMP signaling pathway: acyl ghrelin bound to GHS-R1a on osteoblasts and induced NO production, and, in turn, NO stimulated cGMP production via activating guanylate cyclase (Wang et al., 2009a). Ghrelin increased osteoblast-like cell numbers and DNA synthesis, whereas the proliferative effects of ghrelin were abolished by the GHS-R1a antagonist [D-Lys³]GHRP-6 (Fukushima et al., 2005). In addition, ghrelin increased the expression of osteoblast differentiation markers and calcium accumulation in the matrix and bone mineral density in normal and GH-deficient rats (Fukushima et al., 2005). Furthermore, ghrelin stimulated intramembranous osteogenesis in rats (Deng et al., 2008). In addition to the GHS-R1a pathway, acyl and des-acyl ghrelin were demonstrated to stimulate human osteoblast growth via mitogen-activated protein kinase/phosphoinositide 3-kinase pathways in the absence of GHS-R1a in vitro (Delhanty et al., 2006). In addition to proliferative-stimulating effects, acyl ghrelin promoted differentiation (Kim et al., 2005b; Maccarinelli et al., 2005) and inhibited apoptosis (Kim et al., 2005b) in osteoblastic cells from cell culture experiments. Both immunocytochemistry and radioimmunoassay methods showed that ghrelin was detected mainly in the odontoblasts but also in the pulp of human canines and molars (Aydin et al., 2007b). Hence, ghrelin potentially plays interesting and important physiological roles in teeth. On the other hand, it probably plays a marginal role in the regulation of chondrocyte metabolism and only decreased the metabolic activity of chondrocytes (Lago et al., 2007).

H. Sleep

Intracerebroventricular acyl ghrelin induced increases in behavioral activity, including feeding, exploring, and grooming, and stimulated food and water intake in rats, supporting the roles of acyl ghrelin in the integration of feeding, metabolism, and sleep regulation (Szentirmai et al., 2006a). Acyl ghrelin depolarized laterodorsal tegmental nucleus neurons (Takano et al., 2009) and pedunculopontine tegmental nucleus (Kim et al., 2009) postsynaptically and dose dependently via GHS-R1a, and the ionic mechanisms underlying acyl ghrelin-induced depolarization included a decrease of K⁺ conductance. These findings provide electrophysiological bases to support the implication that laterodorsal tegmental nucleus neurons are involved in the cellular processes through which acyl ghrelin participates in the regulation of sleep-wakefulness. Different effects of exogenous acyl ghrelin and obestatin on sleep in rodents and humans are summarized in Table 4.

Reduced sleep duration and quality seem to be endemic in modern society. Subjects with short sleep had

TABLE 4
Effects of acyl ghrelin and obestatin on sleep

Drugs	Animals Studied	Administration Routes with and without Acting Areas	Effects on Sleep	Reference
Acyl ghrelin	Rats	Microinjection of into hypothalamic sites, such as the lateral hypothalamus, medial preoptic area, and paraventricular nucleus	↑ Wakefulness	Szentirmai et al., 2007a
Acyl ghrelin	Healthy males	i.v.	↑ Slow wave sleep	Weikel et al., 2003
Acyl ghrelin ± GHRH or CRF	Young men	i.v.	↑ Non-REM sleep	Kluge et al., 2008
Acyl ghrelin	Young women	i.v.	↓ REM sleep	
Acyl ghrelin	Elderly men	i.v.	— Sleep pattern	Kluge et al., 2007
			↑ Slow wave sleep and stage 2 sleep	Kluge et al., 2009
			↓ Stage 1 sleep and REM sleep	
Obestatin	Elderly women	i.v.	— Sleep pattern	
	Rats	i.c.v.	↑ NREMS episodes	Szentirmai and Krueger, 2006b
N.A.	Preproghrelin gene KO mice	N.A.	↓ Sleep latency	
			Impaired abilities to manifest and integrate normal sleep to metabolic challenges	Szentirmai et al., 2009

i.c.v., intracerebroventricular; i.v., intravenous; KO, knockout; REM, rapid eye movement; N.A., not applicable.

reduced serum fasting leptin, elevated fasting total ghrelin levels, and increased body mass index (Taheri et al., 2004). Sleep was found to enhance nocturnal plasma total ghrelin surges in healthy subjects (Dzaja et al., 2004), whereas a blunting in the nocturnal rise in plasma total ghrelin concentration characterized obese subjects (Yildiz et al., 2004). The above evidence corroborates the assumption of a tight connection between sleep-wake modulation and metabolic parameters. A single night of sleep deprivation increased total ghrelin levels and feelings of hunger in normal-weight healthy men (Schmid et al., 2008), whereas another study revealed that a single sleep deprivation had relatively weak effect on plasma total ghrelin concentrations (Schüssler et al., 2006). Recurrent bedtime restriction can modify the amount, composition, and distribution of human food intake, and sleeping short hours in an obesity-promoting environment may facilitate the excessive consumption of energy from snacks but not meals (Nedelcheva et al., 2009). These findings illustrate evidence of disturbing influence of sleep loss on endocrine regulation of energy homeostasis, which in the long run may result in altered ingestive behavior, weight gain, and obesity. Patients with chronic insomnia exhibited lower total ghrelin levels across the night (Motivala et al., 2009), indicating that besides short-term experimen-

tal sleep loss, long-term sleep difficulties are associated with altered ghrelin expression. The marked decrease in average sleep duration in the last 50 years, coinciding with the increased prevalence of obesity, together with the observed adverse effects of recurrent partial sleep deprivation on metabolism and hormonal processes, may have important implications for public health (Van Cauter et al., 2008).

I. Memory

Ghrelin was found to be present in the majority of cultured newborn rat neurons (Stoyanova et al., 2009). Circulating acyl ghrelin was found to enter the hippocampus and to bind to neurons of the hippocampal formation, where it promoted dendritic spine synapse formation and generation of long-term potentiation (Diano et al., 2006). These ghrelin-induced synaptic changes were paralleled by enhanced spatial learning and memory. Targeted disruption of the gene encoding ghrelin resulted in decreased numbers of spine synapses in the CA1 region and impaired performance of mice in behavioral memory testing, both of which were rapidly reversed by acyl ghrelin administration (Diano et al., 2006). Effects of exogenous acyl ghrelin and obestatin on memory in rodents and chicks are summarized in Table 5. These observations reveal endogenous functions of

TABLE 5
Effects of acyl ghrelin and obestatin on memory

Drugs	Animals Studied	Administration Routes with and without Acting Areas	Effects on Memory Retention	Reference
Acyl ghrelin	Rats	i.c.v. and microinjected into hippocampus, amygdala, and dorsal raphe nucleus	↑ Memory retention	Carlini et al., 2002, 2004
Acyl ghrelin	Mice	i.c.v.	Reversing decreased memory for novel object recognition in long-term food restriction	Carlini et al., 2008
Acyl ghrelin	Neonatal chicks	i.c.v.	↓ Memory retention	Carvajal et al., 2009
Obestatin	Rats	i.c.v.	↑ Memory retention	Carlini et al., 2007b

i.c.v., intracerebroventricular.

ghrelin gene products that link metabolic control with higher brain functions and suggest novel therapeutic strategies to enhance learning and memory processes.

J. Anxiety Behavior

Short-term psychological stress, such as water avoidance stress, increased plasma adrenocorticotropin and total ghrelin concentration in rats (Kristensson et al., 2006), whereas intracerebroventricular ghrelin augmented hypothalamic noradrenaline and adrenocorticotropin release in a fasting state after food deprivation-induced stress (Kawakami et al., 2008). Effects of exogenous acyl ghrelin and obestatin on anxiety behavior in rodents and chicks are summarized in Table 6. These findings suggest that acyl ghrelin and obestatin may have a role in mediating neuroendocrine and behavioral responses to stressors and that the stomach could play an important role, not only in the regulation of appetite, but also in the regulation of anxiety.

XI. Disturbance of Energy Homeostasis in Various Conditions: Roles of Ghrelin Gene Products in Obesity and Anorexia-Cachexia

A. Obesity

The ghrelin-GHS-R system is imperative in modulating appetite, energy expenditure, and body weight regulation. Given the growing epidemic of obesity, it is important to understand the complex physiological processes that regulate body weight, including the precise role of ghrelin in the involvement of the pathogenesis of overweight and obesity. In addition to inducing hyperphagia, peripheral administration of acyl ghrelin increases fat mass by reducing fat utilization, which results in body weight gain in mice (Tschöp et al., 2000) and obesity in rats (Wren et al., 2001b). These findings underline the role of acyl ghrelin in modulating energy balance in addition to its hyperphagic effect. Weight gain by forced over-feeding in rats (Williams et al., 2006) or short-term over-eating in humans (Robertson et al., 2004) results in the decrease of circulating ghrelin. Obese rodents, including *ob/ob* and *db/db* mice and Zucker fatty rats, exhibited lower fasting total and acyl ghrelin levels than control animals (Ariyasu et al., 2002). Plasma total ghrelin concentration was de-

creased, whereas insulin and leptin concentrations were increased in obese dogs compared with lean control dogs (Jeusette et al., 2005). In humans, fasting plasma total ghrelin level was negatively correlated with body mass index (Ravussin et al., 2001; Tschöp et al., 2001b; Shiiya et al., 2002; Haqq et al., 2003; Purnell et al., 2003; Butler and Bittel, 2007) and body fatness (Ravussin et al., 2001), whereas fasting plasma obestatin level was negatively correlated with body mass index (Guo et al., 2007; Nakahara et al., 2008) and body fatness (Nakahara et al., 2008). However, using a separate measuring method of plasma acyl and des-acyl ghrelin, two independent research groups theorized that plasma total (Barazzoni et al., 2007) and des-acyl ghrelin (Barazzoni et al., 2007; Rodríguez et al., 2009) levels were negatively correlated with body mass index, whereas plasma acyl ghrelin concentration was positively correlated with body mass index (Barazzoni et al., 2007; Rodríguez et al., 2009). Therefore, the assumption that systemic total ghrelin levels reflect acyl ghrelin secretion should be made with caution (Gauna et al., 2007). For instance, fasting plasma total ghrelin (Cummings et al., 2002a; English et al., 2002; Rigamonti et al., 2002; Shiiya et al., 2002; Erdmann et al., 2005; Korner et al., 2005, 2006; le Roux et al., 2005b; Engström et al., 2007; Guo et al., 2007; Vicennati et al., 2007; Huda et al., 2009; Yang et al., 2009), des-acyl ghrelin (Rodríguez et al., 2009), and obestatin (Guo et al., 2007; Nakahara et al., 2008) concentration were lower in obese than lean subjects, whereas acyl ghrelin concentration was higher in obese persons (Rodríguez et al., 2009). In addition, obese persons showed lower expression of GHS-R in omental adipose tissue (Rodríguez et al., 2009). But another study showed that all ghrelin gene products, including acyl ghrelin, des-acyl ghrelin, and obestatin, were all negatively correlated with body mass index and body fatness (Nakahara et al., 2008). Plasma fasting ghrelin concentration was decreased in obese white persons compared with lean white persons, whereas fasting plasma ghrelin was lower in Pima Indians, a population with a very high prevalence of obesity compared with white persons (Tschöp et al., 2001b), indicating that obese subjects possess lower fasting plasma ghrelin levels compared with lean subjects, and different fasting plasma ghrelin

TABLE 6
Effects of acyl ghrelin and obestatin on anxiety behavior

Drugs	Animals Studied	Administration Routes with and without Acting Areas	Effects on Anxiety	Reference
Acyl ghrelin	Mice	i.c.v. and i.p.	↑ Anxiogenic activities in the elevated plus maze test	Asakawa et al., 2001a
Acyl ghrelin	Rats	i.c.v. and microinjected into hippocampus, amygdala, and dorsal raphe nucleus	↑ Anxiety	Carlini et al., 2002, 2004
Acyl ghrelin	Neonatal chicks	i.c.v.	↑ Anxiety	Carvajal et al., 2009
Antisense DNA for acyl ghrelin	Rats	i.c.v.	↑ Anxiolytic effects	Kanehisa et al., 2006
Obestatin	Rats	i.c.v.	↑ Memory retention	Carlini et al., 2007b

i.c.v., intracerebroventricular; i.p., intraperitoneal.

levels exist in different racial groups. These results indicate that fasting plasma total ghrelin is down-regulated as a consequence of excess energy or excess energy storage. Plasma total ghrelin fell to a nadir after eating in lean subjects, but obese humans exhibited a blunted postprandial suppression after normal diet or high-fat meals in both Western and Eastern studied populations, suggesting that food failed to suppress this hunger signal in obese persons, which predisposes to hyperphagia and excess weight gain and reinforces obesity (English et al., 2002; Erdmann et al., 2005; le Roux et al., 2005b; Yang et al., 2009). Postprandial total ghrelin level was elevated in black women compared with white women (Brownley et al., 2004), implying that subnormal postprandial ghrelin suppression may account for overweight and obesity in this ethnic group. An intriguing blunting in the nocturnal rise in plasma total ghrelin concentration was found in obese subjects and may be an important hallmark in the biology of obesity (Yildiz et al., 2004). Taken together, the abnormal low fasting total ghrelin level, the lack of postprandial total ghrelin suppression, and the blunted nocturnal rise in plasma total ghrelin concentration in obese subjects may be involved in the pathophysiology of human obesity. These changes may simply reflect a physiologic response to the decrease in caloric need during obesity. The circulating fasting plasma total ghrelin-to-obestatin ratio was found to be positively correlated with body mass index and was higher in obese than normal-weight Eastern people, suggesting a possible involvement of a high preprandial total ghrelin-to-obestatin ratio in the pathophysiology of human obesity (Guo et al., 2007). In contrast, the circulating fasting plasma obestatin concentration was higher in female obese Western people than in control subjects, whereas the fasting plasma total ghrelin-to-obestatin ratio was lower (Vicennati et al., 2007). The discrepancy could come from different sex and ethnic groups being enrolled.

Transgenic mice that overexpressed acyl ghrelin exhibited hyperphagia without altering long-term body weight gain because of a paradoxical increase in energy expenditure (Bewick et al., 2009). These mice with chronic hyperghrelinemia remained responsive to exogenous acyl ghrelin-induced feeding. Another genetic model of mice overexpressing acyl ghrelin showed no increases in food intake or fat mass and no decrease in energy expenditure (Reed et al., 2008). Different gene promoters used could explain the differences. *Ghrelin*-null [ghrelin(-/-)] mice exhibited neither dwarfs nor anorexia as predicted (Sun et al., 2003), implying that ghrelin may not be critically required for viability, growth, and appetite in rodents. In addition, *ghrelin*-null mice displayed normal response to starvation and diet-induced obesity (Sun et al., 2003). However, a recent study revealed that male *ghrelin*-knockout mice were protected from the rapid weight gain induced by early exposure to a high-fat diet 3 weeks after weaning

(Wortley et al., 2005). This reduced weight gain was linked with decreased adiposity, increased energy expenditure and locomotor activity, and a paradoxical preservation of the GH/IGF-1 axis as the animals aged (Wortley et al., 2005). Another study using *GHS-R1a*-knockout mice showed that *GHS-R1a*-null mice ate less food, stored less of their consumed calories, preferentially used fat as an energy substrate, and accumulated less body weight and adiposity than control mice (Zigman et al., 2005). However, another study showed that *ghrelin*-knockout mice only resisted high-fat diet-induced glucose intolerance but not body weight gain (Dezaki et al., 2006), and yet another study revealed that *GHS-R1a*-knockout mice exhibited blunted central acyl ghrelin-induced feeding response, obese phenotype, hyperleptinemia, hypoinsulinemia, and hypolipidemia (Egecioglu et al., 2006). On the other hand, three independent research groups demonstrated that transgenic mice overexpressing des-acyl ghrelin in their plasma (or in various tissues) showed small phenotype in terms of linear growth (Ariyasu et al., 2005; Asakawa et al., 2005), lower plasma IGF-I level (Ariyasu et al., 2005), decreased fat mass (Asakawa et al., 2005; Zhang et al., 2008a), decreased food intake and body weight (Asakawa et al., 2005), and resistance to high-fat diet-induced obesity (Zhang et al., 2008a). In contrast, a study using rat insulin II promoter-ghrelin transgenic mice overexpressing des-acyl ghrelin in their plasma revealed that des-acyl ghrelin had influence only on glucose metabolism without altering feeding behavior, body weight, and body fat in these transgenic mice (Iwakura et al., 2005). But a study showed that neither *ghrelin*- nor *GHS-R*-knockout mice were resistant to diet-induced obesity (Sun et al., 2008). Collectively, *acyl ghrelin*-knockout and *GHS-R1a*-knockout mice (Wortley et al., 2005; Zigman et al., 2005), as well as des-acyl ghrelin-overexpressing mice (Zhang et al., 2008b), are all resistant to high-fat-diet-induced obesity, indicating the indispensable roles for endogenous ghrelin gene products/GHS-R1a signaling system in the metabolic adaptation to nutrient availability and for the development of the full phenotype of diet-induced obesity. Therefore, antagonizing biological actions of acyl ghrelin may reduce food intake and prevent obesity, especially in the face of a metabolically stressful diet. However, ablation of acyl ghrelin only improved the diabetic but not obese phenotype of *ob/ob* mice (Sun et al., 2006). It is noteworthy that simultaneous deletion of acyl ghrelin and its receptor increased motor activity and energy expenditure in these double-knockout mice, implying the existence of additional as-yet unknown molecular components of the endogenous ghrelin/GHS-R1a system (Pfluger et al., 2008).

Prader-Willi syndrome is the most common and famous prototype of human genetic obesity, involving imprinting disorders of several genes on chromosome 15, and is characterized by severe hyperphagia, GH defi-

ciency, hypogonadism, neonatal hypotonia, dysmorphic features, and cognitive impairment. Marked increased plasma total and acyl ghrelin levels were found in Prader-Willi syndrome, and the elevated ghrelin acted as an orexigenic factor, driving the insatiable appetite and obesity in these patients (Cummings et al., 2002a; DelParigi et al., 2002; Haqq et al., 2003; Paik et al., 2006), whereas plasma des-acyl ghrelin levels remained unchanged compared with obese children who did not have Prader-Willi syndrome (Paik et al., 2006). Fasting plasma obestatin could be unchanged (Park et al., 2007) or elevated (Butler and Bittel, 2007) in subjects with Prader-Willi syndrome. The magnitude of the postprandial total ghrelin suppression was less in patients with Prader-Willi syndrome than in obese and lean subjects (Giménez-Palop et al., 2007).

Fasting gastric acyl ghrelin mRNA expression in mice was increased by a high-fat diet (Asakawa et al., 2003). Lipids suppressed the peripheral ghrelin secretion relatively more weakly than glucose or amino acids in rats (Overduin et al., 2005) and humans (Foster-Schubert et al., 2008), including lean and obese subjects (Yang et al., 2009), whereas dietary fructose attenuated postprandial suppression of total ghrelin (Teff et al., 2004), contributing to the fact that high-fat and high-fructose diets predispose to weight gain. Intravenous administration of acyl ghrelin increased food intake in obese and lean subjects (Druce et al., 2005). However, exogenous acyl ghrelin infusion increased palatability of food in the obese, and obese people were more sensitive to the appetite-stimulating effects of acyl ghrelin than were lean people (Druce et al., 2005), suggesting that inhibition of circulating acyl ghrelin may be a useful therapeutic target in the treatment of obesity. Collectively, in the developed and developing countries (including Western and Eastern societies), humans often consume diets very rich in fat and/or fructose and in overall caloric content even if there is no metabolic need for it. Furthermore, hypertriglyceridemia induced by high-fat diet promoted transport of circulating acyl ghrelin across the blood-brain barrier, which would amplify its orexigenic effect in the brain (Banks et al., 2008). Thus, the inability of their bodies to counteract the actions of acyl ghrelin may be a deteriorating adaptive mechanism, leading to the development of metabolic syndrome and obesity in the modern societies. Therefore, pharmacological inhibition of the action of acyl ghrelin in humans, especially for those people frequently under high-fat and/or high-energy diet, provides a new venue for antiobesity therapy.

Initial studies using antagonism of GHS-R1a or ghrelin's downstream mediator (i.e., AgRP, via injecting melanotan II into the third ventricle) showed reduced acyl ghrelin- and food deprivation-induced food intake, body weight gain, and food hoarding in mice (Asakawa et al., 2003) and hamsters (Keen-Rhinehart and Bartness, 2007). Another study revealed that small-molecule GHS-R1a antagonists improved glucose tolerance, sup-

pressed appetite, and promoted weight loss in rats (Esler et al., 2007), suggesting promising clinical therapeutic value of GHS-R1a antagonism for controlling the obesity pandemic in developed countries. Two novel GHS-R1a antagonists [JMV 3002 and JMV 2959 (Salomé et al., 2009a)] and one partial agonist [JMV 2810 (Salomé et al., 2009a)] have been developed to show in vivo suppression of central acyl ghrelin- and fasting-induced food intake (at the central nervous system level) in rats and in vitro suppression of acyl ghrelin-induced firing rate of the arcuate nucleus (at the single cell level) (Salomé et al., 2009a). Central treatment with acyl ghrelin for 14 days, compared with vehicle-treated control rats, resulted in increases in body weight, lean mass, fat mass (assessed by dual energy X-ray absorptiometry), dissected white fat pad weight, cumulative food intake, food efficiency, and respiratory exchange ratio, and a decrease of energy expenditure in rats, whereas coadministration of the recently developed GHS-R1a antagonist JMV 2959 blocked the majority of these effects, with the notable exception of acyl ghrelin-induced food intake and food efficiency. The results identified the role of GHS-R1a mediating the long-term effects of acyl ghrelin on fat accumulation, which could be at least partly independent of food intake (Salomé et al., 2009b). Because the central ghrelin gene products/GHS-R signaling system has emerged as an important pro-obesity target, antagonism of acyl ghrelin at the central nervous system level is of potential value in the treatment of obesity and clinically related diseases. Recent studies using in vitro-generated biostable RNA-based compounds (i.e., anti-acyl ghrelin Spiegelmer) specifically binding *n*-octanoyl ghrelin have been successfully invented to inhibit acyl ghrelin-stimulated GHS-R activation (Helmling et al., 2004), including in vitro blocking of acyl ghrelin-induced excitation of electrophysiological firing in the medial arcuate nucleus in rats (Becskei et al., 2008) and in vivo suppression of neurostimulatory and orexigenic effects of peripheral acyl ghrelin in rats (Kobelt et al., 2006) and to ameliorate obesity in diet-induced obese mice (Shearman et al., 2006). Other specific monoclonal antibodies, including a high-affinity neutralizing antibody binding acyl ghrelin [i.e., 33A (Lu et al., 2009)] and a catalytic degradation antibody facilitating hydrolysis of the serine octanoate ester moiety of acyl ghrelin (Mayorov et al., 2008), have also been generalized to inhibit in vitro acyl ghrelin-mediated calcium signal and in vivo short-term acyl ghrelin-induced orexigenic effects in mice (Lu et al., 2009), and to maintain greater whole body energy expenditure during fasting and reduce subsequent refeeding in mice (Mayorov et al., 2008). Furthermore, vaccination of rats with acyl ghrelin immunoconjugates resulted in hypophagia and decreases in weight gain and body fat (Zorrilla et al., 2006). Taken together, these findings point out the critical role of acyl ghrelin in body weight regulation and the development of obesity. How-

ever, the anti-acyl ghrelin Spiegelmer did not block neuronal activation of the hypothalamic arcuate nucleus in food-deprived mice (Becskei et al., 2008), whereas long-term administration of 33A did not affect food intake or body weight gain in a mouse model of diet-induced obesity (Lu et al., 2009). Hence, antagonizing the peripheral acyl ghrelin pathway alone may not be sufficient for treating obesity. The sum of other fasting-related signals, such as a drop in glucose, insulin, and leptin concentrations, may play the predominant role in this process. Therefore, further studies using combination therapies that block multiple orexigenic pathways are warranted to provide further insight in better understanding the contributions of acyl ghrelin in overall intake, body weight regulation, and the formation of obesity.

In contrast to conventional pharmacotherapy, bariatric surgery, especially gastric bypass, in which most of the stomach and duodenum are bypassed with the use of a gastrojejunal anastomosis, is the only validated, substantial, sustained, and effective way to provide long-term weight loss and improved lifestyle for patients with morbid obesity (Sjöström et al., 2004). Gastric bypass not only decreases overall mortality compared with conventional treatment (Sjöström et al., 2004) but also demonstrates an advantageous outcome concerning body composition and dietary intake, including an avoidance of fat, compared with vertical banded gastroplasty (Olbbers et al., 2006). Plasma total ghrelin level increased after diet control-induced weight loss (Cummings et al., 2002b; Frühbeck et al., 2004b), whereas 24-h plasma total ghrelin concentration was abnormally low (Cummings et al., 2002b; Frühbeck et al., 2004b) or tended to decrease (Korner et al., 2009) after gastric bypass surgery. The elevated plasma total ghrelin level easily induces hunger sensation for those subjects on diet, and thus hyperphagia occurs, often resulting in body weight regain and leading to failure of diet control. Furthermore, the reduction in circulating total ghrelin concentration in patients receiving Roux-en-Y gastric bypass was not determined by an active weight loss or an improved insulin sensitivity but rather depended on the surgically induced bypass of the ghrelin-producing cell population of the fundus (Frühbeck et al., 2004a,b), because adjustable gastric banding and biliopancreatic diversion, which conserved direct contact of the fundus with ingested food, exhibited higher fasting plasma total ghrelin levels than that in Roux-en-Y gastric bypass (Frühbeck et al., 2004a). Likewise, plasma total ghrelin and obestatin levels were decreased after Roux-en-Y gastric bypass (Suzuki et al., 2005) or microgastric bypass (Stenström et al., 2006) in rats. Most importantly, meal suppression of circulating total (Korner et al., 2006; Engström et al., 2007) and acyl (Korner et al., 2005, 2006) ghrelin was normalized in obese persons after gastric bypass surgery. On the other hand, gastric banding surgery prevented the increase of plasma total

ghrelin level after weight loss in obese Zucker rats, which supported the hypothesis that sustained weight loss observed after gastric banding did not depend solely on food restriction (Monteiro et al., 2007). However, restrictive surgery, such as gastric banding, sometimes resulted in increased (Nijhuis et al., 2004; Korner et al., 2009) or unchanged (Hanusch-Enserer et al., 2003) plasma total ghrelin level after operation in humans. In addition, gastric bypass surgery improved human metabolic and hepatic abnormalities associated with nonalcoholic fatty liver disease (Mattar et al., 2005; Barker et al., 2006; Klein et al., 2006; Furuya et al., 2007). Moreover, because gastrointestinal malignancies may be associated with obesity, pharmacologic and surgical avenues available for treatment of obesity, including lipase inhibitors and gastric or jejunoileal bypass procedures, may set the stage for subsequent gastrointestinal tract cancer.

In brief summary, obesity is the outcome of the dysregulation of a central network of neuropeptidergic and monoaminergic circuits that provide an interface between genetic background and the environment (Yildiz et al., 2004). Nibbling at ghrelin gene products/GHS-R1a system through medical, surgical, or combination therapy, especially manipulating regulation of GOAT at the gastric fundus level, should be therapeutically relevant to morbid obesity and constitute a strategy for the treatment of overeating and obesity.

B. Anorexia and Cachexia

1. Cancer Anorexia-Cachexia Syndrome. Ghrelin is very attractive because it can attenuate cancer-induced anorexia and cachexia. Acyl ghrelin and des-acyl ghrelin were demonstrated in normal tissues and various tumorous tissues, such as human pituitary adenomas (Kim et al., 2001; Korbonits et al., 2001a,b; Martínez-Fuentes et al., 2006), fetal thyroid and follicular tumors (Volante et al., 2003), adrenocortical tumors (Barzon et al., 2005), medullary thyroid carcinoma cell lines (Kanamoto et al., 2001; Morpurgo et al., 2005), gastric endocrine tumors (Papotti et al., 2001; Rindi et al., 2002; Rayhan et al., 2005), intestinal endocrine tumors (Papotti et al., 2001; Rayhan et al., 2005), gastrointestinal stromal tumors (Ekeblad et al., 2006), pancreatic endocrine tumors (Iwakura et al., 2002; Volante et al., 2002; Ekeblad et al., 2007), the small-cell lung cancer cell line (Cassoni et al., 2006), bronchial neuroendocrine tumor (Arnaldi et al., 2003), prostate neoplasms and prostate cancer cell lines (Jeffery et al., 2002; Cassoni et al., 2004), testicular tumors (Gaytan et al., 2004), breast cancer tissues (Jeffery et al., 2005), and leukemic cell lines (De Vriese and Delporte, 2007), using either RT-PCR, immunohistochemistry, or in situ hybridization. Moreover, the promoter region of the ghrelin gene was found in human medullary thyroid carcinoma cell line (Nakai et al., 2004), whereas another novel prepro-ghrelin isoform, exon 3-deleted prepro-ghrelin was de-

tected in prostate cancer (Yeh et al., 2005) and breast cancer (Jeffery et al., 2005). On the other hand, GHS-R1a and GHS-R1b, two splice variants of GHS-R, were detected separately and together in human pituitary tumors (Adams et al., 1998; Skinner et al., 1998; Barlier et al., 1999; Korbonits et al., 2001; Kim et al., 2001, 2003a; Martínez-Fuentes et al., 2006), ovarian tumors (Gaytan et al., 2005), astrocytoma (Dixit et al., 2006), adrenocortical tumors (Barzon et al., 2005), gastrointestinal stromal tumors (Ekeblad et al., 2006), pancreatic endocrine tumors (Volante et al., 2002; Ekeblad et al., 2007), the small-cell lung cancer cell line (Cassoni et al., 2006), bronchial neuroendocrine tumor (Arnaldi et al., 2003), prostate neoplasms and prostate cancer cell lines (Jeffery et al., 2002), testicular tumors (Gaytan et al., 2004), breast cancer tissues (Jeffery et al., 2005), and leukemic cell lines (De Vriese and Delporte, 2007). Obestatin has been demonstrated in many endocrine tumors, such as those of the thyroid, parathyroid, stomach, small intestine, appendix, and pancreas (Volante et al., 2009). The expression of obestatin was positively correlated with that of ghrelin in normal and tumorous samples. An immunohistochemical study demonstrated ghrelin immunoreactivity in enterochromaffin-like cells of human stomach, and the authors speculated that gastric enterochromaffin-like cells acquired the capacity to secrete ghrelin early in their hyperplasia-neoplasia sequence (Srivastava et al., 2004). Collectively, these emerging data suggest that ghrelin gene products and their related coupled receptors may play roles (either promoting or inhibiting) in promoting tumor growth via an autocrine/paracrine pathway. However, controversial evidence exists. Some studies reported that ghrelin was not detected in human somatotroph adenomas (Wasko et al., 2006), mucoepidermoid carcinoma of salivary gland (Aydin et al., 2005), esophageal (Mottershead et al., 2007) and gastric (Aydin et al., 2005; Mottershead et al., 2007) adenocarcinoma, and gastrointestinal endocrine tumors with multiple endocrine neoplasia type 1 (Raffel et al., 2005). The authors indicated that an infiltrating adenocarcinoma could decrease ghrelin production and disrupt local paracrine mechanisms by replacing normal gastric mucosa (Mottershead et al., 2007). They also speculated that the absence of ghrelin in these tumors may be important in loss of appetite in these patients. The reasons for these discrepancies need further evaluation.

In addition to these autocrine/paracrine effects, ghrelin could exert anabolic actions in cancer anorexia-cachexia through an endocrine mechanism. Two new tumor entities of stomach and pancreas, gastric ghrelinoma (Tsolakis et al., 2004) and pancreatic ghrelinoma (Corbetta et al., 2003), with hypersecretion of ghrelin into the blood circulation, were identified and characterized. Consistent data from two independent research groups revealed that serum total and acyl ghrelin remained unaltered in patients with prostate can-

cer (Mungan et al., 2008; Bertaccini et al., 2009), implying that insufficient secretion of ghrelin into serum could be responsible. Plasma fasting ghrelin levels were reported to be unchanged in human medullary thyroid carcinomas (Morpurgo et al., 2005), gastric endocrine tumors (Tsolakis et al., 2008), gastric cancer (An et al., 2007; Huang et al., 2007), colorectal cancer (Huang et al., 2007), pancreatic endocrine tumors (Ekeblad et al., 2007), and lung cancer (Shimizu et al., 2003a). Some studies indicated that circulating ghrelin level was decreased in patients with gastric cancer (Isomoto et al., 2005) and colorectal cancer (D'Onghia et al., 2007). Plasma ghrelin level could be increased, unchanged, or decreased in patients with hepatocellular carcinoma (Tacke et al., 2003; Ataseven et al., 2006; Lin and Yin, 2007). The circulating ghrelin level was negatively correlated with tumor staging and lost negative correlation with body mass index in colorectal cancer (D'Onghia et al., 2007), whereas a strong inverse correlation between ghrelin level and α -fetoprotein was observed in hepatocellular carcinoma (Tacke et al., 2003), suggesting that additional studies are necessary to ascertain the potential role of ghrelin as a predictive and prognostic biomarker in these patients. It is noteworthy that plasma ghrelin level was increased in lung cancer patients with cachexia than without cachexia (Shimizu et al., 2003a; Karapanagiotou et al., 2009), indicating a compensatory mechanism under catabolic-anabolic imbalance in patients with cachexia. Elevated serum ghrelin was also found in patients with metastatic neuroendocrine tumors in liver and was positively correlated with body mass index (Wang et al., 2007). Ghrelin could be coreleased from neuroendocrine tumors and exerted an orexigenic effect in these patients to maintain body mass index despite widely disseminated disease. Furthermore, elevated serum ghrelin levels and decreased serum leptin levels were found in female patients with breast and colon cancer who had cachexia (Wolf et al., 2006). Different cancers causing different rates of body weight loss could explain these differences. Further studies showed that acyl ghrelin level and the acyl-to-total ghrelin ratio was increased in subjects with cancer-induced cachexia compared with cancer and noncancer controls (Garcia et al., 2005). However, appetite score was not increased in these subjects with cachexia despite elevated ghrelin, suggesting a state of ghrelin resistance. We speculate that GOAT was up-regulated or des-acylation became slower in these patients with cachexia, because the acyl-to-total ghrelin ratio was increased. High rather than low serum ghrelin was surprisingly reported to be associated with protection against esophageal adenocarcinoma among overweight subjects (de Martel et al., 2007). This could come from anti-inflammatory effect of ghrelin, because ghrelin was demonstrated to have an inhibitory effect on Barrett's carcinogenesis by its anti-inflammatory actions (Kon-turek et al., 2008). Cachexia has been described as a

GH-resistant state with high GH levels but low IGF-I (Crown et al., 2002). Taken together, cancer cachexia should be a status with both GH and ghrelin resistance, and acyl ghrelin should play a role in signaling and reversing states of energy insufficiency under these circumstances. Future prospective studies using state-of-art techniques to separately measure acyl ghrelin, des-acyl ghrelin, and obestatin are urgently demanded to clarify the differential roles of ghrelin gene products in patients with cancer anorexia and cachexia.

There are few current treatment options for cancer anorexia-cachexia, whereas acyl ghrelin agonist and melanocortin-receptor antagonists are two kinds of promising drugs among them (Bossola et al., 2006). Plasma total ghrelin concentration was increased and leptin concentration was decreased in cachectic nude mice inoculated with two different human melanoma cells (Hanada et al., 2003, 2004) and normal mice implanted with sarcoma (Wang et al., 2006). Ghrelin biosynthesis and secretion were up-regulated with the progression of cachexia, and exogenous administration efficiently counteracted body weight loss in these cachectic mice (Hanada et al., 2003, 2004), suggesting that elevated ghrelin may represent a compensatory mechanism and have a therapeutic ability to ameliorate cancer cachexia. However, a recent study indicated that continuous intravenous infusion of rat acyl ghrelin failed to stimulate feeding in tumor-bearing rats, although acyl ghrelin-infused tumor-bearing rats exhibited elevated hypothalamic NPY and reduced hypothalamic POMC message (Chance et al., 2008). Different ways of drug administration (pulsatile versus continuous dosing) and different species (mice versus rats) could explain the discrepancies, implying that continuous acyl ghrelin infusion might not be an effective treatment for cancer anorexia. Another study using a rat model of cancer cachexia, implanted with methylcholanthrene sarcoma, showed that exogenous injection of human acyl ghrelin and a synthetic acyl ghrelin analog BIM-28131 (DeBoer et al., 2007; DeBoer et al., 2008) efficiently improved food intake and lean body mass in these tumor-bearing rats and accompanied increased hypothalamic expression of orexigenic genes and decreased expression of the transcript of interleukin-1 receptor (DeBoer et al., 2007). Hence, acyl ghrelin and a synthetic ghrelin analog improved cancer anorexia and cachexia through appetite-regulating and anti-inflammatory mechanisms. Wisse et al. (2001) demonstrated that intracerebroventricular injection of melanocortin receptor antagonist SHU9119 completely reversed cancer anorexia in tumor-bearing rats, whereas the orexigenic effects of intracerebroventricular acyl ghrelin and NPY were blunted relative to those in control rats. The orexigenic effect of exogenous acyl ghrelin was partially blunted in tumor-bearing mice compared with normal mice (Wang et al., 2006), suggesting the appearance of acyl ghrelin resistance. Moreover, *GHS-R1a* expression in the hypothalamus of the cachec-

tic mice was up-regulated. Therefore, other factors downstream of the ghrelin-GHS-R1a system should be more important than acyl ghrelin for the pathogenesis of cancer anorexia-cachexia. Taken together, all these implied greater value of melanocortin receptor antagonists in the treatment of cancer anorexia-cachexia syndrome.

In a pilot human trial, intravenous infusion of exogenous human acyl ghrelin successfully stimulated energy intake in seven patients with cancer who had impaired appetite (Neary et al., 2004). A randomized, placebo-controlled, double-blind, double-crossover study confirmed that intravenous administration of human acyl ghrelin was safe and well tolerated in patients with advanced incurable cancer and anorexia/cachexia (Strasser et al., 2008). A phase I, randomized, placebo-controlled, multiple-dose study in healthy volunteers demonstrated that RC-1291 (Garcia and Polvino, 2007), a novel, orally available ghrelin mimetic and GHS, was well tolerated and effective in promoting weight gain with few adverse events (Garcia and Polvino, 2007). The results give hope for the treatment of cancer anorexia-cachexia. Therefore, early detection of cancer-associated anorexia-cachexia syndrome followed by exogenous administration of acyl ghrelin may present an efficient option of combating this deleterious disease. However, acyl ghrelin promoted pancreatic adenocarcinoma cellular proliferation and invasiveness and astrocytoma motility in cell lines (Duxbury et al., 2003; Dixit et al., 2006). A novel association between acyl ghrelin and colorectal cancer has been reported. Ghrelin expression was found to be enhanced in malignant colorectal cells, and it promoted colorectal malignancy by advancing tumor stage through an autocrine/paracrine mechanism (Wassem et al., 2008). Therefore, it is extremely important to determine whether ghrelin is truly carcinogenic or protective against malignancy. We strongly believe that acyl ghrelin will be a new therapeutic target for cancer-related anorexia-cachexia syndrome and/or a new prognostic factor in the next decade.

2. *Noncancerous Anorexia-Cachexia.*

a. Psychogenic: anorexia nervosa. Among psychiatric disorders, anorexia nervosa is a highly morbid pathologic condition with high mortality. These patients experience hunger, but they avoid eating by intense fear of overconsumption and being overweight. Anorexia nervosa often affects young women under 25 years old, and is characterized by weight loss, amenorrhea, and behavioral changes. In addition to marked loss of body weight, this abnormal feeding behavior also leads to metabolic disturbances and is usually life-threatening without proper treatment. Therefore, elucidating the underlying neuroendocrine mechanisms of anorexia nervosa is pivotal for the pharmacotherapy of this disease. In female patients with anorexia nervosa, morning fasting total ghrelin level was significantly higher than those in control (Ariyasu et al., 2001; Nakai et al., 2003; Tolle et al., 2003; Broglio et al., 2004a; Tanaka et al., 2004; Hotta et

al., 2004; Germain et al., 2007; Janas-Kozik et al., 2007) and constitutionally thin subjects (Tolle et al., 2003; Miljic et al., 2006, 2007; Germain et al., 2007). In addition, postprandial decline of plasma total ghrelin response to food intake was lost in these female patients with anorexia nervosa (Nedvídková et al., 2003). These anorectic female subjects also displayed high plasma growth hormone (Tolle et al., 2003; Tanaka et al., 2004; Broglio et al., 2004a; Miljic et al., 2006, 2007; Germain et al., 2007), PYY (Nakahara et al., 2007) and cortisol levels (Tolle et al., 2003; Miljic et al., 2006, 2007; Germain et al., 2007) and low plasma IGF-1 (Tolle et al., 2003; Broglio et al., 2004a; Germain et al., 2007), 17 β -estradiol (Tolle et al., 2003; Germain et al., 2007), triiodothyronine (Tolle et al., 2003; Germain et al., 2007; Nakahara et al., 2007), and leptin levels (Tolle et al., 2003; Miljic et al., 2006; Germain et al., 2007; Nakahara et al., 2007, 2008). In addition, caloric intake, plasma follicle-stimulating hormone levels, and luteinizing hormone levels were lower in patients with anorexia nervosa compared with control and constitutionally thin subjects (Germain et al., 2007). Furthermore, circadian variations of plasma PYY and leptin were blunted in patients with anorexia nervosa (Germain et al., 2007).

Fasting blood glucose tended to be lower in patients with anorexia nervosa, compared with control (Nakai et al., 2003; Tanaka et al., 2004; Janas-Kozik et al., 2007; Nakahara et al., 2007, 2008; Harada et al., 2008) or constitutionally thin subjects (Miljic et al., 2006, 2007). On the other hand, fasting plasma insulin tended to be higher in patients with anorexia nervosa compared with constitutionally thin subjects (Miljic et al., 2006, 2007) but lower compared with controls (Nakahara et al., 2007, 2008). Nutritional rehabilitation completely restored the highly elevated plasma total ghrelin level to normal (Tolle et al., 2003; Tanaka et al., 2004; Janas-Kozik et al., 2007), or partially corrected the abnormal high total ghrelin, GH (Tanaka et al., 2004; Miljic et al., 2006; Nakahara et al., 2007), cortisol and abnormal low leptin (Miljic et al., 2006) levels, in patients with anorexia nervosa. Renutrition from successful treatment of anorexia nervosa also restored insulin secretion and glucose responses to food ingestion in these patients (Nakahara et al., 2007). These observations suggest the possible existence of ghrelin resistance in cachectic states caused by eating disorders, such as anorexia nervosa. The marked elevated fasting total ghrelin and GH concentrations in anorexia nervosa may be up-regulated by a chronic negative energy balance state. Total ghrelin and GH levels in patients with anorexia nervosa may be influenced by acute changes of energy homeostasis during severe emaciation or re-nutrition.

Rapid development of enzyme-linked immunosorbent assay (ELISA) provides us powerful research tools to measure three ghrelin gene products, acyl ghrelin, des-acyl ghrelin, and obestatin, separately. A recent study reported that plasma obestatin, acyl ghrelin, and des-

acyl ghrelin levels were significantly higher in patients with the restrictive type of anorexia nervosa (Harada et al., 2008; Nakahara et al., 2008). All three ghrelin gene products decreased after the oral glucose tolerance test in patient and control groups. The fact that fasting plasma des-acyl ghrelin level was higher in patients with anorexia nervosa and decreased after the oral glucose tolerance test in both patient and control groups, suggesting either a decrease in acylation or an increase in desacylation of ghrelin in anorexia nervosa. Postprandial decline of plasma acyl ghrelin response to the oral glucose tolerance test was blunted in these female patients with anorexia nervosa compared with control subjects (Harada et al., 2008). These imply that ghrelin gene products may serve as markers reflecting both short- and long-term changes of the nutritional state in patients with the restrictive type of anorexia nervosa. However, exogenously intravenous infusion of human acyl ghrelin did not affect GH responses and appetite (in terms of hunger) in patients with anorexia nervosa compared with constitutionally thin subjects but tended to increase sleepiness only in patients with anorexia nervosa (Miljic et al., 2006). In addition, exogenously intravenous infusion of human acyl ghrelin had no effect on glucose metabolism and insulin secretion in patients with anorexia nervosa (Miljic et al., 2007). They concluded that exogenous acyl ghrelin supplementation was unlikely to be effective as a single appetite-stimulatory agent in patients with anorexia nervosa (Miljic et al., 2006). In an independent study, patients with anorexia nervosa exhibited specific reduction in the GH response to exogenous acyl ghrelin administration, despite the hyper-responsiveness to GHRH injection (Broglio et al., 2004a). In contrast, six of nine patients experienced hunger after acyl ghrelin administration (Broglio et al., 2004a). These reflect "desensitization" of the GHS-R1a induced by the chronic elevation of ghrelin level in this pathological condition, and ghrelin itself is unlikely to play a causal role in anorexia nervosa. Further studies may be warranted to investigate the pathogenesis and how to overcome ghrelin resistance in patients with anorexia nervosa for future treatment. Before these, additional studies conducting simultaneous measurement of acyl ghrelin, des-acyl ghrelin, and obestatin should provide a better understanding of metabolic responses to ghrelin in anorexia nervosa.

Future studies are necessary to explore putative molecular mechanisms underlying ghrelin resistance, such as a possible impairment of intracellular GHS-R1a signaling in pathophysiological states presenting with cachexia. On the other hand, plasma ghrelin levels are higher in patients with bulimia nervosa (Tanaka et al., 2002; Kojima et al., 2005), although the body mass index is similar to that of control subjects (Tanaka et al., 2002). Lack of a leptin response to food ingestion was observed in both bulimic and healthy women, compatible with leptin acting as a long-term rather than short-

term modulator of eating behavior (Monteleone et al., 2003). Significantly blunted increase of circulating PYY (Kojima et al., 2005; Monteleone et al., 2005) and reduced suppression of plasma ghrelin (Monteleone et al., 2003, 2005; Kojima et al., 2005) after the test meal were demonstrated in bulimic and healthy women, whereas plasma ghrelin suppression after the meal was significantly correlated with plasma PYY increase in the patients (Monteleone et al., 2005). No significant differences emerged in food-induced plasma insulin and glucose changes between bulimic and healthy women (Monteleone et al., 2005). Moreover, for both patients with anorexia nervosa and those with bulimia nervosa with habitual binge/purge behavior, there were significant correlations among plasma ghrelin values, frequencies of binge/purge cycles and serum amylase values, whereas in bulimia nervosa with nonpurging type, there were no significant correlations among plasma ghrelin values, frequencies of binge-eating episodes and serum amylase values (Tanaka et al., 2003). These results indicated that habitual binge/purge cycles with vomiting may have a greater influence on fasting plasma ghrelin concentration in binge eating behavior, and a gut-hypothalamic pathway involving peripheral signals, especially ghrelin, may be involved in the pathophysiology of bulimia nervosa. However, normal endocrine and metabolic responses to exogenous ghrelin administration were observed patients with purging bulimia nervosa and healthy women (Fassino et al., 2005). A further study revealed that circulating ghrelin increased after modified sham feeding in both groups, with patients with bulimia nervosa exhibiting a greater ghrelin increase that positively correlated with the patients' weekly frequency of binge-purging (Monteleone et al., 2009). Finally, ghrelin concentrations and cardiac vagal tone were decreased after pharmacologic and cognitive-behavioral treatment in patients with bulimia nervosa (Tanaka et al., 2006).

b. Nonpsychogenic: chronic heart failure, chronic obstructive pulmonary disease, liver cirrhosis, and chronic kidney disease.

i. Chronic heart failure. Patients with end-stage chronic heart failure (CHF) are characterized by dysfunction and remodeling of left ventricle and cardiac cachexia (including weight loss and muscle wasting). It is noteworthy that cardiac cachexia is a strong independent risk factor for morbidity and mortality in patients with CHF. Plasma total ghrelin level was determined in a Japanese population (Nagaya et al., 2001b). Plasma total ghrelin level did not differ between patients with CHF and healthy subjects. However, plasma total ghrelin level was higher in patients with CHF and cachexia (defined as those with documented nonedematous and nonintentional weight loss of >7.5% of the previous normal nonedematous weight over a period of at least 6 months) than in those without cachexia. Moreover, circulating GH, TNF- α , norepinephrine, and angiotensin II

levels were also significantly higher in patients with CHF and cachexia. It is noteworthy that the elevated plasma total ghrelin was associated with a decrease in body mass index (Nagaya et al., 2001a). The elevated plasma total ghrelin level may represent a compensatory mechanism in cachectic patients with CHF. However, some study indicated that plasma ghrelin level remained unchanged, whereas plasma adiponectin level was increased irrespective of body mass index in cachectic patients with CHF compared with healthy control subjects (Araújo et al., 2009). In a rat model with doxorubicin-induced heart failure, endogenous plasma total ghrelin level was increased compared with control subjects (Xu et al., 2008). The increased endogenous plasma total ghrelin concentration was positively correlated with cardiac output and high-energy phosphates, suggesting that the increased endogenous plasma total ghrelin level could represent a compensatory self-protective effect by improving cardiac function and retaining myocardial energy reserve in the rats with doxorubicin-induced heart failure. Acyl ghrelin elicits stimulation of feeding (Chen et al., 2005b) and adiposity (Tschöp et al., 2000). Therefore, acyl ghrelin may serve as a rational drug target for ameliorating anorexia, cachexia, and cardiovascular dysfunction in the treatment of CHF. To date, the role of obestatin in CHF remains uninvestigated.

In patients with CHF, short-term intravenous infusion of human acyl ghrelin decreased mean arterial pressure and systemic vascular resistance, increased cardiac index and stroke volume index, and elicited GH, prolactin, adrenocorticotropin, cortisol, and epinephrine secretion but had no renal effects (no change in urine volume, urinary sodium excretion, and creatinine clearance) (Nagaya et al., 2001c). All data obtained from short-term injection of acyl ghrelin suggest that acyl ghrelin may have beneficial effects on CHF.

Controversial results exist regarding long-term administration of acyl ghrelin in the treatment of rat models with CHF. In rats with CHF induced by ligation of the left coronary artery, 3-week intermittent subcutaneous administration (twice a day) of acyl ghrelin improved left ventricular dysfunction and attenuated the development of cardiac cachexia (Nagaya et al., 2001d). Serum GH and IGF-1 levels were higher in both CHF and sham-operated rats treated with acyl ghrelin than in sham-operated rats given with placebo. Rats with CHF exhibited impairment of body weight gain and a significant decrease in the muscle/bone ratio (gastrocnemius muscle weight/tibial length). Long-term injection of acyl ghrelin promoted body weight gain in sham-operated rats and also restored the impaired increase of body weight in rats with CHF. In addition, acyl ghrelin treatment partially restored the decrease in the muscle/bone ratio. In addition, long-term administration of acyl ghrelin improved left ventricular dysfunction, as indicated by increases in cardiac output and stroke volume,

and attenuated the development of left ventricular remodeling, as shown by an increase in posterior wall thickness, inhibition of progressive left ventricular enlargement, and in turn, a reduce in left ventricular wall stress (Nagaya et al., 2001d). However, 1-week intermittent subcutaneous administration (once a day) of acyl ghrelin indicated that acyl ghrelin played only a minor role in controlling rat cardiac function (Torsello et al., 2003). The other rat model with CHF induced by ligation of the left coronary artery revealed that neither 2-week continuous infusion by intravenous pump nor 4-week intermittent subcutaneous injection (three times a day) of acyl ghrelin affected cardiac function (Akashi et al., 2009). However, acyl ghrelin treatment increased body weight, lean mass, and fat mass in rats with CHF. The data indicated that long-term administration of acyl ghrelin did not improve cardiac function, regardless of the application route, but inhibited the development of cachectic status in rats with CHF (Akashi et al., 2009). In a human trial, repeated intravenous administration of human acyl ghrelin (twice a day) for 3 weeks significantly increased left ventricular ejection fraction, decreased left ventricular end-systolic volume, increased peak workload and peak oxygen consumption during exercise, and improved muscle wasting in cachectic patients with CHF (Nagaya et al., 2004). Acyl ghrelin treatment also increased food intake score and decreased plasma norepinephrine, epinephrine, and brain natriuretic peptide levels in cachectic patients with CHF (Nagaya et al., 2004). To date, few therapies other than ghrelin have been shown to be beneficial in cardiac cachexia. Taken together, these data indicate that administration of acyl ghrelin may be a novel therapeutic approach for the treatment of CHF. Further studies may investigate the plasma ghrelin changes in patients with CHF before and after percutaneous transluminal coronary angioplasty or heart transplantation and those in cancer patients with doxorubicin-induced heart failure.

ii. Chronic obstructive pulmonary disease. In Asian patients with chronic obstructive pulmonary disease (COPD), plasma ghrelin level was found to be higher in underweight patients (BMI <20 kg/m²) than in normal weight patients (BMI ≥20 kg/m²) and healthy subjects (Itoh et al., 2004; Ying et al., 2008). Serum concentrations of inflammatory markers, such as TNF- α (Itoh et al., 2004; Luo et al., 2005; Ying et al., 2008), interleukin-6 (Itoh et al., 2004; Ying et al., 2008), and norepinephrine (Itoh et al., 2004), were higher in underweight patients than those in normal weight patients and control subjects. In contrast, the other study reported that only serum high sensitivity C-reactive protein, interleukin-6, and interleukin-8, but not TNF- α , were elevated in patients with COPD compared with control subjects (Piehl-Aulin et al., 2009). The elevation of plasma ghrelin in underweight patients with COPD may reflect the negative energy balance, because plasma ghrelin was negatively correlated with body mass index (Itoh et al.,

2004; Ying et al., 2008) and positively correlated with catabolic factors, such as circulating TNF- α (Itoh et al., 2004; Ying et al., 2008) and norepinephrine (Itoh et al., 2004). Insulin concentration was lower in underweight patients than that in normal-weight patients with COPD and healthy control subjects (Itoh et al., 2004; Ying et al., 2008), whereas IGF-1 concentration was reported to be either increased or decreased in patients with COPD (Itoh et al., 2004; Piehl-Aulin et al., 2009). Furthermore, plasma ghrelin level was positively correlated with percentage of predicted residual volume and residual volume-to-total lung capacity ratio (Itoh et al., 2004) and negatively correlated with forced expiratory volume in 1 s (Ying et al., 2008). The elevation of plasma ghrelin level in these underweight patients is highly linked with a cachectic state and abnormality of pulmonary function (Itoh et al., 2004; Ying et al., 2008). Cachexia is an independent risk factor for mortality in COPD. Thus, pulmonary cachexia should be a therapeutic target in the treatment of COPD. Hence, supplement with exogenous acyl ghrelin may play some roles in ameliorating pulmonary cachexia and abnormal pulmonary functional capacity in underweight patients with COPD. On the other hand, contradictory results also exist. Plasma ghrelin levels were reported to be lower (Luo et al., 2005) or to remain unchanged (Piehl-Aulin et al., 2009) in patients with COPD, whereas plasma leptin levels were either decreased (Luo et al., 2005) or unchanged (Piehl-Aulin et al., 2009). These discrepancies probably result from differences in studied subjects included and distinct ghrelin measuring methods.

In the follow-up study, repeated intravenous administration of human acyl ghrelin (twice a day) in seven cachectic patients with COPD for 3 weeks significantly increased appetite, body weight, lean body mass, hand-grip strength, and maximal inspiratory pressure (Nagaya et al., 2005). In addition, Karnofsky performance status score and the distance walked in 6 min were improved after 3-week therapy of acyl ghrelin injection. Exogenous acyl ghrelin treatment also attenuated the exaggerated sympathetic nerve activity, as indicated by a marked decrease in plasma norepinephrine level (Nagaya et al., 2005). These preliminary results imply that the optimistic of exogenous acyl ghrelin pharmacotherapy in the improvement of body composition, muscle wasting, functional capacity, and amelioration of augmented sympathetic nerve activity in cachectic patients with COPD. However, another prospective study in another ethnic group recruiting a larger patient population with longer treatment period is demanded to validate the efficacy and safety for the treatment of exogenous acyl ghrelin using pharmacologic dose in these patients. To date, the role of obestatin in COPD remains uninvestigated.

iii. Liver cirrhosis. Malnutrition is very common in patients with liver cirrhosis, and the prevalence is reported as high as 80% depending on the severity of liver

disease. Cirrhotic patients often develop anorexia and subsequently exhibit body weight and muscle loss. Because ghrelin is one of the key hormones in modulating feeding behavior and calorie status, biological actions of ghrelin may be highly linked with protein-energy malnutrition in patients with cirrhosis.

On the other hand, patients with liver cirrhosis had a lower energy intake, higher resting energy expenditure (Kalaitzakis et al., 2007), higher fasting sugar (Kalaitzakis et al., 2007, 2009) and leptin levels (Breidert et al., 2004; Ataseven et al., 2006; Kalaitzakis et al., 2007, 2009), and higher insulin resistance (Kalaitzakis et al., 2007, 2009). Plasma fasting total ghrelin levels were found to be either unchanged (Marchesini et al., 2004; Kalaitzakis et al., 2007, 2009), elevated (Tacke et al., 2003; Ataseven et al., 2006), or decreased (Breidert et al., 2004; Diz-Lois et al., 2009) in patients with cirrhosis compared with control subjects. Separate measurement of acyl and des-acyl ghrelin could solve the discrepancy. Plasma postprandial total ghrelin level was reduced in patients with liver cirrhosis (Kalaitzakis et al., 2007, 2009; Diz-Lois et al., 2009). High plasma fasting total ghrelin level was found to be associated with a low calorie intake, the anorexia score, and adjustment of body mass index in patients with cirrhosis (Marchesini et al., 2004). Therefore, in the presence of anorexia, hyperghrelinemia may indicate a compensatory mechanism trying to stimulate food intake, which is nonetheless ineffective in the physiological range in liver cirrhosis. In patients with cirrhosis and hyperghrelinemia, plasma GH level was increased (Diz-Lois et al., 2009), whereas plasma IGF-1 level was decreased (Tacke et al., 2003; Diz-Lois et al., 2009), which also suggests GH resistance in these patients. In patients with liver cirrhosis, plasma fasting total ghrelin level increased in Child class C cirrhosis, linked with complications of chronic liver disease, such as previous gastrointestinal bleeding, ascites, and hepatic encephalopathy (Tacke et al., 2003), and was negatively correlated with plasma leptin (Ataseven et al., 2006) and GH levels (Diz-Lois et al., 2009) and positively correlated with plasma TNF- α level (Ataseven et al., 2006). The reduced postprandial total ghrelin concentration was reported to be associated with delayed gastric emptying in patients with cirrhosis (Kalaitzakis et al., 2009). On the other hand, plasma fasting des-acyl ghrelin, known as another ghrelin gene product, was slightly but not significantly elevated in patients with liver cirrhosis (Takahashi et al., 2006a). Des-acyl ghrelin level was negatively correlated with plasma leptin level, body mass index, arm muscular circumference, triceps skinfold thickness, the substrate oxidation rates of glucose and fat as well as nonprotein respiratory quotients. These data suggest that plasma fasting des-acyl ghrelin level may be a useful indicator reflecting malnutrition in patients with cirrhosis. To date, the role of obestatin in liver cirrhosis remains uninvestigated.

In a rat model with hepatic injury induced by bile duct ligation, exogenously injected acyl ghrelin reversed all oxidant responses, restored impaired liver function, elevated serum cytokines, and elevated total histological scores of liver injury (Içseri et al., 2008). Further clinical trials using ghrelin administration for its orexigenic, anabolic, and anti-inflammatory properties in pharmacological amounts to ameliorate anorexia-cachexia syndromes in patients with liver cirrhosis are strongly required.

iv. Chronic kidney disease. Anorexia is present in approximately 33 to 40% of patients receiving hemodialysis and has detrimental effects on nutritional status, quality of life, and survival (Bossola et al., 2006; Muscaritoli et al., 2007). The role of ghrelin and melanocortin-receptor antagonists (Bossola et al., 2006) seems promising. Therefore, it is interesting to investigate the role of ghrelin in patients with chronic kidney disease (CKD) or end-stage renal disease. The kidney is the primary site of ghrelin clearance, although ghrelin clearance is reduced at the late stage of rat sepsis (Wu et al., 2003). Plasma fasting total (Rodriguez Ayala et al., 2004; Jarkovská et al., 2005a, 2005b; Tentolouris et al., 2005; Iglesias et al., 2006; Chang et al., 2005; Barazzoni et al., 2008; Arbeiter et al., 2009), acyl (Yoshimoto et al., 2002; Pérez-Fontán et al., 2004; Chang et al., 2005; Jarkovská et al., 2005a; Barazzoni et al., 2008), and des-acyl (Yoshimoto et al., 2002; Muscaritoli et al., 2007) ghrelin levels were all elevated in patients with CKD, regardless of the modality of therapy using either hemodialysis or peritoneal dialysis. Plasma GH and IGF-1 levels were also increased, but neither of them was shown to be associated with the elevated plasma ghrelin level in these patients (Jarkovská et al., 2005a). However, the other two studies reported that the plasma GH level was positively correlated with the elevated plasma ghrelin level (Yoshimoto et al., 2002; Iglesias et al., 2006). Age was demonstrated negatively correlated with plasma fasting acyl ghrelin in patients with CKD (Pérez-Fontán et al., 2004). Plasma concentrations of inflammatory markers such as leptin (Jarkovská et al., 2005b) and C-reactive protein (Barazzoni et al., 2008) were also increased in patients with CKD. Plasma total ghrelin, acyl ghrelin, and des-acyl ghrelin levels were effectively removed from the blood after a single course of hemodialysis in patients with CKD (Yoshimoto et al., 2002; Pérez-Fontán et al., 2004). Plasma ghrelin level was negatively correlated with glomerular filtration rate (Arbeiter et al., 2009) and serum creatinine clearance (Ueno et al., 2007) in subjects with and without CKD. Nevertheless, contradictory results showed that only peritoneal dialysis, instead of hemodialysis, were accompanied by a striking decrement of plasma total ghrelin concentration in patients with CKD (Iglesias et al., 2006). In addition, 12-month treatment with peritoneal dialysis decreased the elevated plasma total ghrelin level and increased body fat in patients with CKD (Ro-

driguez Ayala et al., 2004). Taken together, all evidence indicates that the kidney is an important site for clearance and/or degradation of ghrelin and that ghrelin accumulates in patients with CKD.

Self-reports of appetite level have been validated as a useful predictor of outcome in patients with CKD undergoing hemodialysis (Carrero et al., 2007). Anorexia, instead of hyperphagia, was found in patients receiving hemodialysis with a higher area-under-the-curve of plasma acyl ghrelin level. These suggest there is ghrelin resistance (central, peripheral, or both) in patients with CKD. Hyperghrelinemia in uremic patients with anorexia may be a compensatory pathway rather than a causative factor in mediating uremic anorexia and cachexia. Moreover, hyperghrelinemia and poor appetite were found in pediatric patients with CKD, whereas plasma total ghrelin level returned to normal and appetite was improved after renal transplantation (Arbeiter et al., 2009). On the other side, plasma des-acyl ghrelin level was higher in anorexic than nonanorexic patients with CKD undergoing hemodialysis (Muscaritoli et al., 2007), suggesting that des-acyl ghrelin might play a pathogenic role in poor appetite of patients with CKD. A recent report indicated that resting energy expenditure was similar in patients with maintenance hemodialysis and healthy control subjects (Barazzoni et al., 2008). This implies that anorexia plays a more important role than energy expenditure in mediating CKD-related cachexia, which worsens the prognosis in patients with CKD. Hence, additional prospective studies with a larger sample size and a longer observational period are warranted to further clarify the causative role of des-acyl ghrelin in uremia-related anorexia. To date, the role of obestatin in CKD remains uninvestigated.

Recently, a rat model with CKD induced by five sixths nephrectomy resulted in decreased food intake and lean body mass, whereas treatment with acyl ghrelin and small-molecule agonists of GHS-R1a, such as BIM-28125 (Deboer et al., 2008) and BIM-28131, successfully improved them, in part mediated via a decrease in muscle actinomycin degradation (Deboer et al., 2008). Hypothalamic *NPY*, *AgRP*, and *POMC* gene expression was unchanged, whereas prohormone convertase-2 level in the hypothalamus was increased in rats with CKD, which resulted an increase in the processing of *POMC* and subsequently an increase in the secretion of downstream anorexigenic hormones leading to hypophagia, such as α -melanocyte stimulating hormone. Combined pro-inflammatory cytokines were elevated in these rats with CKD, whereas acyl ghrelin and small-molecule ghrelin analogs blocked these elevations. In addition, treatment with acyl ghrelin and small-molecule ghrelin analogs down-regulated *interleukin-1 receptor 1* expression in the brainstem in rats with CKD, which exhibited the anti-inflammatory effect of ghrelin and agonists of GHS-R1a. Thus, acyl ghrelin treatment caused increasing hypothalamic feeding center outflow and an overall

decrease in circulating cytokines to ameliorate cachexia induced by CKD. All together, these data highlight the potential clinical application of small-molecule agonists of GHS-R1a that have a longer half-life and oral bio-availability in future therapeutic use in human CKD. However, another similar study revealed that exogenously injected acyl ghrelin transiently stimulated appetite in young rats with CKD but did not increase daily food intake and improve growth (Alvarez-García et al., 2007). In a human randomized, placebo-controlled trial, subcutaneous single-dose acyl ghrelin administration enhanced short-term food intake in patients receiving dialysis with mild to moderate malnutrition (Wynne et al., 2005). Longer-term studies in humans with CKD are required. Finally, further investigation is urgently needed to better understand the pathogenic mechanisms of CKD-related anorexia-cachexia syndrome and to determine whether amelioration of anorexia and improvement of food intake would result in a long-term benefit in terms of improved quality of life and reduced morbidity and mortality in patients with CKD. A recent study revealed that acyl ghrelin administration in 12 malnourished patients receiving dialysis increased ghrelin levels in circulation, modestly reduced blood pressure for up to 2 h, immediately and significantly increased appetite, and had an increase in energy intake, noted at the first study meal; this effect persisted throughout the week without altering energy expenditure (Ashby et al., 2009). Because malnutrition is a common complication in patients receiving dialysis and carries poor prognosis, direct manipulation of appetite with acyl ghrelin or its analogs represents an attractive and promising therapeutic strategy for this difficult clinical problem. All these efforts provide us useful clues for the potential development of more effective preventive and therapeutic interventions in human CKD.

XII. Therapeutic Approaches to Affecting the Ghrelin System: Endogenous Ghrelin Gene Products, Growth Hormone Secretagogue Receptor 1a, and Ghrelin O-acyltransferase

Pharmacological approaches using affecting endogenous acyl ghrelin and GHS-R1a provide us new strategies for the treatment of obesity and metabolic disorders, because acyl ghrelin possess appetite-stimulating and adipogenetic effects. Central administration of the GHS-R1a antagonist JMV 2959 is promising. Central injection of JMV 2959 abolished fat accumulation without altering acyl ghrelin-induced food intake in rats (Salomé et al., 2009b). However, the primary barrier for JMV 2959 is its administration route, and the effects of peripheral application of JMV 2959 need to be determined. Orally available GHS-R1a antagonists, such as quinazolinone (Rudolph et al., 2007), to promote long-term body weight loss and improve glucose homeostasis, are underdeveloped for the treatment of obesity and

diabetes. In addition to affecting acyl ghrelin and GHS-R1a, gastric electrical stimulation could be another option in the treatment of obesity. Acyl ghrelin induced antral contractions and increased food intake in dogs, whereas gastric electrical stimulation was capable of blocking these excitatory effects of acyl ghrelin (Yin and Chen, 2006). These findings suggest that gastric electrical stimulation may inhibit the resistant effect of acyl ghrelin on weight loss. Finally, diet is an important but easily overlooked factor in our treatment of obesity and metabolic syndrome.

In contrast, analogs of acyl ghrelin or GHS-R1a agonists could combat human anorexia-cachexia such as cancerous and noncancerous states. Acyl ghrelin replacement therapy may alleviate weight loss associated with gastrectomy. Activation of the acyl ghrelin/GHS-R1a system is able to restore the normal feeding pattern and energy balance in the elderly and in cancer patients with impaired appetite. Oral MK-677 increased fat-free mass in human elderly (Nass et al., 2008). In addition, the small-molecule GHS-R1a agonist BIM-28131 and the orally available RC-1291 are very promising. Moreover, traditional herb drugs containing acyl ghrelin mimetics, such as Rikkunshito (or TJ-43) (Takeda et al., 2008; Fujitsuka et al., 2009), which stimulates the release of acyl ghrelin via blockade of serotonin mechanism, are alternatives for analogs of acyl ghrelin or GHS-R1a agonists in the treatment of human anorexia-cachexia.

The nonpeptidergic ghrelin receptor agonists [GSK894490A (Atcha et al., 2009) and CP-464709-18] readily crossed the blood-brain barrier and significantly improved performance in the novel object recognition and modified water maze tests in rats (Atcha et al., 2009). Central obestatin has sleep-promoting and memory-enhancing effects. Disturbance of sleep predisposes to the development of obesity. These observations reveal endogenous functions of ghrelin gene products that link metabolic control with higher brain functions and suggest novel therapeutic strategies to enhance learning and memory processes. Besides improving life quality and maintaining independence, GHS-R1a agonists show potential as interventional agents against aging (Smith et al., 2007). The primary barrier is still the administration route of the developed drugs.

The ability of acyl ghrelin to promote gastrointestinal motility makes it feasible to treat delayed gastrointestinal transit in various pathological conditions. Analogs of acyl ghrelin or stimulators on GHS-R1a may represent new classes of prokinetic agents, such as TZP-101, in future treatments for patients with gastroparesis of either diabetic (Murray et al., 2005; Ejskjaer et al., 2009), neurogenic (Binn et al., 2006), idiopathic (Tack et al., 2005), or symptomatic (Wargin et al., 2009) origin, as well as postoperative ileus (Lasseter et al., 2008). Furthermore, ipamorelin has clinical implication in the treatment of patients with postoperative anorexia and

body weight loss (Venkova et al., 2009). The emerging therapeutics may advance the care of patients with postoperative ileus.

GOAT may be a therapeutic target for eating disorders or other metabolic diseases. Further work on how stomach GOAT is regulated could provide another important step in unraveling the effect of ghrelin on feeding and glucose homeostasis control, which will improve our understanding of obesity and its treatment. Identification of GOAT will facilitate the search for inhibitors that reduce appetite and diminish obesity in humans. First, to date, ghrelin is the only protein to be octanoylated; inhibition of GOAT is unlikely to affect other proteins and may have effects only on the stomach. Second, small-molecule inhibitors of enzymes are generally more feasible to develop than small-molecule mimetics of peptide hormones. Hence, GOAT presents a novel therapeutic target. Future studies using *GOAT*-knockout mice and tissue-specific genetic ablation of *GOAT* will enable clarification of the paracrine roles of ghrelin in different tissues, and particular emphasis should be given to the stomach, pancreas, and hypothalamus (Gualillo et al., 2008).

As loss- and gain-of-function models prove their roles in modulating diet-induced adiposity, modulators of ghrelin-GHS-R-GOAT may be potential novel antiobesity drugs and anticachexia therapeutics. The development of therapy seems attractive and has potential, but it will be costly. Finally, we need to carefully define the "precise indications" for patients most likely to benefit from this treatment.

XIII. Conclusions

During the past decade, numerous gastrointestinally derived peptides have been associated with significant effects on food intake, gut motility, energy balance, and nutrition partition. Among these peptides, the breakthrough discovery of acyl ghrelin, another two ghrelin gene products (des-acyl ghrelin and obestatin), GHS-R1a, and GOAT is amazing and defy several well recognized, traditional biochemical principles. Acyl ghrelin, des-acyl ghrelin, obestatin, GHS-R, and GOAT just look like five pieces of the same puzzle, and investigation of them is extremely attractive and challenging. The ghrelin system has been one of the most extensively investigated gut peptides in the last decade, with more than 3862 publications in 10 years listed on PubMed. Acyl ghrelin not only strongly stimulates GH secretion but is also involved in energy homeostasis by eliciting food intake and promoting adiposity through a GH-independent mechanism. The ghrelin system has been shown to play a role in multiple physiological processes, including appetite regulation, metabolism, anxiety, and, more recently, dendritic spine architecture, long-term potentiation, and cognition.

In summary, acyl ghrelin, des-acyl ghrelin, obestatin, GHS-R, and GOAT may be part of a system with multiple effector elements and constitutes the center of an integrated gut-brain energy axis, modulating appetite, digestion, gut motility, adiposity, and energy partition. Emerging groundbreaking fundamental advances in basic research could translate into potential therapies across many clinical specialties, including endocrinology, gastroenterology, cardiology, oncology, psychiatry, nutrition, and immunology. The investigation of ghrelin gene products and GHS-R1a and GOAT unravels the pathogenesis of mammalian diseases and opens up new paradigms for drugs that can tackle multiple symptoms in various human disorders. Hence, ghrelin gene products, GHS-R, and GOAT may provide a range of therapeutic opportunities to deliver a promising treatment of complex human diseases. Further research will answer and elucidate the biochemical and physiological characteristics of this unique hormone system.

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REFERENCES

- Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschöp MH, et al. (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* **116**:3229–3239.
- Adams EF, Huang B, Buchfelder M, Howard A, Smith RG, Feighner SD, van der Ploeg LH, Bowers CY, and Fahlbusch R (1998) Presence of growth hormone secretagogue receptor messenger ribonucleic acid in human pituitary tumors and rat GH3 cells. *J Clin Endocrinol Metab* **83**:638–642.
- Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, Teramukai S, Seno H, Chiba T, Noma S, et al. (2008) Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. *Eur J Endocrinol* **158**:491–498.
- Akashi YJ, Palus S, Datta R, Halem H, Taylor JE, Thoene-Reineke C, Dong J, Thum T, Culler MD, Anker SD, et al. (2009) No effects of human ghrelin on cardiac function despite profound effects on body composition in a rat model of heart failure. *Int J Cardiol* doi: 10.1016/j.ijcard.2008.06.094.
- Altinova AE, Törner FB, Aktürk M, Elbeğ S, Yetkin I, Cakir N, and Arslan M (2006a) Reduced serum acylated ghrelin levels in patients with hyperthyroidism. *Horm Res* **65**:295–299.
- Altinova AE, Törner F, Karakoc A, Yetkin I, Ayvaz G, Cakir N, and Arslan M (2006b) Serum ghrelin levels in patients with Hashimoto's thyroiditis. *Thyroid* **16**:1259–1264.
- Alvarez-García O, García-López E, Rodríguez J, Gil-Peña H, Molinos I, Carbajo-Pérez E, and Santos F (2007) Administration of ghrelin to young uraemic rats increases food intake transiently, stimulates growth hormone secretion and does not improve longitudinal growth. *Nephrol Dial Transplant* **22**:1309–1313.
- An JY, Choi MG, Noh JH, Sohn TS, Jin DK, and Kim S (2007) Clinical significance of ghrelin concentration of plasma and tumor tissue in patients with gastric cancer. *J Surg Res* **143**:344–349.
- Anderson KA, Ribar TJ, Lin F, Noeldner PK, Green MF, Muehlbauer MJ, Witters LA, Kemp BE, and Means AR (2008) Hypothalamic CaMKK2 contributes to the regulation of energy balance. *Cell Metab* **7**:377–388.
- Andrews ZB, Liu ZW, Wallingford N, Erion DM, Borok E, Friedman JM, Tschöp MH, Shanabrough M, Cline G, Shulman GI, et al. (2008) UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature* **454**:846–851.
- Annemie VD, Debby VD, Valentijn V, Bart de S, Walter L, Liliane S, and Peter Paul de D (2009) Central administration of obestatin fails to show inhibitory effects on food and water intake in mice. *Regul Pept* **156**:77–82.
- Araújo JP, Lourenço P, Rocha-Gonçalves F, Ferreira A, and Bettencourt P (2009) Adiponectin is increased in cardiac cachexia irrespective of body mass index. *Eur J Heart Fail* **11**:567–572.
- Arbeiter AK, Büscher R, Petersenn S, Hauffa BP, Mann K, and Hoyer PF (2009) Ghrelin and other appetite-regulating hormones in paediatric patients with chronic renal failure during dialysis and following kidney transplantation. *Nephrol Dial Transplant* **24**:643–646.
- Arnaldi G, Mancini T, Kola B, Appolloni G, Freddi S, Concettoni C, Bearzi I, Masini A, Boscaro M, and Mantero F (2003) Cyclical Cushing's syndrome in a patient with a bronchial neuroendocrine tumor (typical carcinoid) expressing ghrelin and growth hormone secretagogue receptors. *J Clin Endocrinol Metab* **88**:5834–5840.
- Ariga H, Imai K, Chen C, Mantyh C, Pappas TN, and Takahashi T (2008a) Fixed feeding potentiates interdigestive gastric motor activity in rats: importance of eating habits for maintaining interdigestive MMC. *Am J Physiol Gastrointest Liver Physiol* **294**:G655–G659.
- Ariga H, Nakade Y, Tsukamoto K, Imai K, Chen C, Mantyh C, Pappas TN, and Takahashi T (2008c) Ghrelin accelerates gastric emptying via early manifestation of antro-pyloric coordination in conscious rats. *Regul Pept* **146**:112–116.
- Ariga H, Tsukamoto K, Chen C, Mantyh C, Pappas TN, and Takahashi T (2007) Endogenous acyl ghrelin is involved in mediating spontaneous phase III-like contractions of the rat stomach. *Neurogastroenterol Motil* **19**:675–680.
- Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K, Ogawa Y, Hosoda K, Akamizu T, Kojima M, et al. (2002) Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. *Endocrinology* **143**:3341–3350.
- Ariyasu H, Takaya K, Iwakura H, Hosoda H, Akamizu T, Arai Y, Kangawa K, and Nakao K (2005) Transgenic mice overexpressing des-acyl ghrelin show small phenotype. *Endocrinology* **146**:355–364.
- Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, et al. (2001) Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* **86**:4753–4758.
- Arnold M, Mura A, Langhans W, and Geary N (2006) Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. *J Neurosci* **26**:11052–60.
- Arosio M, Ronchi CL, Gebbia C, Cappiello V, Beck-Peccoz P, and Peracchi M (2003) Stimulatory effects of ghrelin on circulating somatostatin and pancreatic polypeptide levels. *J Clin Endocrinol Metab* **88**:701–704.
- Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, and Kasuga M (2005) Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* **54**:18–24.
- Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, and Kasuga M (2003) Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* **52**:947–952.
- Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, Katsuura G, Makino S, Fujino MA, and Kasuga M (2001a) A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* **74**:143–147.
- Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Nijijima A, Fujino MA, et al. (2001b) Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* **120**:337–345.
- Ashby DR, Ford HE, Wynne KJ, Wren AM, Murphy KG, Busbridge M, Brown EA, Taube DH, Ghatei MA, Tam FW, et al. (2009) Sustained appetite improvement in malnourished dialysis patients by daily ghrelin treatment. *Kidney Int* **76**:199–206.
- Ataka K, Inui A, Asakawa A, Kato I, and Fujimiya M (2008) Obestatin inhibits motor activity in the antrum and duodenum in the fed state of conscious rats. *Am J Physiol Gastrointest Liver Physiol* **294**:G1210–G1218.
- Ataseven H, Bahecioglu IH, Kuzu N, Yalniz M, Celebi S, Erensoy A, and Ustundag B (2006) The levels of ghrelin, leptin, TNF-alpha, and IL-6 in liver cirrhosis and hepatocellular carcinoma due to HBV and HDV infection. *Mediators Inflamm* **2006**:78380.
- Atcha Z, Chen WS, Ong AB, Wong FK, Neo A, Browne ER, Witherington J, and Pemberton DJ (2009) Cognitive enhancing effects of ghrelin receptor agonists. *Psychopharmacology (Berl)* **206**:415–427.
- Aydin S, Ozercan IH, Geckil H, Dagli F, Aydin S, Kumru S, Kilic N, Sahin I, and Ozercan MR (2007b) Ghrelin is present in teeth. *J Biochem Mol Biol* **40**:368–372.
- Aydin S, Ozercan IH, Dagli F, Aydin S, Dogru O, Celebi S, Akin O, and Guzel SP (2005) Ghrelin immunohistochemistry of gastric adenocarcinoma and mucocypidermoid carcinoma of salivary gland. *Biotech Histochem* **80**:163–168.
- Bagnasco M, Tulipano G, Melis MR, Argiolas A, Cocchi D, and Muller EE (2003) Endogenous ghrelin is an orexigenic peptide acting in the arcuate nucleus in response to fasting. *Regul Pept* **111**:161–167.
- Balasubramaniam A, Wood S, Joshi R, Su C, Friend LA, Sheriff S, and James JH (2006) Ghrelin stimulates food intake and growth hormone release in rats with thermal injury: synthesis of ghrelin. *Peptides* **27**:1624–1631.
- Baldanzi G, Filigheddu N, Cutrupi S, Catapano F, Bonisconi S, Fubini A, Malan D, Baj G, Granata R, Broglio F, et al. (2002) 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.
- Banks WA, Burney BO, and Robinson SM (2008) Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. *Peptides* **29**:2061–2065.
- Banks WA, Tschöp M, Robinson SM, and Heiman ML (2002) Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* **302**:822–827.
- Barazzoni R, Bosutti A, Stebel M, Cattin MR, Roder E, Visintin L, Cattin L, Biolo G, Zanetti M, and Guarnieri G (2005) Ghrelin regulates mitochondrial-lipid metabolism gene expression and tissue fat distribution in liver and skeletal muscle. *Am J Physiol Endocrinol Metab* **288**:E228–E235.
- Barazzoni R, Zanetti M, Ferreira C, Vinci P, Pirulli A, Mucci M, Dore F, Fonda M, Ciochi B, Cattin L, et al. (2007) Relationships between desacylated and acylated ghrelin and insulin sensitivity in the metabolic syndrome. *J Clin Endocrinol Metab* **92**:3935–3940.
- Barazzoni R, Zanetti M, Stulle M, Mucci MP, Pirulli A, Dore F, Panzetta G, Vasile A, Biolo G, and Guarnieri G (2008) Higher total ghrelin levels are associated with higher insulin-mediated glucose disposal in non-diabetic maintenance hemodialysis patients. *Clin Nutr* **27**:142–149.
- Barker KB, Palekar NA, Bowers SP, Goldberg JE, Pulcini JP, and Harrison SA (2006) Non-alcoholic steatohepatitis: effect of Roux-en-Y gastric bypass surgery. *Am J Gastroenterol* **101**:368–373.
- Barlier A, Zamora AJ, Grino M, Gunz G, Pellegrini-Boulier I, Morange-Ramos I, Figarella-Branger D, Dufour H, Jaquet P, and Enjalbert A (1999) Expression of functional growth hormone secretagogue receptors in human pituitary adenomas: polymerase chain reaction, triple in-situ hybridization and cell culture studies. *J Neuroendocrinol* **11**:491–502.

- Barzon L, Pacenti M, Masi G, Stefani AL, Fincati K, and Palù G (2005) Loss of growth hormone secretagogue receptor 1a and overexpression of type 1b receptor transcripts in human adrenocortical tumors. *Oncology* **68**:414–421.
- Bassil AK, Häglund Y, Brown J, Rudholm T, Hellström PM, Näslund E, Lee K, and Sanger GJ (2007a) Little or no ability of obestatin to interact with ghrelin or modify motility in the rat gastrointestinal tract. *Br J Pharmacol* **150**:58–64.
- Becskei C, Bilik KU, Klussmann S, Jarosch F, Lutz TA, and Riediger T (2008) The anti-ghrelin Spiegelmer NOX-B11-3 blocks ghrelin- but not fasting-induced neuronal activation in the hypothalamic arcuate nucleus. *J Neuroendocrinol* **20**:85–92.
- Bedendi I, Alloati G, Marcantoni A, Malan D, Catapano F, Ghé C, Deghenghi R, Ghigo E, and Muccioli G (2003) Cardiac effects of ghrelin and its endogenous derivatives des-octanoyl ghrelin and des-Gln¹⁴-ghrelin. *Eur J Pharmacol* **476**:87–95.
- Bericik P, Verdú EF, Foster JA, Lu J, Scharringa A, Kean I, Wang L, Blennerhassett P, and Collins SM (2009) Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of *Helicobacter pylori* infection. *Am J Physiol Regul Integr Comp Physiol* **296**:R587–R594.
- Bertaccini A, Perneti R, Marchiori D, Pagotto U, Palladaro F, Palmieri F, Vitullo G, Guidi M, and Martorana G (2009) Variations in blood ghrelin levels in prostate cancer patients submitted to hormone suppressive treatment. *Anticancer Res* **29**:1345–1348.
- Bewick GA, Gardiner JV, Dhillon WS, Kent AS, White NE, Webster Z, Ghatei MA, and Bloom SR (2005) Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype. *FASEB J* **19**:1680–1682.
- Bewick GA, Kent A, Campbell D, Patterson M, Ghatei MA, Bloom SR, and Gardiner JV (2009) Mice with hyperghrelinemia are hyperphagic and glucose intolerant and have reduced leptin sensitivity. *Diabetes* **58**:840–846.
- Binn M, Albert C, Gougeon A, Maerki H, Coulie B, Lemoine M, Rabasa Lhoret R, Tomasetto C, and Poitras P (2006) Ghrelin gastrokintetic action in patients with neurogenic gastroparesis. *Peptides* **27**:1603–1606.
- Bomberg EM, Grace MK, Wirth MM, Levine AS, and Olszewski PK (2007) Central ghrelin induces feeding driven by energy needs not by reward. *Neuroreport* **18**:591–595.
- Bossola M, Tazza L, Giungi S, and Luciani G (2006) Anorexia in hemodialysis patients: an update. *Kidney Int* **70**:417–422.
- Braclik M, Marcisz C, Giebel S, and Orzel A (2008) Serum leptin and ghrelin levels in premenopausal women with stable body mass index during treatment of thyroid dysfunction. *Thyroid* **18**:545–550.
- Breider M, Zimmermann TF, Schneider R, Ehninger G, and Brabant G (2004) Ghrelin/Leptin-imbalance in patients with primary biliary cirrhosis. *Exp Clin Endocrinol Diabetes* **112**:123–126.
- Bresciani E, Rapetti D, Donà F, Bulgarelli I, Tamiazzo L, Locatelli V, and Torsello A (2006) Obestatin inhibits feeding but does not modulate GH and corticosterone secretion in the rat. *J Endocrinol Invest* **29**:RC16–RC18.
- Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, and Ghigo E (2001) Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab* **86**:5083–5086.
- Broglio F, Benso A, Castiglioni C, Gottero C, Prodram F, Destefanis S, Gauna C, van der Lely AJ, Deghenghi R, Bo M, et al. (2003a) The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. *J Clin Endocrinol Metab* **88**:1537–1542.
- Broglio F, Gianotti L, Destefanis S, Fassino S, Abbate Daga G, Mondelli V, Lanfranco F, Gottero C, Gauna C, Hofland L, et al. (2004a) The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state. *Clin Endocrinol (Oxf)* **60**:592–599.
- Broglio F, Gottero C, Benso A, Prodram F, Destefanis S, Gauna C, Maccario M, Deghenghi R, van der Lely AJ, and Ghigo E (2003b) Effects of ghrelin on the insulin and glycemic responses to glucose, arginine, or free fatty acids load in humans. *J Clin Endocrinol Metab* **88**:4268–4272.
- Broglio F, Gottero C, Prodram F, Destefanis S, Gauna C, Me E, Riganti F, Vivenza D, Rapa A, Martina V, et al. (2004b) Ghrelin secretion is inhibited by glucose load and insulin-induced hypoglycemia but unaffected by glucagon and arginine in humans. *Clin Endocrinol (Oxf)* **61**:503–509.
- Broglio F, Gottero C, Prodram F, Gauna C, Muccioli G, Papotti M, Aribat T, Van Der Lely AJ, and Ghigo E (2004c) Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. *J Clin Endocrinol Metab* **89**:3062–3065.
- Broglio F, Koetsveld P, Benso A, Gottero C, Prodram F, Papotti M, Muccioli G, Gauna C, Hofland L, Deghenghi R, et al. (2002b) Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. *J Clin Endocrinol Metab* **87**:4829–4832.
- Broglio F, Prodram F, Riganti F, Gottero C, Destefanis S, Granata R, Muccioli G, Aribat T, van der Lely AJ, and Ghigo E (2008) The continuous infusion of acylated ghrelin enhances growth hormone secretion and worsens glucose metabolism in humans. *J Endocrinol Invest* **31**:788–794.
- Brownley KA, Light KC, Grewen KM, Bragdon EE, Hinderliter AL, and West SG (2004) Postprandial ghrelin is elevated in black compared with white women. *J Clin Endocrinol Metab* **89**:4457–4463.
- Brzozowski T, Konturek PC, Konturek SJ, Kwiecień S, Drodzowicz D, Bielanski W, Pajdo R, Ptak A, Nikiforuk A, Pawlik WW, et al. (2004) Exogenous and endogenous ghrelin in gastroprotection against stress-induced gastric damage. *Regul Pept* **120**:39–51.
- Brzozowski T, Konturek PC, Sliwowski Z, Pajdo R, Drodzowicz D, Kwiecień S, Burnat G, Konturek SJ, and Pawlik WW (2006) Prostaglandin/cyclooxygenase pathway in ghrelin-induced gastroprotection against ischemia-reperfusion injury. *J Pharmacol Exp Ther* **319**:477–487.
- Butler MG and Bittel DC (2007) Plasma obestatin and ghrelin levels in subjects with Prader-Willi syndrome. *Am J Med Genet A* **143**:415–421.
- Callahan HS, Cummings DE, Pepe MS, Breen PA, Matthys CC, and Weigle DS (2004) Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. *J Clin Endocrinol Metab* **89**:1319–1324.
- Camiña JP, Campos JF, Caminos JE, Dieguez C, and Casanueva FF (2007) Obestatin-mediated proliferation of human retinal pigment epithelial cells: regulatory mechanisms. *J Cell Physiol* **211**:1–9.
- Caminos JE, Gualillo O, Lago F, Otero M, Blanco M, Gallego R, Garcia-Caballero T, Goldring MB, Casanueva FF, Gomez-Reino JJ, et al. (2005) The endogenous growth hormone secretagogue (ghrelin) is synthesized and secreted by chondrocytes. *Endocrinology* **146**:1285–1292.
- Caminos JE, Seoane LM, Tovar SA, Casanueva FF, and Dieguez C (2002) Influence of thyroid status and growth hormone deficiency on ghrelin. *Eur J Endocrinol* **147**:159–163.
- Carlini VP, Gaydou RC, Schiöth HB, and de Barioglio SR (2007a) Selective serotonin reuptake inhibitor (fluoxetine) decreases the effects of ghrelin on memory retention and food intake. *Regul Pept* **140**:65–73.
- Carlini VP, Martini AC, Schiöth HB, Ruiz RD, Fiol de Cuneo M, and de Barioglio SR (2008) Decreased memory for novel object recognition in chronically food-restricted mice is reversed by acute ghrelin administration. *Neuroscience* **153**:929–934.
- Carlini VP, Monzón ME, Varas MM, Cragolini AB, Schiöth HB, Scimonelli TN, and de Barioglio SR (2002) Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem Biophys Res Commun* **299**:739–743.
- Carlini VP, Schiöth HB, and Debarioglio SR (2007b) Obestatin improves memory performance and causes anxiolytic effects in rats. *Biochem Biophys Res Commun* **352**:907–912.
- Carlini VP, Varas MM, Cragolini AB, Schiöth HB, Scimonelli TN, and de Barioglio SR (2004) Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem Biophys Res Commun* **313**:635–641.
- Carpino PA, Lefker BA, Toler SM, Pan LC, Haddock JR, Murray MC, Cook ER, DiBriño JN, DeNinno SL, Chidsey-Frink, et al. (2002) Discovery and biological characterization of capromorelin analogues with extended half-lives. *Bioorg Med Chem Lett* **12**:3279–3282.
- Carrero JJ, Qureshi AR, Axelsson J, Avesani CM, Suliman ME, Kato S, Bárány P, Snaedal-Jonsdóttir S, Alvestrand A, Heimbürger O, et al. (2007) Comparison of nutritional and inflammatory markers in dialysis patients with reduced appetite. *Am J Clin Nutr* **85**:695–701.
- Carvajal P, Carlini VP, Schiöth HB, de Barioglio SR, and Salvatierra NA (2009) Central ghrelin increases anxiety in the Open Field test and impairs retention memory in a passive avoidance task in neonatal chicks. *Neurobiol Learn Mem* **91**:402–407.
- Cassoni P, Allia E, Marrocco T, Ghé C, Ghigo E, Muccioli G, and Papotti M (2006) Ghrelin and cortistatin in lung cancer: expression of peptides and related receptors in human primary tumors and in vitro effect on the H345 small cell carcinoma cell line. *J Endocrinol Invest* **29**:781–790.
- Cassoni P, Ghé C, Marrocco T, Tarabra E, Allia E, Catapano F, Deghenghi R, Ghigo E, Papotti M, and Muccioli G (2004) Expression of ghrelin and biological activity of specific receptors for ghrelin and des-acyl ghrelin in human prostate neoplasms and related cell lines. *Eur J Endocrinol* **150**:173–184.
- Chance WT, Dayal R, Friend LA, Thomas I, and Sheriff S (2008) Continuous intravenous infusion of ghrelin does not stimulate feeding in tumor-bearing rats. *Nutr Cancer* **60**:75–90.
- Chang CC, Hung CH, Yen CS, Hwang KL, and Lin CY (2005) The relationship of plasma ghrelin level to energy regulation, feeding and left ventricular function in non-diabetic haemodialysis patients. *Nephrol Dial Transplant* **20**:2172–2177.
- Chang L, Zhao J, Li GZ, Geng B, Pan CS, Qi YF, and Tang CS (2004) Ghrelin protects myocardium from isoproterenol-induced injury in rats. *Acta Pharmacol Sin* **25**:1131–1137.
- Chartrel N, Alvear-Perez R, Leprince J, Iturriz X, Reaux-Le Goazigo A, Audinot V, Chomar P, Coge F, Nosjean O, Rodriguez M, et al. (2007) Comment on “obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin’s effects on food intake”. *Science* **315**:766; author reply 766.
- Chen CY, Chao Y, Chang FY, Chien EJ, Lee SD, and Doong ML (2005a) Intracerebral des-acyl ghrelin inhibits food intake and non-nutrient gastric emptying in conscious rats. *Int J Mol Med* **16**:695–699.
- Chen CY, Chien EJ, Chang FY, Lu CL, Luo JC, and Lee SD (2008a) Impacts of peripheral obestatin on colonic motility and secretion in conscious fed rats. *Peptides* **29**:1603–1608.
- Chen CY, Doong ML, Chien EJ, Luo JC, Lu CL, Lin HC, Chang FY, and Lee SD (2008b) Intracerebroventricular ghrelin enhances non-nutrient semiliquid gastric emptying in fasted conscious rats. *Gastroenterol J Taiwan* **25**:242–248.
- Chen CY, Fujimiya M, Asakawa A, Chang FY, Cheng JT, Lee SD, and Inui A (2009) At the cutting edge: ghrelin gene products in food intake and gut motility. *Neuroendocrinology* **89**:9–17.
- Chen CY, Fujimiya M, Asakawa A, Lee SD, and Inui A (2008c) Regulation of ingestive behavior, the upper gastrointestinal motility and gastric acid secretion by ghrelin in mammals. *Curr Nutr Food Sci* **4**:32–40.
- Chen CY, Inui A, Asakawa A, Fujino K, Kato I, Chen CC, Ueno N, and Fujimiya M (2005b) Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. *Gastroenterology* **129**:8–25.
- Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, et al. (2004) Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* **145**:2607–2612.
- Choe YH, Jin DK, Kim SE, Song SY, Paik KH, Park HY, Oh YJ, Kim AH, Kim JS, Kim CW, et al. (2005) Hyperghrelinemia does not accelerate gastric emptying in Prader-Willi syndrome patients. *J Clin Endocrinol Metab* **90**:3367–3370.
- Coiro V, Saccani-Jotti G, Minelli R, Melani A, Milli B, Manfredi G, Volpi R, and Chiodera P (2005) Adrenocorticotropin/cortisol and arginine-vasopressin secretory patterns in response to ghrelin in normal men. *Neuroendocrinology* **81**:103–106.
- Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, and Low MJ (2001) The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int J Obes Relat Metab Disord* **25** (Suppl 5):S63–S67.
- Corbetta S, Peracchi M, Cappiello V, Lania A, Lauri E, Vago L, Beck-Peccoz P, and Spada A (2003) Circulating ghrelin levels in patients with pancreatic and gastro-

- intestinal neuroendocrine tumors: identification of one pancreatic ghrelinoma. *J Clin Endocrinol Metab* **88**:3117–3120.
- Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, Strassburger CJ, Bidlingmaier M, Esterman M, Heiman ML, et al. (2003) The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* **37**:649–661.
- Crown AL, Cottle K, Lightman SL, Falk S, Mohamed-Ali V, Armstrong L, Millar AB, and Holly JM (2002) What is the role of the insulin-like growth factor system in the pathophysiology of cancer cachexia, and how is it regulated? *Clin Endocrinol (Oxf)* **56**:723–733.
- Cui C, Ohnuma H, Daimon M, Susa S, Yamaguchi H, Kameda W, Jimbu Y, Oizumi T, and Kato T (2008) Ghrelin infused into the portal vein inhibits glucose-stimulated insulin secretion in Wistar rats. *Peptides* **29**:1241–1246.
- Cui RJ and Appleyard SM (2009) Ghrelin inhibits visceral afferent activation of catecholamine neurons in the solitary tract nucleus. *Appetite* **52**:824.
- Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A, and Weigle DS (2002a) Elevated plasma ghrelin levels in Prader Willi syndrome. *Nat Med* **8**:643–644.
- Cummings DE, Frayo RS, Marmonier C, Aubert R, and Chapelot D (2004) Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* **287**:E297–E304.
- Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, and Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* **50**:1714–1719.
- Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, and Purnell JQ (2002b) Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* **346**:1623–1630.
- Currie PJ, Mirza A, Fuld R, Park D, and Vasselli JR (2005) Ghrelin is an orexigenic and metabolic signaling peptide in the arcuate and paraventricular nuclei. *Am J Physiol Regul Integr Comp Physiol* **289**:R353–R358.
- Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, and Nakazato M (2000) 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.
- Date Y, Murakami N, Toshinai K, Matsukura S, Nijijima A, Matsuo H, Kangawa K, and Nakazato M (2002) The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* **123**:1120–1128.
- Date Y, Shimbara T, Koda S, Toshinai K, Ida T, Murakami N, Miyazato M, Kokame K, Ishizuka Y, Ishida Y, et al. (2006) Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. *Cell Metab* **4**:323–331.
- Date Y, Toshinai K, Koda S, Miyazato M, Shimbara T, Tsuruta T, Nijijima A, Kangawa K, and Nakazato M (2005) Peripheral interaction of ghrelin with cholecystokinin on feeding regulation. *Endocrinology* **146**:3518–3525.
- Davenport AP, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, Spedding M, Kojima M, and Kangawa K (2005) International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. *Pharmacol Rev* **57**:541–546.
- Davies JS, Kotokorpi P, Eccles SR, Barnes SK, Tokarczuk PF, Allen SK, Whitworth HS, Guschina IA, Evans BA, Mode A, et al. (2009) Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. *Mol Endocrinol* **23**:914–924.
- Deboer MD, Zhu X, Levasseur PR, Inui A, Hu Z, Han G, Mitch WE, Taylor JE, Halem HA, Dong JZ, et al. (2008) Ghrelin treatment of chronic kidney disease: improvements in lean body mass and cytokine profile. *Endocrinology* **149**:827–835.
- DeBoer MD, Zhu XX, Levasseur P, Meguid MM, Suzuki S, Inui A, Taylor JE, Halem HA, Dong JZ, Datta R, et al. (2007) Ghrelin treatment causes increased food intake and retention of lean body mass in a rat model of cancer cachexia. *Endocrinology* **148**:3004–3012.
- Delhanty PJ, van der Eerden BC, van der Velde M, Gauna C, Pols HA, Jahr H, Chiba H, van der Lely AJ, and van Leeuwen JP (2006) Ghrelin and unacylated ghrelin stimulate human osteoblast growth via mitogen-activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K) pathways in the absence of GHS-R1a. *J Endocrinol* **188**:37–47.
- DelParigi A, Tschöp M, Heiman ML, Salbe AD, Vozarova B, Sell SM, Bunt JC, and Tataranni PA (2002) High circulating ghrelin: a potential cause for hyperphagia and obesity in prader-will syndrome. *J Clin Endocrinol Metab* **87**:5461–5464.
- Deng F, Ling J, Ma J, Liu C, and Zhang W (2008) Stimulation of intramembranous bone repair in rats by ghrelin. *Exp Physiol* **93**:872–879.
- Depoortere I, De Winter B, Thijs T, De Man J, Pelckmans P, and Peeters T (2005) Comparison of the gastroprokinetic effects of ghrelin, GHRP-6 and motilin in rats in vivo and in vitro. *Eur J Pharmacol* **515**:160–168.
- Depoortere I, Thijs T, Moehars D, De Smet B, Ver Donck L, and Peeters TL (2008) Effect of peripheral obestatin on food intake and gastric emptying in ghrelin-knockout mice. *Br J Pharmacol* **153**:1550–1557.
- Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, and Yada T (2004) Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signaling in beta-cells: implication in the glycemic control in rodents. *Diabetes* **53**:3142–3151.
- Dezaki K, Kakei M, and Yada T (2007) Ghrelin uses Galphai2 and activates voltage-dependent K^{+} channels to attenuate glucose-induced Ca^{2+} signaling and insulin release in islet beta-cells: novel signal transduction of ghrelin. *Diabetes* **56**:2319–2327.
- Dezaki K, Sone H, Koizumi M, Nakata M, Kakei M, Nagai H, Hosoda H, Kangawa K, and Yada T (2006) Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* **55**:3486–3493.
- de Martel C, Haggerty TD, Corley DA, Vogelmann JH, Orentreich N, and Parsonnet J (2007) Serum ghrelin levels and risk of subsequent adenocarcinoma of the esophagus. *Am J Gastroenterol* **102**:1166–1172.
- De Smet B, Thijs T, Peeters TL, and Depoortere I (2007) Effect of peripheral obestatin on gastric emptying and intestinal contractility in rodents. *Neurogastroenterol Motil* **19**:211–217.
- De Spiegeleer B, Vergote V, Pezeshki A, Peremans K, and Burvenich C (2008) Impurity profiling quality control testing of synthetic peptides using liquid chromatography-photodiode array-fluorescence and liquid chromatography-electrospray ionization-mass spectrometry: the obestatin case. *Anal Biochem* **376**:229–234.
- De Vriese C and Delpote C (2007) Autocrine proliferative effect of ghrelin on leukemic HL-60 and THP-1 cells. *J Endocrinol* **192**:199–205.
- De Winter BY, De Man JG, Seerden TC, Depoortere I, Herman AG, Peeters TL, and Pelckmans PA (2004) Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. *Neurogastroenterol Motil* **16**:439–446.
- Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, Gaskin FS, Nonaka N, Jaeger LB, Banks WA, et al. (2006) Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* **9**:381–388.
- Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakhthivel SK, Palaniappan R, Lillard JW Jr., and Taub DD (2004) Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* **114**:57–66.
- Dixit VD, Weeraratna AT, Yang H, Bertak D, Cooper-Jenkins A, Riggins GJ, Eberhart CG, and Taub DD (2006) Ghrelin and the growth hormone secretagogue receptor constitute a novel autocrine pathway in astrocytoma motility. *J Biol Chem* **281**:16681–16690.
- Dixit VD, Yang H, Cooper-Jenkins A, Giri BB, Patel K, and Taub DD (2009) Reduction of T cell-derived ghrelin enhances proinflammatory cytokine expression: implications for age-associated increases in inflammation. *Blood* **113**:5202–5205.
- Dixit VD, Yang H, Sun Y, Weeraratna AT, Youm YH, Smith RG, and Taub DD (2007) Ghrelin promotes thymopoiesis during aging. *J Clin Invest* **117**:2778–2790.
- Diz-Lois MT, Garcia-Buela J, Suarez F, Sangiao-Alvarellos S, Vidal O, and Cordido F (2009) Fasting and postprandial plasma ghrelin levels are decreased in patients with liver failure previous to liver transplantation. *Endocrine* **35**:467–476.
- Doi A, Shono T, Nishi M, Furuta H, Sasaki H, and Nanjo K (2006) IA-2beta, but not IA-2, is induced by ghrelin and inhibits glucose-stimulated insulin secretion. *Proc Natl Acad Sci U S A* **103**:885–890.
- D'Onghia V, Leocini R, Carli R, Santoro A, Gigliani S, Sorbellini F, Marzocca G, Bernini A, Campagna S, Marinello E, et al. (2007) Circulating gastrin and ghrelin levels in patients with colorectal cancer: correlation with tumor stage, Helicobacter pylori infection and BMI. *Biomed Pharmacother* **61**:137–141.
- Dornonville de la Cour C, Lindström E, Norlén P, and Håkanson R (2004) Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells. *Regul Pept* **120**:23–32.
- Dornonville de la Cour C, Lindqvist A, Eggecioglu E, Tung YC, Surve V, Ohlsson C, Jansson JO, Erlanson-Albertsson C, Dickson SL, and Håkanson R (2005) Ghrelin treatment reverses the reduction in weight gain and body fat in gastrectomized mice. *Gut* **54**:907–913.
- Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, and Woods SC (2006) Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology* **147**:23–30.
- Druce MR, Neary NM, Small C, Milton J, Monteiro M, Patterson M, Gbatei MA, and Bloom SR (2006) Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *Int J Obes (Lond)* **30**:293–296.
- Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Gbatei MA, Small C, and Bloom SR (2005) Ghrelin increases food intake in obese as well as lean subjects. *Int J Obes (Lond)* **29**:1130–1136.
- Dun SL, Brailoiu GC, Brailoiu E, Yang J, Chang JK, and Dun NJ (2006) Distribution and biological activity of obestatin in the rat. *J Endocrinol* **191**:481–489.
- Duxbury MS, Waseem T, Ito H, Robinson MK, Zinner MJ, Ashley SW, and Whang EE (2003) Ghrelin promotes pancreatic adenocarcinoma cellular proliferation and invasiveness. *Biochem Biophys Res Commun* **309**:464–468.
- Dzaja A, Dalal MA, Himmerich H, Uhr M, Pollmächer T, and Schulz A (2004) Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *Am J Physiol Endocrinol Metab* **286**:E963–E967.
- Edholm T, Levin F, Hellström PM, and Schmidt PT (2004) Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. *Regul Pept* **121**:25–30.
- Egcecioglu E, Bjursell M, Ljunberg A, Dickson SL, Kopchick JJ, Bergström G, Svensson L, Oscarsson J, Törnell J, and Bohlöly-Y M (2006) Growth hormone receptor deficiency results in blunted ghrelin feeding response, obesity, and hypolipidemia in mice. *Am J Physiol Endocrinol Metab* **290**:E317–E325.
- Egido EM, Hernández R, Marco J, and Silvestre RA (2009) Effect of obestatin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas. *Regul Pept* **152**:61–66.
- Ejksjaer N, Vestergaard ET, Hellström PM, Gormsen LC, Madsbad S, Madsen JL, Jensen TA, Pezzullo JC, Christiansen JS, Shaughnessy L, et al. (2009) Ghrelin receptor agonist (TZP-101) accelerates gastric emptying in adults with diabetes and symptomatic gastroparesis. *Aliment Pharmacol Ther* **29**:1179–1187.
- Ekeblad S, Lejonklou MH, Grimfjärd P, Johansson T, Eriksson B, Grmelius L, Stridsberg M, Ståhlberg P, and Skogseid B (2007) Co-expression of ghrelin and its receptor in pancreatic endocrine tumours. *Clin Endocrinol (Oxf)* **66**:115–122.
- Ekeblad S, Nilsson B, Lejonklou MH, Johansson T, Ståhlberg P, Nilsson O, Ahlman H, and Skogseid B (2006) Gastrointestinal stromal tumors express the orexigen ghrelin. *Endocr Relat Cancer* **13**:963–970.
- Emanuel AJ, Dihn TT, and Ritter S (2009) Hindbrain catecholamine neurons contribute to the growth hormone but not the feeding response to ghrelin. *Appetite* **52**:829.
- English PJ, Ashcroft A, Patterson M, Dovey TM, Halford JC, Harrison J, Eccleston D, Bloom SR, Gbatei MA, and Wilding JP (2007) Metformin prolongs the postprandial fall in plasma ghrelin concentrations in type 2 diabetes. *Diabetes Metab Res Rev* **23**:299–303.

- English PJ, Ghatei MA, Malik IA, Bloom SR, and Wilding JP (2002) Food fails to suppress ghrelin levels in obese humans. *J Clin Endocrinol Metab* **87**:2984–2987.
- Engström BE, Ohrvall M, Sundbom M, Lind L, and Karlsson FA (2007) Meal suppression of circulating ghrelin is normalized in obese individuals following gastric bypass surgery. *Int J Obes (Lond)* **31**:476–480.
- Enomoto M, Nagaya N, Uematsu M, Okumura H, Nakagawa E, Ono F, Hosoda H, Oya H, Kojima M, Kamatsuse K, et al. (2003) Cardiovascular and hormonal effects of subcutaneous administration of ghrelin, a novel growth hormone-releasing peptide, in healthy humans. *Clin Sci (Lond)* **105**:431–435.
- Erdmann J, Lippl F, Wagenfeil S, and Schusdziarra V (2005) Differential association of basal and postprandial plasma ghrelin with leptin, insulin, and type 2 diabetes. *Diabetes* **54**:1371–1378.
- Esler WP, Rudolph J, Claus TH, Tang W, Barucci N, Brown SE, Bullock W, Daly M, Decarr L, Li Y, et al. (2007) Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss. *Endocrinology* **148**:5175–5185.
- Fassino S, Daga GA, Mondelli V, Pierò A, Broglio F, Picu A, Giordano R, Baldi M, Arvat E, Ghigo E, et al. (2005) Hormonal and metabolic responses to acute ghrelin administration in patients with bulimia nervosa. *Psychoneuroendocrinology* **30**:534–540.
- Faulconbridge LF, Cummings DE, Kaplan JM, and Grill HJ (2003) Hyperphagic effects of brainstem ghrelin administration. *Diabetes* **52**:2260–2265.
- Filigheddu N, Gnocchi VF, Coscia M, Cappelli M, Porporato PE, Taulli R, Traini S, Baldanzi G, Chianale F, Cutrupi S, et al. (2007) Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells. *Mol Biol Cell* **18**:986–994.
- Foster-Schubert KE, Overduin J, Prudom CE, Liu J, Callahan HS, Gaylinn BD, Thorne MO, and Cummings DE (2008) Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. *J Clin Endocrinol Metab* **93**:1971–1979.
- Fraser GL, Venkova K, Hoveyda HR, Thomas H, and Greenwood-Van Meerveld B (2009) Effect of the ghrelin receptor agonist TZP-101 on colonic transit in a rat model of postoperative ileus. *Eur J Pharmacol* **604**:132–137.
- Frühbeck G, Diez-Caballero A, Gil MJ, Montero I, Gómez-Ambrosi J, Salvador J, and Cienfuegos JA (2004a) The decrease in plasma ghrelin concentrations following bariatric surgery depends on the functional integrity of the fundus. *Obes Surg* **14**:606–612.
- Frühbeck G, Rotellar F, Hernández-Lizoain JL, Gil MJ, Gómez-Ambrosi J, Salvador J, and Cienfuegos JA (2004b) Fasting plasma ghrelin concentrations 6 months after gastric bypass are not determined by weight loss or changes in insulinemia. *Obes Surg* **14**:1208–1215.
- Fry M and Ferguson AV (2009) Ghrelin modulates electrical activity of area postrema neurons. *Am J Physiol Regul Integr Comp Physiol* **296**:R485–R492.
- Fukuda H, Mizuta Y, Isomoto H, Takeshima F, Ohnita K, Ohba K, Omagari K, Taniyama K, and Kohno S (2004) Ghrelin enhances gastric motility through direct stimulation of intrinsic neural pathways and capsaicin-sensitive afferent neurones in rats. *Scand J Gastroenterol* **39**:1209–1214.
- Fujimiya M, Asakawa A, Ataka K, Kato I, and Inui A (2008) Different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility in conscious rats. *World J Gastroenterol* **14**:6318–6326.
- Fujino K, Inui A, Asakawa A, Kihara N, Fujimura M, and Fujimiya M (2003) Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol* **550**:227–240.
- Fujitsuka N, Asakawa A, Hayashi M, Sameshima M, Amitani H, Kojima S, Fujimiya M, and Inui A (2009) Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT_{2c} receptor and acyl ghrelin. *Biol Psychiatry* **65**:748–759.
- Fukuhara S, Suzuki H, Masaoka T, Arakawa M, Hosoda H, Minegishi Y, Kangawa K, Ishii H, Kitajima M, and Hibi T (2005) Enhanced ghrelin secretion in rats with cysteamine-induced duodenal ulcers. *Am J Physiol Gastrointest Liver Physiol* **289**:G138–G145.
- Fukushima N, Hanada R, Teranishi H, Fukue Y, Tachibana T, Ishikawa H, Takeda S, Takeuchi Y, Fukumoto S, Kangawa K, et al. (2005) Ghrelin directly regulates bone formation. *J Bone Miner Res* **20**:790–798.
- Furuya CK Jr, de Oliveira CP, de Mello ES, Faintuch J, Raskovski A, Matsuda M, Veazzoo DC, Halpern A, Garrido AB Jr, Alves VA, and Carrilho FJ (2007) Effects of bariatric surgery on nonalcoholic fatty liver disease: preliminary findings after 2 years. *J Gastroenterol Hepatol* **22**:510–514.
- Garcia JM, Garcia-Touza M, Hijazi RA, Taffet G, Epner D, Mann D, Smith RG, Cunningham GR, and Marcelli M (2005) Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. *J Clin Endocrinol Metab* **90**:2920–2926.
- Garcia JM and Polvino WJ (2007) Effect on body weight and safety of RC-1291, a novel, orally available ghrelin mimetic and growth hormone secretagogue: results of a phase I, randomized, placebo-controlled, multiple-dose study in healthy volunteers. *Oncologist* **12**:594–600.
- Gardiner J and Bloom S (2008) Ghrelin gets its GOAT. *Cell Metab* **7**:193–194.
- Gauna C, Delhanty PJ, Hofland LJ, Janssen JA, Broglio F, Ross RJ, Ghigo E, and van der Lely AJ (2005) Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. *J Clin Endocrinol Metab* **90**:1055–1060.
- Gauna C, Kiewiet RM, Janssen JA, van de Zande B, Delhanty PJ, Ghigo E, Hofland LJ, Themmen AP, and van der Lely AJ (2007a) Unacylated ghrelin acts as a potent insulin secretagogue in glucose-stimulated conditions. *Am J Physiol Endocrinol Metab* **293**:E697–E704.
- Gauna C, Meyler FM, Janssen JA, Delhanty PJ, Abribat T, van Koetsveld P, Hofland LJ, Broglio F, Ghigo E, and van der Lely AJ (2007b) 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.
- Gauna C, Uitterlinden P, Kramer P, Kiewiet RM, Janssen JA, Delhanty PJ, van Aken MO, Ghigo E, Hofland LJ, Themmen AP, et al. (2007b) Intravenous glucose administration in fasting rats has differential effects on acylated and unacylated ghrelin in the portal and systemic circulation: a comparison between portal and peripheral concentrations in anesthetized rats. *Endocrinology* **148**:5278–5287.
- Gaytan F, Barreiro ML, Caminos JE, Chopin LK, Herington AC, Morales C, Pinilla L, Paniagua R, Nistal M, Casanueva FF, et al. (2004) Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. *J Clin Endocrinol Metab* **89**:400–409.
- Gaytan F, Morales C, Barreiro ML, Jeffery P, Chopin LK, Herington AC, Casanueva FF, Aguilar E, Dieguez C, and Tena-Sempere M (2005) Expression of growth hormone secretagogue receptor type 1a, the functional ghrelin receptor, in human ovarian surface epithelium, müllerian duct derivatives, and ovarian tumors. *J Clin Endocrinol Metab* **90**:1798–1804.
- Germain N, Galusca B, Le Roux CW, Bossu C, Ghatei MA, Lang F, Bloom SR, and Estour B (2007) Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin. *Am J Clin Nutr* **85**:967–971.
- Gilg S and Lutz TA (2006) The orexigenic effect of peripheral ghrelin differs between rats of different age and with different baseline food intake, and it may in part be mediated by the area postrema. *Physiol Behav* **87**:353–359.
- Giménez-Palop O, Giménez-Pérez G, Mauricio D, Berlanga E, Potau N, Vilardell C, Arroyo J, González-Clemente JM, and Caixàs A (2005) Circulating ghrelin in thyroid dysfunction is related to insulin resistance and not to hunger, food intake or anthropometric changes. *Eur J Endocrinol* **153**:73–79.
- Giménez-Palop O, Giménez-Pérez G, Mauricio D, González-Clemente JM, Potau N, Berlanga E, Trallero R, Laferrère B, and Caixàs A (2007) A lesser postprandial suppression of plasma ghrelin in Prader-Willi syndrome is associated with low fasting and a blunted postprandial PYY response. *Clin Endocrinol (Oxf)* **66**:198–204.
- Gjedde S, Vestergaard ET, Gormsen LC, Riis AL, Rungby J, Møller N, Weeke J, and Jørgensen JO (2008) Serum ghrelin levels are increased in hypothyroid patients and become normalized by L-thyroxine treatment. *J Clin Endocrinol Metab* **93**:2277–2280.
- Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, and Korbonits M (2002) The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* **87**:2988–2991.
- Gómez R, Lago F, Gómez-Reino JJ, Dieguez C, and Gualillo O (2009) Expression and modulation of ghrelin O-acyltransferase in cultured chondrocytes. *Arthritis Rheum* **60**:1704–1709.
- Gonnelli S, Caffarelli C, Del Santo K, Cadirni A, Guerriero C, Lucani B, Franci B, and Nuti R (2008) The relationship of ghrelin and adiponectin with bone mineral density and bone turnover markers in elderly men. *Calcif Tissue Int* **83**:55–60.
- González CR, Vázquez MJ, López M, and Dieguez C (2008) Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *J Mol Endocrinol* **41**:415–421.
- Gormsen LC, Gjedde S, Gjedde S, Vestergaard ET, Christiansen JS, Jørgensen JO, Nielsen S, and Møller N (2006) Free fatty acids decrease circulating ghrelin concentrations in humans. *Eur J Endocrinol* **154**:667–673.
- Gormsen LC, Nielsen C, Gjedde S, Gjedde S, Vestergaard ET, Christiansen JS, Jørgensen JO, and Møller N (2007) Effects of free fatty acids, growth hormone and growth hormone receptor blockade on serum ghrelin levels in humans. *Clin Endocrinol (Oxf)* **66**:641–645.
- Goto M, Arima H, Watanabe M, Hayashi M, Banno R, Sato I, Nagasaki H, and Oiso Y (2006) Ghrelin increases neuropeptide Y and agouti-related peptide gene expression in the arcuate nucleus in rat hypothalamic organotypic cultures. *Endocrinology* **147**:5102–5109.
- Gourcerol G, Coskun T, Craft LS, Mayer JP, Heiman ML, Wang L, Million M, St-Pierre DH, and Taché Y (2007) Preproghrelin-derived peptide, obestatin, fails to influence food intake in lean or obese rodents. *Obesity* **15**:2643–2652.
- Granata R, Settanni F, Biancone L, Trovato L, Nano R, Bertuzzi F, Destefanis S, Annunziata M, Martinetti M, Catapano F, et al. (2007) Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3',5'-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidylinositol 3-kinase/Akt signaling. *Endocrinology* **148**:512–529.
- Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, Nano R, Annunziata M, Campiglia P, Armoletti E, et al. (2008) Obestatin promotes survival of pancreatic beta-cells and human islets and induces expression of genes involved in the regulation of beta-cell mass and function. *Diabetes* **57**:967–979.
- Granata R, Settanni F, Trovato L, Destefanis S, Gallo D, Martinetti M, Ghigo E, and Muccioli G (2006) Unacylated as well as acylated ghrelin promotes cell survival and inhibit apoptosis in HIT-T15 pancreatic beta-cells. *J Endocrinol Invest* **29**:RC19–RC22.
- Green BD, Irwin N, and Flatt PR (2007) Direct and indirect effects of obestatin peptides on food intake and the regulation of glucose homeostasis and insulin secretion in mice. *Peptides* **28**:981–987.
- Greenwood-Van Meerveld B (2007) Emerging drugs for postoperative ileus. *Expert Opin Emerg Drugs* **12**:619–626.
- Grossini E, Molinari C, Mary DA, Ghigo E, Bona G, and Vacca G (2007) Intracoronary ghrelin infusion decreases coronary blood flow in anesthetized pigs. *Endocrinology* **148**:806–812.
- Gualillo O, Lago F, and Dieguez C (2008) Introducing GOAT: a target for obesity and anti-diabetic drugs? *Trends Pharmacol Sci* **29**:398–401.
- Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, and Howard AD (1997) Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* **48**:23–29.
- Guo ZF, Ren AJ, Zheng X, Qin YW, Cheng F, Zhang J, Wu H, Yuan WJ, and Zou L (2008) Different responses of circulating ghrelin, obestatin levels to fasting, re-feeding and different food compositions, and their local expressions in rats. *Peptides* **29**:1247–1254.
- Guo ZF, Zheng X, Qin YW, Hu JQ, Chen SP, and Zhang Z (2007) Circulating

- preprandial ghrelin to obestatin ratio is increased in human obesity. *J Clin Endocrinol Metab* **92**:1875–1880.
- Gutiérrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, Witcher DR, Luo S, Onyia JE, and Hale JE (2008) Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci U S A* **105**:6320–6325.
- Hajnal A, Nyland JE, Acharya NK, and Kessler DA (2009) Sucrose is more potent to prevent habituation of accumbens dopamine release than corn oil. *Appetite* **52**:835.
- Hanada T, Toshinai K, Date Y, Kajimura N, Tsukada T, Hayashi Y, Kangawa K, and Nakazato M (2004) Upregulation of ghrelin expression in cachectic nude mice bearing human melanoma cells. *Metabolism* **53**:84–88.
- Hanada T, Toshinai K, Kajimura N, Nara-Ashizawa N, Tsukada T, Hayashi Y, Osuye K, Kangawa K, Matsukura S, and Nakazato M (2003) Anti-cachectic effect of ghrelin in nude mice bearing human melanoma cells. *Biochem Biophys Res Commun* **301**:275–279.
- Hanusch-Enserer U, Brabant G, and Roden M (2003) Ghrelin concentrations in morbidly obese patients after adjustable gastric banding. *N Engl J Med* **348**:2159–2160.
- Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, and Purnell JQ (2003) Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. *J Clin Endocrinol Metab* **88**:174–178.
- Harada T, Nakahara T, Yasuhara D, Kojima S, Sagiya K, Amitani H, Laviano A, Naruo T, and Inui A (2008) Obestatin, acyl ghrelin, and des-acyl ghrelin responses to an oral glucose tolerance test in the restricting type of anorexia nervosa. *Biol Psychiatry* **63**:245–247.
- Hashimoto H, Fujihara H, Kawasaki M, Saito T, Shibata M, Otsubo H, Takei Y, and Ueta Y (2007) Centrally and peripherally administered ghrelin potentially inhibits water intake in rats. *Endocrinology* **148**:1638–1647.
- Hattori N, Saito T, Yagyu T, Jiang BH, Kitagawa K, and Inagaki C (2001) GH, GH receptor, GH secretagogue receptor, and ghrelin expression in human T cells, B cells, and neutrophils. *J Clin Endocrinol Metab* **86**:4284–4291.
- Helmling S, Maasch C, Eulberg D, Buchner K, Schröder W, Lange C, Vonhoff S, Wolzka B, Tschöp MH, Rosewicz S, et al. (2004) Inhibition of ghrelin action in vitro and in vivo by an RNA-Spiegelmer. *Proc Natl Acad Sci U S A* **101**:13174–13179.
- Hewson AK and Dickson SL (2000) Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. *J Neuroendocrinol* **12**:1047–1049.
- Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-Sickinger AG, et al. (2007) GPR39 signaling is stimulated by zinc ions but not by obestatin. *Endocrinology* **148**:13–20.
- Hosoda H and Kangawa K (2008) The autonomic nervous system regulates gastric ghrelin secretion in rats. *Regul Pept* **146**:12–18.
- Hosoda H, Kojima M, Matsuo H, and Kangawa K (2000a) Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. *J Biol Chem* **275**:21995–22000.
- Hosoda H, Kojima M, Matsuo H, and Kangawa K (2000b) Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun* **279**:909–913.
- Hotta M, Ohwada R, Katakami H, Shibasaki T, Hizuka N, and Takano K (2004) Plasma levels of intact and degraded ghrelin and their responses to glucose infusion in anorexia nervosa. *J Clin Endocrinol Metab* **89**:5707–5712.
- Howard AD, Feighner SD, Cully DF, Arena JP, Liberators PA, Rosenblum CI, Hameelin M, Hreniuk DL, Palyha OC, Anderson J, et al. (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* **273**:974–977.
- Hoybye C, Barkeling B, Naslund E, Thorén M, and Hellstrom PM (2007) Eating behavior and gastric emptying in adults with Prader-Willi syndrome. *Ann Nutr Metab* **51**:264–269.
- Huang Q, Fan YZ, Ge BJ, Zhu Q, and Tu ZY (2007) Circulating ghrelin in patients with gastric or colorectal cancer. *Dig Dis Sci* **52**:803–809.
- Huda MS, Dovey T, Wong SP, English PJ, Halford J, McCulloch P, Cleator J, Martin B, Cashen J, Hayden K, et al. (2009) Ghrelin restores 'lean-type' hunger and energy expenditure profiles in morbidly obese subjects but has no effect on post-gastrostomy subjects. *Int J Obes (Lond)* **33**:317–325.
- Huda MS, Durham BH, Wong SP, Deepak D, Kerrigan D, McCulloch P, Ranganath L, Pinkney J, and Wilding JP (2008) Plasma obestatin levels are lower in obese and post-gastrostomy subjects, but do not change in response to a meal. *Int J Obes (Lond)* **32**:129–135.
- Huda MS, Durham BH, Wong SP, Dovey TM, McCulloch P, Kerrigan D, Pinkney JH, Fraser WD, and Wilding JP (2007) Lack of an acute effect of ghrelin on markers of bone turnover in healthy controls and post-gastrostomy subjects. *Bone* **41**:406–413.
- Iantomo M, Chen H, Kim JA, Tesauro M, Lauro D, Cardillo C, and Quon MJ (2007) Ghrelin has novel vascular actions that mimic PI 3-kinase-dependent actions of insulin to stimulate production of NO from endothelial cells. *Am J Physiol Endocrinol Metab* **292**:E756–E764.
- Iglesias MJ, Salgado A, Piñeiro R, Rodiño BK, Otero MF, Grigorian L, Gallego R, Diéguez C, Gualillo O, González-Juanatey JR, et al. (2007) Lack of effect of the ghrelin gene-derived peptide obestatin on cardiomyocyte viability and metabolism. *J Endocrinol Invest* **30**:470–476.
- Iglesias J, Diez JJ, Fernández-Reyes MJ, Codocero R, Alvarez-Fidalgo P, Bajo MA, Aguilera A, and Selgas R (2006) Serum ghrelin concentrations in patients with chronic renal failure undergoing dialysis. *Clin Endocrinol (Oxf)* **64**:68–73.
- Inhoff T, Mönnikes H, Noetzel S, Stengel A, Goebel M, Dinh QT, Riedl A, Bannert N, Wisser AS, Wiedenmann B, et al. (2008) Desacyl ghrelin inhibits the orexigenic effect of peripherally injected ghrelin in rats. *Peptides* **29**:2159–2168.
- İçseri SO, Sener G, Saglam B, Ercan F, Gedik N, and Yeğen BC (2008) Ghrelin alleviates biliary obstruction-induced chronic hepatic injury in rats. *Regul Pept* **146**:73–79.
- Ishii S, Kamegai J, Tamura H, Shimizu T, Sugihara H, and Oikawa S (2003) Hypothalamic neuropeptide Y/Y1 receptor pathway activated by a reduction in circulating leptin, but not by an increase in circulating ghrelin, contributes to hyperphagia associated with triiodothyronine-induced thyrotoxicosis. *Neuroendocrinology* **78**:321–330.
- Ishizaki S, Murase T, Sugimura Y, Kakiya S, Yokoi H, Tachikawa K, Arima H, Miura Y, and Oiso Y (2002) Role of ghrelin in the regulation of vasopressin release in conscious rats. *Endocrinology* **143**:1589–1593.
- Isomoto H, Ueno H, Nishi Y, Yasutake T, Tanaka K, Kawano N, Ohnita K, Mizuta Y, Inoue K, Nakazato M, et al. (2005) Circulating ghrelin levels in patients with various upper gastrointestinal diseases. *Dig Dis Sci* **50**:833–838.
- Itoh T, Nagaya N, Yoshikawa M, Fukuoka A, Takenaka H, Shimizu Y, Haruta Y, Oya H, Yamagishi M, Hosoda H, et al. (2004) Elevated plasma ghrelin level in underweight patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **170**:879–882.
- Iwakura H, Hosoda K, Doi R, Komoto I, Nishimura H, Son C, Fujikura J, Tomita T, Takaya K, Ogawa Y, et al. (2002) Ghrelin expression in islet cell tumors: augmented expression of ghrelin in a case of glucagonoma with multiple endocrine neoplasm type I. *J Clin Endocrinol Metab* **87**:4885–4888.
- Iwakura H, Hosoda K, Son C, Fujikura J, Tomita T, Noguchi M, Ariyasu H, Takaya K, Masuzaki H, Ogawa Y, et al. (2005) Analysis of rat insulin II promoter-ghrelin transgenic mice and rat glucagon promoter-ghrelin transgenic mice. *J Biol Chem* **280**:15247–15256.
- Jackson VR, Nothacker HP, and Civelli O (2006) GPR39 receptor expression in the mouse brain. *Neuroreport* **17**:813–816.
- Janas-Kozik M, Krupka-Matuszczyk I, Malinowska-Kolodziej I, and Lewin-Kowalik J (2007) Total ghrelin plasma level in patients with the restrictive type of anorexia nervosa. *Regul Pept* **140**:43–46.
- Jarkovská Z, Hodková M, Szamová M, Rosická M, Dusilová-Sulková S, Marek J, Justová V, Lacinová Z, Haluzík M, Haas T, et al. (2005a) Plasma levels of active and total ghrelin in renal failure: a relationship with GH/IGF-I axis. *Growth Horm IGF Res* **15**:369–376.
- Jarkovská Z, Rosická M, Krsek M, Sulková S, Haluzík M, Justová V, Lacinová Z, and Marek J (2005b) Plasma ghrelin levels in patients with end-stage renal disease. *Physiol Res* **54**:403–408.
- Jeffery PL, Herington AC, and Chopin LK (2002) Expression and action of the growth hormone releasing peptide ghrelin and its receptor in prostate cancer cell lines. *J Endocrinol* **172**:R7–R11.
- Jeffery PL, Murray RE, Yeh AH, McNamara JF, Duncan RP, Francis GD, Herington AC, and Chopin LK (2005) Expression and function of the ghrelin axis, including a novel preproghrelin isoform, in human breast cancer tissues and cell lines. *Endocr Relat Cancer* **12**:839–850.
- Jeon TY, Lee S, Kim HH, Kim YJ, Son HC, Kim DH, and Sim MS (2004) Changes in plasma ghrelin concentration immediately after gastrostomy in patients with early gastric cancer. *J Clin Endocrinol Metab* **89**:5392–5396.
- Jerlhag E (2008) Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. *Addict Biol* **13**:358–363.
- Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, and Engel JA (2007) Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict Biol* **12**:6–16.
- Jerlhag E, Egecioglu E, Landgren S, Salomé N, Heilig M, Moechars D, Datta R, Perrissoud D, Dickson SL, and Engel JA (2009) Requirement of central ghrelin signaling for alcohol reward. *Proc Natl Acad Sci U S A* **106**:11318–11323.
- Jeusette IC, Lhoest ET, Istasse LP, and Diez MO (2005) Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. *Am J Vet Res* **66**:81–86.
- Johansson I, Destefanis S, Aberg ND, Aberg MA, Blomgren K, Zhu C, Ghè C, Granata R, Ghigo E, Muccioli G, et al. (2008) Proliferative and protective effects of growth hormone secretagogues on adult rat hippocampal progenitor cells. *Endocrinology* **149**:2191–2199.
- Johnson AW, Dailey MJ, Moran TH, and Holland PC (2009) Ghrelin alters the appetitive and consummatory response to learned cues associated with food. *Appetite* **52**:839.
- Jürimäe J, Lätt E, Haljaste K, Purge P, Cicchella A, and Jürimäe T (2009a) Influence of puberty on ghrelin and BMD in athletes. *Int J Sports Med* **30**:403–407.
- Jürimäe J, Pomerants T, Tillmann V, and Jürimäe T (2009b) Bone metabolism markers and ghrelin in boys at different stages of sexual maturity. *Acta Paediatr* **98**:892–896.
- Kalaitzakis E, Bosaeus I, Ohman L, and Björnsson E (2007) Altered postprandial glucose, insulin, leptin, and ghrelin in liver cirrhosis: correlations with energy intake and resting energy expenditure. *Am J Clin Nutr* **85**:808–815.
- Kalaitzakis E, Sadik R, Holst JJ, Ohman L, and Björnsson E (2009) Gut transit is associated with gastrointestinal symptoms and gut hormone profile in patients with cirrhosis. *Clin Gastroenterol Hepatol* **7**:346–352.
- Kalra SP and Kalra PS (2004) Overlapping and interactive pathways regulating appetite and craving. *J Addict Dis* **23**:5–21.
- Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, and Wakabayashi I (2000) Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* **141**:4797–4800.
- Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, and Wakabayashi I (2001) Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes* **50**:2438–2443.
- Kanamoto N, Akamizu T, Hosoda H, Hataya Y, Ariyasu H, Takaya K, Hosoda K, Saijo M, Moriyama K, Shimatsu A, et al. (2001) Substantial production of ghrelin by a human medullary thyroid carcinoma cell line. *J Clin Endocrinol Metab* **86**:4984–4990.
- Kanehisa M, Akiyoshi J, Kitaichi T, Matsushita H, Tanaka E, Kodama K, Hanada H, and Isogawa K (2006) Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **30**:1403–1407.
- Kapica M, Laubitz D, Puzio I, Jankowska A, and Zabielski R (2006) The ghrelin

- pentapeptide inhibits the secretion of pancreatic juice in rats. *J Physiol Pharmacol* **57**:691–700.
- Kapica M, Zabielska M, Puzio I, Jankowska A, Kato I, Kuwahara A, and Zabielski R (2007) Obestatin stimulates the secretion of pancreatic juice enzymes through a vagal pathway in anaesthetized rats—preliminary results. *J Physiol Pharmacol* **58** (Suppl 3):123–130.
- Karapanagiotou EM, Polyzos A, Dilana KD, Gratsias I, Boura P, Gkiozos I, and Syrigos KN (2009) Increased serum levels of ghrelin at diagnosis mediate body weight loss in non-small cell lung cancer (NSCLC) patients. *Lung Cancer* **66**:393–398.
- Kawakami A, Okada N, Rokkaku K, Honda K, Ishibashi S, and Onaka T (2008) Leptin inhibits and ghrelin augments hypothalamic noradrenaline release after stress. *Stress* **11**:363–369.
- Kawczynska-Drozd A, Olszanecki R, Jawien J, Brzozowski T, Pawlik WW, Korbut R, and Guzik TJ (2006) Ghrelin inhibits vascular superoxide production in spontaneously hypertensive rats. *Am J Hypertens* **19**:764–767.
- Keen-Rhinehart E and Bartness TJ (2007) MTH attenuates ghrelin- and food deprivation-induced increases in food hoarding and food intake. *Horm Behav* **52**:612–620.
- Kelley AE and Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci* **22**:3306–3311.
- Kellokoski E, Kunnari A, Jokela M, Mäkelä S, Kesäniemi YA, and Hörkö S (2009) Ghrelin and obestatin modulate early atherogenic processes on cells: enhancement of monocyte adhesion and oxidized low-density lipoprotein binding. *Metabolism* **58**:1572–1580.
- Kiewiet RM, Gauna C, van Aken MO, van de Zande B, and van der Lely AJ (2008) Bolus administration of obestatin does not change glucose and insulin levels neither in the systemic nor in the portal circulation of the rat. *Peptides* **29**:2144–2149.
- Kiewiet RM, van Aken MO, van der Weerd K, Uitterlinden P, Themmen AP, Hofland LJ, de Rijke YB, Delhanty PJ, Ghigo E, Aribat T, et al. (2009) Effects of acute administration of acylated and unacylated ghrelin on glucose and insulin concentrations in morbidly obese subjects without overt diabetes. *Eur J Endocrinol* **161**:567–573.
- Kim DJ, Yoon SJ, Choi B, Kim TS, Woo YS, Kim W, Myrick H, Peterson BS, Choi YB, Kim YK, et al. (2005a) Increased fasting plasma ghrelin levels during alcohol abstinence. *Alcohol Alcohol* **40**:76–79.
- Kim J, Nakajima K, Oomura Y, Wayner MJ, and Sasaki K (2009) Electrophysiological effects of ghrelin on pedunculopontine tegmental neurons in rats: An in vitro study. *Peptides* **30**:745–757.
- Kim K, Arai K, Sanno N, Osamura RY, Teramoto A, and Shibasaki T (2001) Ghrelin and growth hormone (GH) secretagogue receptor (GHSR) mRNA expression in human pituitary adenomas. *Clin Endocrinol (Oxf)* **54**:759–768.
- Kim K, Sanno N, Arai K, Takano K, Yasufuku-Takano J, Teramoto A, and Shibasaki T (2003a) Ghrelin mRNA and GH secretagogue receptor mRNA in human GH-producing pituitary adenomas is affected by mutations in the alpha subunit of G protein. *Clin Endocrinol (Oxf)* **59**:630–636.
- Kim MS, Yoon CY, Park KH, Shin CS, Park KS, Kim SY, Cho BY, and Lee HK (2003b) Changes in ghrelin and ghrelin receptor expression according to feeding status. *Neuroreport* **14**:1317–1320.
- Kim SW, Her SJ, Park SJ, Kim D, Park KS, Lee HK, Han BH, Kim MS, Shin CS, and Kim SY (2005b) Ghrelin stimulates proliferation and differentiation and inhibits apoptosis in osteoblastic MC3T3-E1 cells. *Bone* **37**:359–369.
- Kirchner H, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, Schürmann A, Joost HG, Jandacek RJ, Hale JE, et al. (2009) GOAT links dietary lipids with the endocrine control of energy balance. *Nat Med* **15**:741–745.
- Kitazawa T, De Smet B, Verbeke K, Depoortere I, and Peeters TL (2005) Gastric motor effects of peptide and non-peptide ghrelin agonists in mice in vivo and in vitro. *Gut* **54**:1078–1084.
- Klein S, Mittendorfer B, Eagon JC, Patterson B, Grant L, Feirt N, Seki E, Brenner D, Korenblat K, and McCrea J (2006) Gastric bypass surgery improves metabolic and hepatic abnormalities associated with nonalcoholic fatty liver disease. *Gastroenterology* **130**:1564–1572.
- Kleinz MJ, Maguire JJ, Skepper JN, and Davenport AP (2006) Functional and immunocytochemical evidence for a role of ghrelin and des-octanoyl ghrelin in the regulation of vascular tone in man. *Cardiovasc Res* **69**:227–235.
- Kluge M, Gazea M, Schüssler P, Genzel L, Dresler M, Kleyer S, Uhr M, Yassouridis A, and Steiger A (2009) Ghrelin increases slow wave sleep and stage 2 sleep and decreases stage 1 sleep and REM sleep in elderly men but does not affect sleep in elderly women. *Psychoneuroendocrinology* doi: 10.1016/j.psyneuen.2009.07.007.
- Kluge M, Schüssler P, Bleninger P, Kleyer S, Uhr M, Weikel JC, Yassouridis A, Zuber V, and Steiger A (2008) Ghrelin alone or co-administered with GHRH or CRH increases non-REM sleep and decreases REM sleep in young males. *Psychoneuroendocrinology* **33**:497–506.
- Kluge M, Schüssler P, Zuber V, Kleyer S, Yassouridis A, Dresler M, Uhr M, and Steiger A (2007) Ghrelin enhances the nocturnal secretion of cortisol and growth hormone in young females without influencing sleep. *Psychoneuroendocrinology* **32**:1079–1085.
- Kobelt P, Helmling S, Stengel A, Wlotzka B, Andresen V, Klapp BF, Wiedenmann B, Klusmann S, and Mönnikes H (2006) Anti-ghrelin Spiegelmer NOX-B11 inhibits neurostimulatory and orexigenic effects of peripheral ghrelin in rats. *Gut* **55**:788–792.
- Kobelt P, Tebbe JJ, Tjandra I, Stengel A, Bae HG, Andresen V, van der Voort IR, Veh RW, Werner CR, Klapp BF, et al. (2005) CCK inhibits the orexigenic effect of peripheral ghrelin. *Am J Physiol Regul Integr Comp Physiol* **288**:R751–R758.
- Kobelt P, Wissner AS, Stengel A, Goebel M, Bannert N, Gourcerol G, Inhoff T, Noetzel S, Wiedenmann B, Klapp BF, et al. (2008) Peripheral obestatin has no effect on feeding behavior and brain Fos expression in rodents. *Peptides* **29**:1018–1027.
- Kohno D, Gao HZ, Muroya S, Kikuyama S, and Yada T (2003) Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca²⁺ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* **52**:948–956.
- Kohno D, Sone H, Minokoshi Y, and Yada T (2008) Ghrelin raises [Ca²⁺]_i via AMPK in hypothalamic arcuate nucleus NPY neurons. *Biochem Biophys Res Commun* **366**:388–392.
- Kojima M (2008) The discovery of ghrelin—a personal memory. *Regul Pept* **145**:2–6.
- Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, and Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**:656–660.
- Kojima M and Kangawa K (2005) Ghrelin: structure and function. *Physiol Rev* **85**:495–522.
- Kojima S, Nakahara T, Nagai N, Muranaga T, Tanaka M, Yasuhara D, Masuda A, Date Y, Ueno H, Nakazato M, et al. (2005) Altered ghrelin and peptide YY responses to meals in bulimia nervosa. *Clin Endocrinol (Oxf)* **62**:74–78.
- Kola B, Farkas I, Christ-Crain M, Wittmann G, Lolli F, Amin F, Harvey-White J, Liposits Z, Kunos G, Grossman AB, et al. (2008) The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS One* **3**:e1797.
- Kola B, Hubina E, Tucci SA, Kirkham TC, Garcia EA, Mitchell SE, Williams LM, Hawley SA, Hardie DG, Grossman AB, et al. (2005) Cannabinoids and ghrelin have both central and peripheral metabolic and cardiac effects via AMP-activated protein kinase. *J Biol Chem* **280**:25196–25201.
- Konturek PC, Brzozowski T, Pajdo R, Nikiforuk A, Kwiecien S, Harsch I, Drozdowicz D, Hahn EG, and Konturek SJ (2004) Ghrelin—a new gastroprotective factor in gastric mucosa. *J Physiol Pharmacol* **55**:325–336.
- Konturek PC, Brzozowski T, Walter B, Burnat G, Hess T, Hahn EG, and Konturek SJ (2006) Ghrelin-induced gastroprotection against ischemia-reperfusion injury involves an activation of sensory afferent nerves and hyperemia mediated by nitric oxide. *Eur J Pharmacol* **536**:171–181.
- Konturek PC, Burnat G, Rau T, Hahn EG, and Konturek S (2008) Effect of adiponectin and ghrelin on apoptosis of Barrett adenocarcinoma cell line. *Dig Dis Sci* **53**:597–605.
- Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, Kangawa K, and Grossman AB (2001a) The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. *J Clin Endocrinol Metab* **86**:881–887.
- Korbonits M, Kojima M, Kangawa K, and Grossman AB (2001b) Presence of ghrelin in normal and adenomatous human pituitary. *Endocrine* **14**:101–104.
- Korner J, Bessler M, Cirilo LJ, Conwell IM, Daud A, Restuccia NL, and Wardlaw SL (2005) Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. *J Clin Endocrinol Metab* **90**:359–365.
- Korner J, Inabnet W, Conwell IM, Taveras C, Daud A, Olivero-Rivera L, Restuccia NL, and Bessler M (2006) Differential effects of gastric bypass and banding on circulating gut hormone and leptin levels. *Obesity (Silver Spring)* **14**:1553–1561.
- Korner J, Inabnet W, Febres G, Conwell IM, McMahon DJ, Salas R, Taveras C, Schrope B, and Bessler M (2009) Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass. *Int J Obes (Lond)* **33**:786–795.
- Kos K, Harte AL, O'Hare PJ, Kumar S, and McTernan PG (2009) Ghrelin and the differential regulation of des-acyl (DSG) and oct-anoyl ghrelin (OTG) in human adipose tissue (AT). *Clin Endocrinol (Oxf)* **70**:383–389.
- Kristensson E, Sundqvist M, Astin M, Kjerling M, Mattsson H, Dornonville de la Cour C, Håkanson R, and Lindström E (2006) Acute psychological stress raises plasma ghrelin in the rat. *Regul Pept* **134**:114–117.
- Kurose Y, Iqbal J, Rao A, Murata Y, Hasegawa Y, Terashima Y, Kojima M, Kangawa K, and Clarke IJ (2005) Changes in expression of the genes for the leptin receptor and the growth hormone-releasing peptide/ghrelin receptor in the hypothalamic arcuate nucleus with long-term manipulation of adiposity by dietary means. *J Neuroendocrinol* **17**:331–340.
- Lago R, Gomez R, Dieguez C, Gomez-Reino JJ, Lago F, and Gualillo O (2007) Unlike ghrelin, obestatin does not exert any relevant activity in chondrocytes. *Ann Rheum Dis* **66**:1399–1400.
- Lasseter KC, Shaughnessy L, Cummings D, Pezzullo JC, Wargin W, Gagnon R, Oliva J, and Kosutic G (2008) Ghrelin agonist (TZP-101): safety, pharmacokinetics and pharmacodynamic evaluation in healthy volunteers: a phase I, first-in-human study. *J Clin Pharmacol* **48**:193–202.
- Lauwers E, Landuyt B, Arkens L, Schoofs L, and Luyten W (2006) Obestatin does not activate orphan G protein-coupled receptor GPR39. *Biochem Biophys Res Commun* **351**:21–25.
- le Roux CW, Neary NM, Halsey TJ, Small CJ, Martinez-Isla AM, Ghatei MA, Theodorou NA, and Bloom SR (2005a) Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. *J Clin Endocrinol Metab* **90**:4521–4524.
- le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, and Bloom SR (2005b) Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. *J Clin Endocrinol Metab* **90**:1068–1071.
- Lee KJ, Cha DY, Cheon SJ, Yeo M, and Cho SW (2009) Plasma ghrelin levels and their relationship with gastric emptying in patients with dysmotility-like functional dyspepsia. *Digestion* **80**:58–63.
- Leidy HJ, Mattes RD, and Campbell WW (2007) Effects of acute and chronic protein intake on metabolism, appetite, and ghrelin during weight loss. *Obesity (Silver Spring)* **15**:1215–1225.
- Levin F, Edholm T, Ehrström M, Wallin B, Schmidt PT, Kirchgessner AM, Hilsted LM, Hellström PM, and Näslund E (2005) Effect of peripherally administered ghrelin on gastric emptying and acid secretion in the rat. *Regul Pept* **131**:59–65.
- Levin F, Edholm T, Schmidt PT, Gryback P, Jacobsson H, Degerblad M, Höybye C, Holst JJ, Rehfeld JF, Hellström PM, et al. (2006) Ghrelin stimulates gastric emptying and hunger in normal-weight humans. *J Clin Endocrinol Metab* **91**:3296–3302.
- Li L, Zhang LK, Pang YZ, Pan CS, Qi YF, Chen L, Wang X, Tang CS, and Zhang J

- (2006) Cardioprotective effects of ghrelin and des-octanoyl ghrelin on myocardial injury induced by isoproterenol in rats. *Acta Pharmacol Sin* **27**:527–535.
- Li WG, Favril D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormick ML, Sigmund CD, Tang C, and Weintraub NL (2004) Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. *Circulation* **109**:2221–2226.
- Li ZF, Song SW, Qin YW, Zhang JL, Zhao XX, Zhang BL, Ren AJ, Guo ZF, and Zheng X (2009) Bolus intravenous injection of obestatin does not change blood pressure level of spontaneously hypertensive rat. *Peptides* **30**:1928–1930.
- Lin CC and Yin MC (2007) B vitamins deficiency and decreased anti-oxidative state in patients with liver cancer. *Eur J Nutr* **46**:293–299.
- Lin Y, Matsumura K, Fukuhara M, Kagiya S, Fujii K, and Iida M (2004) Ghrelin acts at the nucleus of the solitary tract to decrease arterial pressure in rats. *Hypertension* **43**:977–982.
- Lindqvist A, de la Cour CD, Stegmark A, Håkanson R, and Erlanson-Albertsson C (2005) Overeating of palatable food is associated with blunted leptin and ghrelin responses. *Regul Pept* **130**:123–132.
- Liu J, Prudom CE, Nass R, Pezzoli SS, Oliveri MC, Johnson ML, Veldhuis P, Gordon DA, Howard AD, Witcher DR, et al. (2008) Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J Clin Endocrinol Metab* **93**:1980–1987.
- Longo KA, Charoenthongtrakul S, Giuliana DJ, Govek EK, McDonagh T, Qi Y, DiStefano PS, and Geddes BJ (2008) Improved insulin sensitivity and metabolic flexibility in ghrelin receptor knockout mice. *Regul Pept* **150**:55–61.
- López M, Lage R, Saha AK, Pérez-Tilve D, Vázquez MJ, Varela L, Sangiao-Alvarellos S, Tovar S, Raghay K, Rodríguez-Cuenca S, et al. (2008) Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab* **7**:389–399.
- Lu SC, Xu J, Chinooswong N, Liu S, Steavenson S, Gegg C, Brankow D, Lindberg R, Véniant M, and Gu W (2009) An acyl-ghrelin-specific neutralizing antibody inhibits the acute ghrelin-mediated orexigenic effects in mice. *Mol Pharmacol* **75**:901–907.
- Luo FM, Liu XJ, Li SQ, Wang ZL, Liu CT, and Yuan YM (2005) Circulating ghrelin in patients with chronic obstructive pulmonary disease. *Nutrition* **21**:793–798.
- Luquet S, Phillips CT, and Palmiter RD (2007) NPY/AgRP neurons are not essential for feeding responses to glucoprivation. *Peptides* **28**:214–225.
- Maccarielli G, Sibilia V, Torsello A, Raimondo F, Pitto M, Giustina A, Netti C, and Cocchi D (2005) Ghrelin regulates proliferation and differentiation of osteoblastic cells. *J Endocrinol* **184**:249–256.
- Mackelvie KJ, Meneilly GS, Elahi D, Wong AC, Barr SI, and Chanoine JP (2007) Regulation of appetite in lean and obese adolescents after exercise: role of acylated and desacyl ghrelin. *J Clin Endocrinol Metab* **92**:648–654.
- Malik NM, Moore GB, Kaur R, Liu YL, Wood SL, Morrow RW, Sanger GJ, and Andrews PL (2008a) Adaptive upregulation of gastric and hypothalamic ghrelin receptors and increased plasma ghrelin in a model of cancer chemotherapy-induced dyspepsia. *Regul Pept* **148**:33–38.
- Malik S, McGlone F, Bedrossian D, and Dagher A (2008b) Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell Metab* **7**:400–409.
- Marchesini G, Bianchi G, Lucidi P, Villanova N, Zoli M, and De Feo P (2004) Plasma ghrelin concentrations, food intake, and anorexia in liver failure. *J Clin Endocrinol Metab* **89**:2136–2141.
- Martínez-Fuentes AJ, Moreno-Fernández J, Vázquez-Martínez R, Durán-Prado M, de la Riva A, Tena-Sempere M, Diéguez C, Jiménez-Reina L, Webb SM, Pumar A, et al. (2006) Ghrelin is produced by and directly activates corticotrope cells from adrenocorticotropin-secreting adenomas. *J Clin Endocrinol Metab* **91**:2225–2231.
- Masaoka T, Suzuki H, Hosoda H, Ota T, Minegishi Y, Nagata H, Kangawa K, and Ishii H (2003) Enhanced plasma ghrelin levels in rats with streptozotocin-induced diabetes. *FEBS Lett* **541**:64–68.
- Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, and Kangawa K (2000) Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* **276**:905–908.
- Matsuda K, Miura T, Kaiya H, Maruyama K, Shimakura S, Uchiyama M, Kangawa K, and Shioda S (2006) Regulation of food intake by acyl and des-acyl ghrelins in the goldfish. *Peptides* **27**:2321–2325.
- Matsumura K, Tsuchihashi T, Fujii K, Abe I, and Iida M (2002) Central ghrelin modulates sympathetic activity in conscious rabbits. *Hypertension* **40**:694–699.
- Mattar SG, Velcu LM, Rabinovitz M, Demetris AJ, Krasinskas AM, Barinas-Mitchell E, Eid GM, Ramanathan R, Taylor DS, and Schauer PR (2005) Surgically-induced weight loss significantly improves nonalcoholic fatty liver disease and the metabolic syndrome. *Ann Surg* **242**:610–617.
- Mayorov AV, Amara N, Chang JY, Moss JA, Hixon MS, Ruiz DI, Meijler MM, Zorrilla EP, and Janda KD (2008) Catalytic antibody degradation of ghrelin increases whole-body metabolic rate and reduces refeeding in fasting mice. *Proc Natl Acad Sci U S A* **105**:17487–17492.
- McKee KK, Tan CP, Palyha OC, Liu J, Feighner SD, Hreniuk DL, Smith RG, Howard AD, and Van der Ploeg LH (1997) Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics* **46**:426–434.
- Mietlicki EG, Nowak EL, and Daniels D (2009) The effect of ghrelin on water intake during dipsogenic conditions. *Physiol Behav* **96**:37–43.
- Miljic D, Pekic S, Djurovic M, Doknic M, Milic N, Casanueva FF, Ghatei M, and Popovic V (2006) Ghrelin has partial or no effect on appetite, growth hormone, prolactin, and cortisol release in patients with anorexia nervosa. *J Clin Endocrinol Metab* **91**:1491–1495.
- Miljic D, Djurovic M, Pekic S, Doknic M, Stojanovic M, Milic N, Casanueva FF, Ghatei M, and Popovic V (2007) Glucose metabolism during ghrelin infusion in patients with anorexia nervosa. *J Endocrinol Invest* **30**:771–775.
- Mizutani M, Atsuchi K, Asakawa A, Matsuda N, Fujimura M, Inui A, Kato I, and Fujimiyama M (2009) Localization of acyl ghrelin- and des-acyl ghrelin-immunoreactive cells in the rat stomach and their responses to intragastric pH. *Am J Physiol Gastrointest Liver Physiol* **297**:G974–G980.
- Mondal MS, Date Y, Yamaguchi H, Toshinai K, Tsuruta T, Kangawa K, and Nakazato M (2005) Identification of ghrelin and its receptor in neurons of the rat arcuate nucleus. *Regul Pept* **126**:55–59.
- Mondal MS, Toshinai K, Ueno H, Koshinaka K, and Nakazato M (2008) Characterization of obestatin in rat and human stomach and plasma, and its lack of acute effect on feeding behavior in rodents. *J Endocrinol* **198**:339–346.
- Monteiro MP, Ribeiro AH, Nunes AF, Sousa MM, Monteiro JD, Aguiar AP, and Cardoso MH (2007) Increase in ghrelin levels after weight loss in obese Zucker rats is prevented by gastric banding. *Obes Surg* **17**:1599–1607.
- Monteleone P, Martiadis V, Fabrazzo M, Serritella C, and Maj M (2003) Ghrelin and leptin responses to food ingestion in bulimia nervosa: implications for binge-eating and compensatory behaviours. *Psychol Med* **33**:1387–1394.
- Monteleone P, Martiadis V, Rigamonti AE, Fabrazzo M, Giordani C, Muller EE, and Maj M (2005) Investigation of peptide YY and ghrelin responses to a test meal in bulimia nervosa. *Biol Psychiatry* **57**:926–931.
- Monteleone P, Serritella C, Scognamiglio P, and Maj M (2009) Enhanced ghrelin secretion in the cephalic phase of food ingestion in women with bulimia nervosa. *Psychoneuroendocrinology* doi: 10.1016/j.psyneuen.2009.07.001.
- Morpurgo PS, Cappiello V, Verga U, Vicentini L, Vaghi I, Lauri E, Nebuloni M, Beck-Peccoz P, and Spada A (2005) Ghrelin in human medullary thyroid carcinomas. *Clin Endocrinol (Oxf)* **63**:437–441.
- Motivala SJ, Tomiyama AJ, Ziegler M, Khandrika S, and Irwin MR (2009) Nocturnal levels of ghrelin and leptin and sleep in chronic insomnia. *Psychoneuroendocrinology* **34**:540–545.
- Mottershead M, Karteris E, Barclay JY, Suortamo S, Newbold M, Randeve H, and Nwokolo CU (2007) Immunohistochemical and quantitative mRNA assessment of ghrelin expression in gastric and oesophageal adenocarcinoma. *J Clin Pathol* **60**:405–409.
- Mozid AM, Tringali G, Forsling ML, Hendricks MS, Ajodha S, Edwards R, Navarra P, Grossman AB, and Korbonits M (2003) Ghrelin is released from rat hypothalamic explants and stimulates corticotrophin-releasing hormone and arginine-vasopressin. *Horm Metab Res* **35**:455–459.
- Muccioli G, Baragli A, Granata R, Papotti M, and Ghigo E (2007) Heterogeneity of ghrelin/growth hormone secretagogue receptors. Toward the understanding of the molecular identity of novel ghrelin/GHS receptors. *Neuroendocrinology* **86**:147–164.
- Muccioli G, Pons N, Ghè C, Catapano F, Granata R, and Ghigo E (2004) Ghrelin and des-acyl ghrelin both inhibit isoproterenol-induced lipolysis in rat adipocytes via a non-type 1A growth hormone secretagogue receptor. *Eur J Pharmacol* **498**:27–35.
- Mundinger TO, Cummings DE, and Taborsky GJ Jr (2006) Direct stimulation of ghrelin secretion by sympathetic nerves. *Endocrinology* **147**:2893–2901.
- Mungan NA, Eminferzane S, Mungan AG, Yesilli C, Seckiner I, Can M, Ayoglu F, and Akduman B (2008) Diagnostic value of serum ghrelin levels in prostate cancer. *Urol Int* **80**:245–248.
- Murata M, Okimura Y, Iida K, Matsumoto M, Sowa H, Kaji H, Kojima M, Kangawa K, and Chihara K (2002) Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. *J Biol Chem* **277**:5667–5674.
- Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, Johnston C, Bloom SR, and Emmanuel AV (2005) Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut* **54**:1693–1698.
- Muscariotti M, Molino A, Chiappini MG, Laviano A, Ammann T, Spinsanti P, Melchiorri D, Inui A, Alegiani F, and Rossi Fanelli F (2007) Anorexia in hemodialysis patients: the possible role of des-acyl ghrelin. *Am J Nephrol* **27**:360–365.
- Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, Hayashi Y, and Kangawa K (2001a) Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regul Integr Comp Physiol* **280**:R1483–R1487.
- Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, Hosoda H, Shimizu W, Yamagishi M, Oya H, et al. (2001b) Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation* **104**:2034–2038.
- Nagaya N, Miyatake K, Uematsu M, Oya H, Shimizu W, Hosoda H, Kojima M, Nakanishi N, Mori H, and Kangawa K (2001c) Hemodynamic, renal, and hormonal effects of ghrelin infusion in patients with chronic heart failure. *J Clin Endocrinol Metab* **86**:5854–5859.
- Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, and Kangawa K (2004) Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. *Circulation* **110**:3674–3679.
- Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, Hosoda H, Hirota Y, Ishida H, Mori H, et al. (2001d) Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation* **104**:1430–1435.
- Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, and Kangawa K (2005) Treatment of cachexia with ghrelin in patients with COPD. *Chest* **128**:1187–1193.
- Nagaraj S, Peddha MS, and Manjappa UV (2008) Fragments of obestatin as modulators of feed intake, circulating lipids, and stored fat. *Biochem Biophys Res Commun* **366**:731–737.
- Nakagawa E, Nagaya N, Okumura H, Enomoto M, Oya H, Ono F, Hosoda H, Kojima M, and Kangawa K (2002) Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: responses to the intravenous and oral administration of glucose. *Clin Sci (Lond)* **103**:325–328.
- Nakahara T, Kojima S, Tanaka M, Yasuhara D, Harada T, Sagiya K, Muranaga T, Nagai N, Nakazato M, Nozoe S, et al. (2007) Incomplete restoration of the secretion of ghrelin and PYY compared to insulin after food ingestion following weight gain in anorexia nervosa. *J Psychiatr Res* **41**:814–820.
- Nakahara T, Harada T, Yasuhara D, Shimada N, Amitani H, Sakoguchi T, Kamiji MM, Asakawa A, and Inui A (2008) 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.
- Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, Akamizu T, and Kangawa K (2003)

- Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. *Eur J Endocrinol* **149**:R1–R3.
- Nakai N, Kaneko M, Nakao N, Fujikawa T, Nakashima K, Ogata M, and Tanaka M (2004) Identification of promoter region of ghrelin gene in human medullary thyroid carcinoma cell line. *Life Sci* **75**:2193–2201.
- Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, and Matsukura S (2001) A role for ghrelin in the central regulation of feeding. *Nature* **409**:194–198.
- Naleid AM, Grace MK, Cummings DE, and Levine AS (2005) Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* **26**:2274–2279.
- Nanzer AM, Khalaf S, Mozdil AM, Fowkes RC, Patel MV, Burrin JM, Grossman AB, and Korbonits M (2004) Ghrelin exerts a proliferative effect on a rat pituitary somatotroph cell line via the mitogen-activated protein kinase pathway. *Eur J Endocrinol* **151**:233–240.
- Nascif SO, Correa-Silva SR, Silva MR, and Lengyel AM (2007) Decreased ghrelin-induced GH release in thyrotoxicosis: comparison with GH-releasing peptide-6 (GHRP-6) and GHRH. *Pituitary* **10**:27–33.
- Nascif SO, Molica P, Correa-Silva SR, Silva MR, and Lengyel AM (2009) Ghrelin and GHRP-6-induced ACTH and cortisol release in thyrotoxicosis. *Pituitary* **12**:315–321.
- Nass R, Pezzoli SS, Oliveri MC, Patrie JT, Harrell FE Jr, Clasey JL, Heymsfield SB, Bach MA, Vance ML, and Thorne MO (2008) Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. *Ann Intern Med* **149**:601–611.
- Natalucci G, Riedl S, Gleiss A, Zidek T, and Frisch H (2005) Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern. *Eur J Endocrinol* **152**:845–850.
- Nearly NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC, and Bloom SR (2004) Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* **89**:2832–2836.
- Nearly NM, Druce MR, Small CJ, and Bloom SR (2006) Acylated ghrelin stimulates food intake in the fed and fasted states but desacylated ghrelin has no effect. *Gut* **55**:135.
- Nedeltcheva AV, Kilkus JM, Imperial J, Kasza K, Schoeller DA, and Penev PD (2009) Sleep curtailment is accompanied by increased intake of calories from snacks. *Am J Clin Nutr* **89**:126–133.
- Nedvídková J, Krykorková I, Barták V, Papezová H, Gold PW, Alessi S, and Pacak K (2003) Loss of meal-induced decrease in plasma ghrelin levels in patients with anorexia nervosa. *J Clin Endocrinol Metab* **88**:1678–1682.
- Nijhuis J, van Dieën FM, Buurman WA, and Greve JW (2004) Ghrelin, leptin and insulin levels after restrictive surgery: a 2-year follow-up study. *Obes Surg* **14**:783–787.
- Nishizawa T, Suzuki H, Nomoto Y, Masaoka T, Hosoda H, Mori M, Ohara T, Morishita T, Kangawa K, and Hibi T (2006) Enhanced plasma ghrelin levels in patients with functional dyspepsia. *Aliment Pharmacol Ther* **24** (Suppl 4):104–110.
- Nogueiras R, Pfluger P, Tovar S, Arnold M, Mitchell S, Morris A, Perez-Tilve D, Vazquez MJ, Wiedmer P, Castañeda TR, et al. (2007) Effects of obestatin on energy balance and growth hormone secretion in rodents. *Endocrinology* **148**:21–26.
- Nogueiras R, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C, and Williams LM (2004) Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. *Diabetes* **53**:2552–2558.
- Nonogaki K, Ohashi-Nozue K, and Oka Y (2006) A negative feedback system between brain serotonin systems and plasma active ghrelin levels in mice. *Biochem Biophys Res Commun* **341**:703–707.
- Ohgusu H, Shirouzu K, Nakamura Y, Nakashima Y, Ida T, Sato T, and Kojima M (2009) Ghrelin O-acyltransferase (GOAT) has a preference for n-hexanoyl-CoA over n-octanoyl-CoA as an acyl donor. *Biochem Biophys Res Commun* **386**:153–158.
- Ohno T, Kamiyama Y, Aihara R, Nakabayashi T, Mochiki E, Asao T, and Kuwano H (2006) Ghrelin does not stimulate gastrointestinal motility and gastric emptying: an experimental study of conscious dogs. *Neurogastroenterol Motil* **18**:129–135.
- Okumura H, Nagaya N, Enomoto M, Nakagawa E, Oya H, and Kangawa K (2002) Vasodilatory effect of ghrelin, an endogenous peptide from the stomach. *J Cardiovasc Pharmacol* **39**:779–783.
- Olbers T, Björkman S, Lindroos A, Maleckas A, Lönn L, Sjöström L, and Lönnroth H (2006) Body composition, dietary intake, and energy expenditure after laparoscopic Roux-en-Y gastric bypass and laparoscopic vertical banded gastroplasty: a randomized clinical trial. *Ann Surg* **244**:715–722.
- Olszewski PK, Bomberg EM, Martell A, Grace MK, and Levine AS (2007) Intraventricular ghrelin activates oxytocin neurons: implications in feeding behavior. *Neuroreport* **18**:499–503.
- Olszewski PK, Grace MK, Billington CJ, and Levine AS (2003a) Hypothalamic paraventricular injections of ghrelin: effect on feeding and c-Fos immunoreactivity. *Peptides* **24**:919–923.
- Olszewski PK, Li D, Grace MK, Billington CJ, Kotz CM, and Levine AS (2003b) Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. *Peptides* **24**:597–602.
- Ott V, Fasshauer M, Dalski A, Meier B, Perwitz N, Klein HH, Tschöp M, and Klein J (2002) Direct peripheral effects of ghrelin include suppression of adiponectin expression. *Horm Metab Res* **34**:640–645.
- Overduin J, Frayo RS, Grill HJ, Kaplan JM, and Cummings DE (2005) Role of the duodenum and macronutrient type in ghrelin regulation. *Endocrinology* **146**:845–850.
- Pacifico L, Anania C, Poggiogalle E, Osborn JF, Prossomariti G, Martino F, and Chiesa C (2009) Relationships of acylated and des-acyl ghrelin levels to bone mineralization in obese children and adolescents. *Bone* **45**:274–279.
- Paik KH, Choe YH, Park WH, Oh YJ, Kim AH, Chu SH, Kim SW, Kwon EK, Han SJ, Shon WY, et al. (2006) Suppression of acylated ghrelin during oral glucose tolerance test is correlated with whole-body insulin sensitivity in children with Prader-Willi syndrome. *J Clin Endocrinol Metab* **91**:1876–1881.
- Pan W, Tu H, and Kastin AJ (2006) Differential BBB interactions of three ingestive peptides: obestatin, ghrelin, and adiponectin. *Peptides* **27**:911–916.
- Papotti M, Cassoni P, Volante M, Deghenghi R, Muccioli G, and Ghigo E (2001) Ghrelin-producing endocrine tumors of the stomach and intestine. *J Clin Endocrinol Metab* **86**:5052–5059.
- Park WH, Oh YJ, Kim GY, Kim SE, Paik KH, Han SJ, Kim AH, Chu SH, Kwon EK, Kim SW, et al. (2007) Obestatin is not elevated or correlated with insulin in children with Prader-Willi syndrome. *J Clin Endocrinol Metab* **92**:229–234.
- Paulino G, Barbier de la Serre C, Knotts TA, Oort PJ, Newman JW, Adams SH, and Raybould HE (2009) Increased expression of receptors for orexigenic factors in nodose ganglion of diet-induced obese rats. *Am J Physiol Endocrinol Metab* **296**:E898–E903.
- Pemberton CJ, Tokola H, Bagi Z, Koller A, Pöntinen J, Ola A, Vuolteenaho O, Szokodi I, and Ruskoaho H (2004) Ghrelin induces vasoconstriction in the rat coronary vasculature without altering cardiac peptide secretion. *Am J Physiol Heart Circ Physiol* **287**:H1522–H1529.
- Pérez-Fontán M, Córdido F, Rodríguez-Carmona A, Peteiro J, García-Naveiro R, and García-Buela J (2004) Plasma ghrelin levels in patients undergoing haemodialysis and peritoneal dialysis. *Nephrol Dial Transplant* **19**:2095–2100.
- Pettersson I, Muccioli G, Granata R, Deghenghi R, Ghigo E, Ohlsson C, and Isgaard J (2002) Natural (ghrelin) and synthetic (hexarelin) GH secretagogues stimulate H9c2 cardiomyocyte cell proliferation. *J Endocrinol* **175**:201–209.
- Pfluger PT, Kirchner H, Günzel S, Schrott B, Perez-Tilve D, Fu S, Benoit SC, Horvath T, Joost HG, Wortley KE, et al. (2008) Simultaneous deletion of ghrelin and its receptor increases motor activity and energy expenditure. *Am J Physiol Gastrointest Liver Physiol* **294**:G610–G618.
- Piehl-Aulin K, Jones I, Lindvall B, Magnuson A, and Abdel-Halim SM (2009) Increased serum inflammatory markers in the absence of clinical and skeletal muscle inflammation in patients with chronic obstructive pulmonary disease. *Respiration* **78**:191–196.
- Pilichiewicz AN, Feltrin KL, Horowitz M, Holtmann G, Wishart JM, Jones KL, Talley NJ, and Feinle-Bisset C (2008) Functional dyspepsia is associated with a greater symptomatic response to fat but not carbohydrate, increased fasting and postprandial CCK, and diminished PYY. *Am J Gastroenterol* **103**:2613–2623.
- Poitras P, Polvino WJ, and Rocheleau B (2005) Gastrokinetic effect of ghrelin analog RC-1139 in the rat. Effect on post-operative and on morphine induced ileus. *Peptides* **26**:1598–1601.
- Pöykkö SM, Kellokoski E, Ukkola O, Kauma H, Päivänsalo M, Kesäniemi YA, and Hörkö S (2006) Plasma ghrelin concentrations are positively associated with carotid artery atherosclerosis in males. *J Intern Med* **260**:43–52.
- Purnell JQ, Weigle DS, Breen P, and Cummings DE (2003) Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *J Clin Endocrinol Metab* **88**:5747–5752.
- Qader SS, Håkanson R, Rehfeld JF, Lundquist I, and Salehi A (2008) Proghrelin-derived peptides influence the secretion of insulin, glucagon, pancreatic polypeptide and somatostatin: a study on isolated islets from mouse and rat pancreas. *Regul Pept* **146**:230–237.
- Qi X, Reed JT, Wang G, Han S, Englander EW, and Greeley GH Jr (2008) Ghrelin secretion is not reduced by increased fat mass during diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* **295**:R429–R435.
- Quarta D, Di Francesco C, Melotto S, Mangiarini L, Heidbreder C, and Hedou G (2009) Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. *Neurochem Int* **54**:89–94.
- Raffel A, Krausch M, Cupisti K, Gerharz CD, Eisenberger CF, and Knoefel WT (2005) Ghrelin expression in neuroendocrine tumours of the gastrointestinal tract with multiple endocrine neoplasia type 1. *Horm Metab Res* **37**:653–655.
- Ravussin E, Tschöp M, Morales S, Bouchard C, and Heiman ML (2001) Plasma ghrelin concentration and energy balance: overfeeding and negative energy balance studies in twins. *J Clin Endocrinol Metab* **86**:4547–4551.
- Rayhan N, Sano T, Qian ZR, Obari AK, and Hirokawa M (2005) Histological and immunohistochemical study of composite neuroendocrine-exocrine carcinomas of the stomach. *J Med Invest* **52**:191–202.
- Reed JA, Benoit SC, Pfluger PT, Tschöp MH, D'Alessio DA, and Seeley RJ (2008) Mice with chronically increased circulating ghrelin develop age-related glucose intolerance. *Am J Physiol Endocrinol Metab* **294**:E752–E760.
- Reimer MK, Pacini G, and Ahren B (2003) Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology* **144**:916–921.
- Ren AJ, Guo ZF, Wang YK, Wang LG, Wang WZ, Lin L, Zheng X, and Yuan WJ (2008) Inhibitory effect of obestatin on glucose-induced insulin secretion in rats. *Biochem Biophys Res Commun* **369**:969–972.
- Rigamonti AE, Giordani C, Bonomo SM, Cella SG, and Müller EE (2006) Early tolerance to the hypophagic effect of the cannabinoid receptor antagonist SR141716 does not impede blockade of an orexigenic stimulus. *Eur J Pharmacol* **542**:116–120.
- Rigamonti AE, Pincelli AI, Corrà B, Viarengo R, Bonomo SM, Galimberti D, Scacchi M, Scarpini E, Cavagnini F, and Müller EE (2002) Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. *J Endocrinol* **175**:R1–R5.
- Riis AL, Hansen TK, Møller N, Weeke J, and Jørgensen JO (2003) Hyperthyroidism is associated with suppressed circulating ghrelin levels. *J Clin Endocrinol Metab* **88**:853–857.
- Rindi G, Savio A, Torsello A, Zoli M, Locatelli V, Cocchi D, Paolotti D, and Solcia E (2002) Ghrelin expression in gut endocrine growths. *Histochem Cell Biol* **117**:521–525.
- Robertson MD, Henderson RA, Vist GE, and Rumsey RD (2004) Plasma ghrelin response following a period of acute overfeeding in normal weight men. *Int J Obes Relat Metab Disord* **28**:727–733.

- Rodríguez A, Gómez-Ambrosi J, Catalán V, Gil MJ, Becerril S, Sáinz N, Silva C, Salvador J, Colina I, and Frühbeck G (2009) Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes. *Int J Obes (Lond)* **33**:541–552.
- Rodríguez Ayala E, Pecoits-Filho R, Heimbürger O, Lindholm B, Nordfors L, and Stenvinkel P (2004) Associations between plasma ghrelin levels and body composition in end-stage renal disease: a longitudinal study. *Nephrol Dial Transplant* **19**:421–426.
- Röjdmärk S, Calissendorff J, Danielsson O, and Brismar K (2005) Hunger-satiety signals in patients with Graves' thyrotoxicosis before, during, and after long-term pharmacological treatment. *Endocrine* **27**:55–61.
- Rossi F, Castelli A, Bianco MJ, Bertone C, Brama M, and Santiemva V (2009) Ghrelin inhibits contraction and proliferation of human aortic smooth muscle cells by cAMP/PKA pathway activation. *Atherosclerosis* **203**:97–104.
- Rucinski M, Ziolkowska A, Tyczewska M, and Malendowicz LK (2009) Expression of prepro-ghrelin and related receptor genes in the rat adrenal gland and evidences that ghrelin exerts a potent stimulating effect on corticosterone secretion by cultured rat adrenocortical cells. *Peptides* **30**:1448–1455.
- Rudolph J, Esler WP, O'Connor S, Coish PD, Wickens PL, Brands M, Bierer DE, Bloomquist BT, Bondar G, Chen L, et al. (2007) Quinazolinone derivatives as orally available ghrelin receptor antagonists for the treatment of diabetes and obesity. *J Med Chem* **50**:5202–5216.
- Sakata I, Nakamura K, Yamazaki M, Matsubara M, Hayashi Y, Kangawa K, and Sakai T (2002) Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract. *Peptides* **23**:531–536.
- Sakata I, Yamazaki M, Inoue K, Hayashi Y, Kangawa K, and Sakai T (2003) Growth hormone secretagogue receptor expression in the cells of the stomach-projected afferent nerve in the rat nodose ganglion. *Neurosci Lett* **342**:183–186.
- Sakata I, Yang J, Lee CE, Osborne-Lawrence S, Rovinsky SA, Elmquist JK, and Zigman JM (2009) Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells. *Am J Physiol Endocrinol Metab* **297**:E134–E141.
- Sallam HS, Oliveira HM, Gan HT, Herndon DN, and Chen JD (2007) Ghrelin improves burn-induced delayed gastrointestinal transit in rats. *Am J Physiol Regul Integr Comp Physiol* **292**:R253–R257.
- Salomé N, Haage D, Perrissoud D, Moulin A, Demange L, Egecioglu E, Fehrentz JA, Martinez J, and Dickson SL (2009a) Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats. *Eur J Pharmacol* **612**:167–173.
- Salomé N, Hansson C, Taube M, Gustafsson-Ericson L, Egecioglu E, Karlsson-Lindahl L, Fehrentz JA, Martinez J, Perrissoud D, and Dickson SL (2009b) On the central mechanism underlying ghrelin's chronic pro-obesity effects in rats: new insights from studies exploiting a potent ghrelin receptor antagonist. *J Neuroendocrinol* **21**:777–785.
- Samson WK, White MM, Price C, and Ferguson AV (2007) Obestatin acts in brain to inhibit thirst. *Am J Physiol Regul Integr Comp Physiol* **292**:R637–R643.
- Samson WK, Yosten GL, Chang JK, Ferguson AV, and White MM (2008) Obestatin inhibits vasopressin secretion: evidence for a physiological action in the control of fluid homeostasis. *J Endocrinol* **196**:559–564.
- Sangiao-Alvarellos S, Vázquez MJ, Varela L, Nogueiras R, Saha AK, Córdido F, López M, and Diéguez C (2009) Central ghrelin regulates peripheral lipid metabolism in a growth hormone-independent fashion. *Endocrinology* **150**:4562–4574.
- Sato T, Fukue Y, Teranishi H, Yoshida Y, and Kojima M (2005) Molecular forms of hypothalamic ghrelin and its regulation by fasting and 2-deoxy-d-glucose administration. *Endocrinology* **146**:2510–2516.
- Schmid SM, Hallschmid M, Jauch-Chara K, Born J, and Schultes B (2008) A single night of sleep deprivation increases ghrelin levels and feelings of hunger in normal-weight healthy men. *J Sleep Res* **17**:331–334.
- Schüssler P, Uhr M, Ising M, Weikel JC, Schmid DA, Held K, Mathias S, and Steiger A (2006) Nocturnal ghrelin, ACTH, GH and cortisol secretion after sleep deprivation in humans. *Psychoneuroendocrinology* **31**:915–923.
- Schwartz MW, Woods SC, Porte D Jr., Seeley RJ, and Baskin DG (2000) Central nervous system control of food intake. *Nature* **404**:661–671.
- Schwenke DO, Tokudome T, Kishimoto I, Horio T, Shirai M, Cragg PA, and Kangawa K (2008a) Early ghrelin treatment after myocardial infarction prevents an increase in cardiac sympathetic tone and reduces mortality. *Endocrinology* **149**:5172–5176.
- Schwenke DO, Tokudome T, Shirai M, Hosoda H, Horio T, Kishimoto I, and Kangawa K (2008b) Exogenous ghrelin attenuates the progression of chronic hypoxia-induced pulmonary hypertension in conscious rats. *Endocrinology* **149**:237–244.
- Seoane LM, Al-Massadi O, Barreiro F, Dieguez C, and Casanueva FF (2007a) Growth hormone and somatostatin directly inhibit gastric ghrelin secretion. An in vitro organ culture system. *J Endocrinol Invest* **30**:RC22–RC25.
- Seoane LM, Al-Massadi O, Caminos JE, Tovar SA, Dieguez C, and Casanueva FF (2007b) Sensory stimuli directly acting at the central nervous system regulate gastric ghrelin secretion. An ex vivo organ culture study. *Endocrinology* **148**:3998–4006.
- Seoane LM, Al-Massadi O, Pazos Y, Pagotto U, and Casanueva FF (2006) Central obestatin administration does not modify either spontaneous or ghrelin-induced food intake in rats. *J Endocrinol Invest* **29**:RC13–RC15.
- Seoane LM, López M, Tovar S, Casanueva FF, Señaris R, and Diéguez C (2003) Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. *Endocrinology* **144**:544–551.
- Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF, and Dieguez C (2000) Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *Eur J Endocrinol* **143**:R7–R9.
- Shafton AD, Sanger GJ, Witherington J, Brown JD, Muir A, Butler S, Abberley L, Shimizu Y, and Furness JB (2009) Oral administration of a centrally acting ghrelin receptor agonist to conscious rats triggers defecation. *Neurogastroenterol Motil* **21**:71–77.
- Shaw AM, Irani BG, Moore MC, Haskell-Luevano C, and Millard WJ (2005) Ghrelin-induced food intake and growth hormone secretion are altered in melanocortin 3 and 4 receptor knockout mice. *Peptides* **26**:1720–1727.
- Shearman LP, Wang SP, Helmling S, Stribling DS, Mazur P, Ge L, Wang L, Klusmann S, Macintyre DE, Howard AD, et al. (2006) Ghrelin neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice. *Endocrinology* **147**:1517–1526.
- Shiiba T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, and Matsukura S (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* **87**:240–244.
- Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H, Date Y, and Nakazato M (2004) Central administration of ghrelin preferentially enhances fat ingestion. *Neurosci Lett* **369**:75–79.
- Shimizu Y, Chang EC, Shafton AD, Ferens DM, Sanger GJ, Witherington J, and Furness JB (2006) Evidence that stimulation of ghrelin receptors in the spinal cord initiates propulsive activity in the colon of the rat. *J Physiol* **576**:329–338.
- Shimizu Y, Nagaya N, Isoe T, Imazu M, Okumura H, Hosoda H, Kojima M, Kangawa K, and Kohno N (2003a) Increased plasma ghrelin level in lung cancer cachexia. *Clin Cancer Res* **9**:774–778.
- Shimizu Y, Nagaya N, Teranishi Y, Imazu M, Yamamoto H, Shokawa T, Kangawa K, Kohno N, and Yoshizumi M (2003b) Ghrelin improves endothelial dysfunction through growth hormone-independent mechanisms in rats. *Biochem Biophys Res Commun* **310**:830–835.
- Shinomiya T, Fukunaga M, Akamizu T, Irako T, Yokode M, Kangawa K, Nakai Y, and Nakai Y (2005) Plasma acylated ghrelin levels correlate with subjective symptoms of functional dyspepsia in female patients. *Scand J Gastroenterol* **40**:648–653.
- Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyayama F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, et al. (2001) Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide YY1 receptor pathway. *Diabetes* **50**:227–232.
- Sibilia V, Bresciani E, Lattuada N, Rapetti D, Locatelli V, De Luca V, Donà F, Netti C, Torsello A, and Guidobono F (2006) Intracerebroventricular acute and chronic administration of obestatin minimally affect food intake but not weight gain in the rat. *J Endocrinol Invest* **29**:RC31–RC34.
- Sibilia V, Rindi G, Pagani F, Rapetti D, Locatelli V, Torsello A, Campanini N, Deghenghi R, and Netti C (2003) Ghrelin protects against ethanol-induced gastric ulcers in rats: studies on the mechanisms of action. *Endocrinology* **144**:353–359.
- Sjöström L, Lindroos AK, Peltonen M, Torgerson J, Bouhard C, Carlsson B, Dahlgren S, Larsson B, Narbro K, Sjöström CD, et al. (2004) Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* **351**:2683–2693.
- Skinner MM, Nass R, Lopes B, Laws ER, and Thorner MO (1998) Growth hormone secretagogue receptor expression in human pituitary tumors. *J Clin Endocrinol Metab* **83**:4314–4320.
- Smith RG, Sun Y, Jiang H, Albarran-Zeckler R, and Timchenko N (2007) Ghrelin receptor (GHS-R1A) agonists show potential as interventional agents during aging. *Ann N Y Acad Sci* **1119**:147–164.
- Smith RJ, Tahsili-Fahadan P, and Aston-Jones G (2010) Orexin/hypocretin is necessary for context-driven cocaine-seeking. *Neuropharmacology* **58**:179–184.
- Soares JB and Leite-Moreira AF (2008) Ghrelin, des-acyl ghrelin and obestatin: three pieces of the same puzzle. *Peptides* **29**:1255–1270.
- Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, Hosoda H, and Kangawa K (2008) Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. *Am J Physiol Heart Circ Physiol* **294**:H426–H432.
- Srivastava A, Kamath A, Barry SA, and Dayal Y (2004) Ghrelin expression in hyperplastic and neoplastic proliferations of the enterochromaffin-like (ECL) cells. *Endocr Pathol* **15**:47–54.
- Stengel A, Wissler A, Kobelt P, Goebel M, Wiedenmann B, Klapp BF, Taché Y, and Mönnikes H (2008) Des-acyl ghrelin suppresses food intake and body weight gain in adult obese Zucker rats (Abstract). *Gastroenterology* **134** (Suppl 1):A-148.
- Stenström B, Furnes MW, Tømmerås K, Syversen U, Zhao CM, and Chen D (2006) Mechanism of gastric bypass-induced body weight loss: one-year follow-up after micro-gastric bypass in rats. *J Gastrointest Surg* **10**:1384–1391.
- Stoyanova II, Wiertz RW, and Rutten WL (2009) Time-dependent changes in ghrelin-immunoreactivity in dissociated neuronal cultures of the newborn rat neocortex. *Regul Pept* **158**:86–90.
- Strasser F, Lutz TA, Maeder MT, Thuerlimann B, Bueche D, Tschöp M, Kaufmann K, Holst B, Brändle M, von Moos R, et al. (2008) Safety, tolerability and pharmacokinetics of intravenous ghrelin for cancer-related anorexia/cachexia: a randomized, placebo-controlled, double-blind, double-crossover study. *Br J Cancer* **98**:300–308.
- Sun Y, Ahmed S, and Smith RG (2003) Deletion of ghrelin impairs neither growth nor appetite. *Mol Cell Biol* **23**:7973–7981.
- Sun Y, Asnicar M, Saha PK, Chan L, and Smith RG (2006) Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metab* **3**:379–386.
- Sun Y, Butte NF, Garcia JM, and Smith RG (2008) Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology* **149**:843–850.
- Sun Y, Garcia JM, and Smith RG (2007) Ghrelin and growth hormone secretagogue receptor expression in mice during aging. *Endocrinology* **148**:1323–1329.
- Suzuki S, Ramos EJ, Goncalves CG, Chen C, and Meguid MM (2005) Changes in GI hormones and their effect on gastric emptying and transit times after Roux-en-Y gastric bypass in rat model. *Surgery* **138**:283–290.
- Szentirmai E, Hajdu I, Obal F Jr., and Krueger JM (2006a) Ghrelin-induced sleep responses in ad libitum fed and food-restricted rats. *Brain Res* **1088**:131–140.
- Szentirmai E, Kapás L, and Krueger JM (2007a) Ghrelin microinjection into fore-

- brain sites induces wakefulness and feeding in rats. *Am J Physiol Regul Integr Comp Physiol* **292**:R575–R585.
- Szentirmai E, Kapás L, Sun Y, Smith RG, and Krueger JM (2009) The preproghrelin gene is required for the normal integration of thermoregulation and sleep in mice. *Proc Natl Acad Sci U S A* **106**:14069–14074.
- Szentirmai E and Krueger JM (2006b) Obestatin alters sleep in rats. *Neurosci Lett* **404**:222–226.
- Tachibana T, Kaiya H, Denbow DM, Kangawa K, and Furuse M (2006) Central ghrelin acts as an anti-dipsogenic peptide in chicks. *Neurosci Lett* **405**:241–245.
- Tack J, Depoortere I, Bisschops R, Delpoite C, Coulie B, Meulemans A, Janssens J, and Peeters T (2006) Influence of ghrelin on interdigestive gastrointestinal motility in humans. *Gut* **55**:327–333.
- Tack J, Depoortere I, Bisschops R, Verbeke K, Janssens J, and Peeters T (2005) Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Aliment Pharmacol Ther* **22**:847–853.
- Tacke F, Brabant G, Kruck E, Horn R, Schöffski P, Hecker H, Manns MP, and Trautwein C (2003) Ghrelin in chronic liver disease. *J Hepatol* **38**:447–454.
- Taheri S, Lin L, Austin D, Young T, and Mignot E (2004) Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med* **1**:e62.
- Takahashi H, Kato A, Onodera K, and Suzuki K (2006a) Fasting plasma ghrelin levels reflect malnutrition state in patients with liver cirrhosis. *Hepatol Res* **34**:117–123.
- Takahashi K, Furukawa C, Takano A, Ishikawa N, Kato T, Hayama S, Suzuki C, Yasui W, Inai K, Sone S, et al. (2006b) The neuromedin U-growth hormone secretagogue receptor 1b/neurotensin receptor 1 oncogenic signaling pathway as a therapeutic target for lung cancer. *Cancer Res* **66**:9408–9419.
- Takamori K, Mizuta Y, Takeshima F, Akazawa Y, Isomoto H, Ohnita K, Ohba K, Omagari K, Shikuwa S, and Kohno S (2007) Relation among plasma ghrelin level, gastric emptying, and psychologic condition in patients with functional dyspepsia. *J Clin Gastroenterol* **41**:477–483.
- Takano S, Kim J, Ikari Y, Ogaya M, Nakajima K, Oomura Y, Wayner MJ, and Sasaki K (2009) Electrophysiological effects of ghrelin on laterodorsal tegmental neurons in rats: an in vitro study. *Peptides* **30**:1901–1908.
- Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, et al. (2000) Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* **85**:4908–4911.
- Takeda H, Sadakane C, Hattori T, Katsurada T, Ohkawara T, Nagai K, and Asaka M (2008) Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT₂ receptor antagonism. *Gastroenterology* **134**:2004–2013.
- Tamura H, Kamegai J, Shimizu T, Ishii S, Sugihara H, and Oikawa S (2002) Ghrelin stimulates GH but not food intake in arcuate nucleus ablated rats. *Endocrinology* **143**:3268–3275.
- Tanaka M, Nakahara T, Kojima S, Nakano T, Muranaga T, Nagai N, Ueno H, Nakazato M, Nozoe S, and Naruo T (2004) Effect of nutritional rehabilitation on circulating ghrelin and growth hormone levels in patients with anorexia nervosa. *Regul Pept* **122**:163–168.
- Tanaka M, Nakahara T, Muranaga T, Kojima S, Yasuhara D, Ueno H, Nakazato M, and Inui A (2006) Ghrelin concentrations and cardiac vagal tone are decreased after pharmacologic and cognitive-behavioral treatment in patients with bulimia nervosa. *Horm Behav* **50**:261–265.
- Tanaka M, Naruo T, Muranaga T, Yasuhara D, Shiiya T, Nakazato M, Matsukura S, and Nozoe S (2002) Increased fasting plasma ghrelin levels in patients with bulimia nervosa. *Eur J Endocrinol* **146**:R1–R3.
- Tanaka M, Naruo T, Nagai N, Kuroki N, Shiiya T, Nakazato M, Matsukura S, and Nozoe S (2003) Habitual binge/purge behavior influences circulating ghrelin levels in eating disorders. *J Psychiatr Res* **37**:17–22.
- Tanaka-Shintani M and Watanabe M (2005) Distribution of ghrelin-immunoreactive cells in human gastric mucosa: comparison with that of parietal cells. *J Gastroenterol* **40**:345–349.
- Tanda ML, Lombardi V, Genovesi M, Ultimieri F, Lai A, Gandolfo M, Dalle Mule I, Grasso L, Bogazzi F, Broglio F, et al. (2009) Plasma total and acylated Ghrelin concentrations in patients with clinical and subclinical thyroid dysfunction. *J Endocrinol Invest* **32**:74–78.
- Taniguchi H, Ariga H, Zheng J, Ludwig K, Mantyh C, Pappas TN, and Takahashi T (2008) Endogenous ghrelin and 5-HT regulate interdigestive gastrointestinal contractions in conscious rats. *Am J Physiol Gastrointest Liver Physiol* **295**:G403–G411.
- Tannous dit El Khoury D, Obeid O, Azar ST, and Hwalla N (2006) Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. *Ann Nutr Metab* **50**:260–269.
- Tatara MR, Krupski W, Sliwa E, Maciejewski R, and Dabrowski A (2007) Fundectomy-evoked osteopenia in pigs is mediated by the gastric-hypothalamic-pituitary axis. *Exp Biol Med (Maywood)* **232**:1449–1457.
- Tebbe JJ, Mronga S, Tebbe CG, Ortmann E, Arnold R, and Schäfer MK (2005a) Ghrelin-induced stimulation of colonic propulsion is dependent on hypothalamic neuropeptide Y1- and corticotrophin-releasing factor 1 receptor activation. *J Neuroendocrinol* **17**:570–576.
- Tebbe JJ, Tebbe CG, Mronga S, Ritter M, and Schäfer MK (2005b) Central neuropeptide Y receptors are involved in 3rd ventricular ghrelin induced alteration of colonic transit time in conscious fed rats. *BMC Gastroenterol* **5**:5.
- Teff KL, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, Townsend RR, Keim NL, D'Alessio D, and Havel PJ (2004) Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab* **89**:2963–2972.
- Tentolouris N, Makrilakis K, Doulergakis D, Moysakakis I, Kokkinos A, Kyriaki D, Georgoulas C, Stathakis C, and Katsilambros N (2005) Increased plasma ghrelin levels in chronic renal failure are not associated with hemodynamic parameters. *Horm Metab Res* **37**:646–652.
- Tesaro M, Schinzari F, Iantorno M, Rizza S, Melina D, Lauro D, and Cardillo C (2005) Ghrelin improves endothelial function in patients with metabolic syndrome. *Circulation* **112**:2986–2992.
- Theander-Carrillo C, Wiedmer P, Cettour-Rose P, Nogueiras R, Perez-Tilve D, Pfluger P, Castaneda TR, Muzzin P, Schürmann A, Szanto I, et al. (2006) Ghrelin action in the brain controls adipocyte metabolism. *J Clin Invest* **116**:1983–1993.
- Theodoropoulou A, Psyrrianni A, Metallinos IC, Habeos I, Vgenakis AG, and Kyriazopoulou V (2009) Ghrelin response to oral glucose load in hyperthyroidism, before and after treatment with antithyroid drugs. *J Endocrinol Invest* **32**:94–97.
- Thompson NM, Gill DA, Davies R, Loveridge N, Houston PA, Robinson IC, and Wells T (2004) Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. *Endocrinology* **145**:234–242.
- Tolle V, Kadem M, Bluet-Pajot MT, Frere D, Foulon C, Bossu C, Dardennes R, Mounier C, Zizzari P, Lang F, et al. (2003) Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. *J Clin Endocrinol Metab* **88**:109–116.
- Tong Q, Ye CP, Jones JE, Elmquist JK, and Lowell BB (2008) Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. *Nat Neurosci* **11**:998–1000.
- Torsello A, Bresciani E, Rossoni G, Avallone R, Tulipano G, Cocchi D, Bulgarelli I, Degenghi R, Berti F, and Locatelli V (2003) Ghrelin plays a minor role in the physiological control of cardiac function in the rat. *Endocrinology* **144**:1787–1792.
- Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, et al. (2003) Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* **144**:1506–1512.
- Toshinai K, Yamaguchi H, Sun Y, Smith RG, Yamanaka A, Sakurai T, Date Y, Mondal MS, Shimbara T, Kawagoe T, et al. (2006) Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. *Endocrinology* **147**:2306–2314.
- Trudel L, Bouin M, Tomasetto C, Eberling P, St-Pierre S, Bannion P, L'Heureux MC, and Poiras P (2003) Two new peptides to improve post-operative gastric ileus in dog. *Peptides* **24**:531–534.
- Trudel L, Tomasetto C, Rio MC, Bouin M, Plourde V, Eberling P, and Poiras P (2002) Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat. *Am J Physiol Gastrointest Liver Physiol* **282**:G948–G952.
- Tschöp M, Flora DB, Mayer JP, and Heiman ML (2002) Hypophysectomy prevents ghrelin-induced adiposity and increases gastric ghrelin secretion in rats. *Obes Res* **10**:991–999.
- Tschöp M, Smiley DL, and Heiman ML (2000) Ghrelin induces adiposity in rodents. *Nature* **407**:908–913.
- Tschöp M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, and Folwaczny C (2001a) Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* **24**:RC19–RC21.
- Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, and Heiman ML (2001b) Circulating ghrelin levels are decreased in human obesity. *Diabetes* **50**:707–709.
- Tsolakis AV, Portela-Gomes GM, Stridsberg M, Grimelius L, Sundin A, Eriksson BK, Oberg KE, and Janson ET (2004) Malignant gastric ghrelinoma with hyperghrelinemia. *J Clin Endocrinol Metab* **89**:3739–3744.
- Tsolakis AV, Stridsberg M, Grimelius L, Portela-Gomes GM, Falkmer SE, Waldum HL, and Janson ET (2008) Ghrelin immunoreactive cells in gastric endocrine tumors and their relation to plasma ghrelin concentration. *J Clin Gastroenterol* **42**:381–388.
- Tsubone T, Masaki T, Katsuragi I, Tanaka K, Kakuma T, and Yoshimatsu H (2005) Ghrelin regulates adiposity in white adipose tissue and UCP1 mRNA expression in brown adipose tissue in mice. *Regul Pept* **130**:97–103.
- Tsubota Y, Owada-Makabe K, Yukawa K, and Maeda M (2005) Hypotensive effect of des-acyl ghrelin at nucleus tractus solitarius of rat. *Neuroreport* **16**:163–166.
- Tümer C, Ofllazoglu HD, Obay BD, Kelle M, and Taşdemir E (2008) Effect of ghrelin on gastric myoelectric activity and gastric emptying in rats. *Regul Pept* **146**:26–32.
- Ueberberg B, Unger N, Saeger W, Mann K, and Petersenn S (2009) Expression of ghrelin and its receptor in human tissues. *Horm Metab Res* **41**:814–821.
- Ueno H, Shiiya T, Mizuta M, Mondal SM, and Nakazato M (2007) Plasma ghrelin concentrations in different clinical stages of diabetic complications and glycemic control in Japanese diabetics. *Endocr J* **54**:895–902.
- Unniappan S, Speck M, and Kieffer TJ (2008) Metabolic effects of chronic obestatin infusion in rats. *Peptides* **29**:1354–1361.
- Van Cauter E, Spiegel K, Tasali E, and Leproult R (2008) Metabolic consequences of sleep and sleep loss. *Sleep Med* **9** (Suppl 1):S23–S28.
- van den Top M, Lee K, Whyment AD, Blanks AM, and Spanswick D (2004) Orexin-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nat Neurosci* **7**:493–494.
- van den Top M, Lyons DJ, Lee K, Coderre E, Renaud LP, and Spanswick D (2007) Pharmacological and molecular characterization of ATP-sensitive K(+) conductances in CART and NPY/AgRP expressing neurons of the hypothalamic arcuate nucleus. *Neuroscience* **144**:815–824.
- Venkova K, Fraser G, Hoveyda HR, and Greenwood-Van Meerveld B (2007) Prokinetic effects of a new ghrelin receptor agonist TZP-101 in a rat model of postoperative ileus. *Dig Dis Sci* **52**:2241–2248.
- Venkova K, Mann W, Nelson R, and Greenwood-Van Meerveld B (2009) Efficacy of ipamorelin, a novel ghrelin mimetic, in a rodent model of postoperative ileus. *J Pharmacol Exp Ther* **329**:1110–1116.
- Vergote V, Van Dorpe S, Peremans K, Burvenich C, and De Spiegeleer B (2008) In vitro metabolic stability of obestatin: kinetics and identification of cleavage products. *Peptides* **29**:1740–1748.
- Vestergaard ET, Andersen NH, Hansen TK, Rasmussen LM, Moller N, Sorensen KE, Sloth E, and Jorgensen JO (2007a) Cardiovascular effects of intravenous ghrelin infusion in healthy young men. *Am J Physiol Heart Circ Physiol* **293**:H3020–H3026.
- Vestergaard ET, Hansen TK, Gormsen LC, Jakobsen P, Moller N, Christiansen JS,

- and Jorgensen JO (2007b) Constant intravenous ghrelin infusion in healthy young men: clinical pharmacokinetics and metabolic effects. *Am J Physiol Endocrinol Metab* **292**:E1829–E1836.
- Vetter ML, Cardillo S, Rickels MR, and Iqbal N (2009) Narrative review: effect of bariatric surgery on type 2 diabetes mellitus. *Ann Intern Med* **150**:94–103.
- Vicennati V, Genghini S, De Iasio R, Pasqui F, Pagotto U, and Pasquali R (2007) Circulating obestatin levels and the ghrelin/obestatin ratio in obese women. *Eur J Endocrinol* **157**:295–301.
- Vlasova MA, Järvinen K, and Herzig KH (2009) Cardiovascular effects of ghrelin antagonist in conscious rats. *Regul Pept* **156**:72–76.
- Volante M, Allia E, Fulcheri E, Cassoni P, Ghigo E, Muccioli G, and Papotti M (2003) Ghrelin in fetal thyroid and follicular tumors and cell lines: expression and effects on tumor growth. *Am J Pathol* **162**:645–654.
- Volante M, Allia E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, Muccioli G, Ghigo E, and Papotti M (2002) Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *J Clin Endocrinol Metab* **87**:1300–1308.
- Volante M, Rosas R, Ceppi P, Rapa I, Cassoni P, Wiedenmann B, Settanni F, Granata R, and Papotti M (2009) Obestatin in human neuroendocrine tissues and tumors: expression and effect on tumor growth. *J Pathol* **218**:458–466.
- Wang DH, Hu YS, Du JJ, Hu YY, Zhong WD, and Qin WJ (2009a) Ghrelin stimulates proliferation of human osteoblastic TE85 cells via NO/cGMP signaling pathway. *Endocrine* **35**:112–117.
- Wang HS, Oh DS, Ohning GV, and Pisegna JR (2007) Elevated serum ghrelin exerts an orexigenic effect that may maintain body mass index in patients with metastatic neuroendocrine tumors. *J Mol Neurosci* **33**:225–231.
- Wang W, Andersson M, Iresjö BM, Lönnroth C, and Lundholm K (2006) Effects of ghrelin on anorexia in tumor-bearing mice with eicosanoid-related cachexia. *Int J Oncol* **28**:1393–1400.
- Wang WG, Chen X, Jiang H, and Jiang ZY (2008) Effects of ghrelin on glucose-sensing and gastric distension sensitive neurons in rat dorsal vagal complex. *Regul Pept* **146**:169–175.
- Wang Y and Liu J (2009) Plasma ghrelin modulation in gastric band operation and sleeve gastrectomy. *Obes Surg* **19**:357–362.
- Wargin W, Thomas H, Clohs L, St-Louis C, Ejskjaer N, Gutierrez M, Shaughnessy L, and Kosutic G (2009) Contribution of protein binding to the pharmacokinetics of the ghrelin receptor agonist TZP-101 in healthy volunteers and adults with symptomatic gastroparesis: two randomized, double-blind studies and a binding profile study. *Clin Drug Investig* **29**:409–418.
- Waseem T, Javaid-Ur-Rehman, Ahmad F, Azam M, and Qureshi MA (2008) Role of ghrelin axis in colorectal cancer: a novel association. *Peptides* **29**:1369–1376.
- Wasko R, Jaskuła M, Plewa R, Komarowska H, Poreba E, Goździcka-Józefiak A, Liebert W, Bednarek J, and Sowiński J (2006) The evaluation of ghrelin mRNA expression in human somatotroph adenomas. *Neuro Endocrinol Lett* **27**:169–173.
- Wei W, Qi X, Reed J, Ceci J, Wang HQ, Wang G, Englander EW, and Greeley GH Jr (2006) Effect of chronic hyperghrelinemia on ingestive action of ghrelin. *Am J Physiol Regul Integr Comp Physiol* **290**:R803–R808.
- Weikel JC, Wichniak A, Ising M, Brunner H, Friess E, Held K, Mathias S, Schmid DA, Uhr M, and Steiger A (2003) Ghrelin promotes slow-wave sleep in humans. *Am J Physiol Endocrinol Metab* **284**:E407–E415.
- Wellman PJ, Bellinger LL, Cepeda-Benito A, Susabda A, Ho DH, and Davis KW (2005) Meal patterns and body weight after nicotine in male rats as a function of chow or high-fat diet. *Pharmacol Biochem Behav* **82**:627–634.
- Wettschureck N, Moers A, Wallenwein B, Parlow AF, Maser-Gluth C, and Offermanns S (2005) Loss of Gq/11 family G proteins in the nervous system causes pituitary somatotroph hypoplasia and dwarfism in mice. *Mol Cell Biol* **25**:1942–1948.
- Wiley KE and Davenport AP (2002) Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological antagonist of endothelin-1. *Br J Pharmacol* **136**:1146–1152.
- Willesen MG, Kristensen P, and Rømer J (1999) Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* **70**:306–316.
- Williams DL, Cummings DE, Grill HJ, and Kaplan JM (2003a) Meal-related ghrelin suppression requires postgastric feedback. *Endocrinology* **144**:2765–2767.
- Williams DL, Grill HJ, Cummings DE, and Kaplan JM (2003b) Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology* **144**:5184–5187.
- Williams DL, Grill HJ, Cummings DE, and Kaplan JM (2006) Overfeeding-induced weight gain suppresses plasma ghrelin levels in rats. *J Endocrinol Invest* **29**:863–868.
- Wisse BE, Frayo RS, Schwartz MW, and Cummings DE (2001) Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. *Endocrinology* **142**:3292–3301.
- Wolf I, Sadetzki S, Kanety H, Kundel Y, Pariente C, Epstein N, Oberman B, Catane R, Kaufman B, and Shimon I (2006) Adiponectin, ghrelin, and leptin in cancer cachexia in breast and colon cancer patients. *Cancer* **106**:966–973.
- Woods SC (2004) Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. *Am J Physiol Gastrointest Liver Physiol* **286**:G7–G13.
- Wortley KE, del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO, and Sleeman MW (2005) Absence of ghrelin protects against early-onset obesity. *J Clin Invest* **115**:3573–3578.
- Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillon WS, Ghatei MA, and Bloom SR (2001a) Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* **86**:5992.
- Wren AM, Small CJ, Abbott CR, Dhillon WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, et al. (2001b) Ghrelin causes hyperphagia and obesity in rats. *Diabetes* **50**:2540–2547.
- Wren AM, Small CJ, Fribbens CV, Neary NM, Ward HL, Seal LJ, Ghatei MA, and Bloom SR (2002) The hypothalamic mechanisms of the hypophysiotropic action of ghrelin. *Neuroendocrinology* **76**:316–324.
- Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, et al. (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* **141**:4325–4328.
- Wu R, Zhou M, Cui X, Simms HH, and Wang P (2003) Ghrelin clearance is reduced at the late stage of polymicrobial sepsis. *Int J Mol Med* **12**:777–781.
- Wynne K, Giannitsopoulou K, Small CJ, Patterson M, Frost G, Ghatei MA, Brown EA, Bloom SR, and Choi P (2005) Subcutaneous ghrelin enhances acute food intake in malnourished patients who receive maintenance peritoneal dialysis: a randomized, placebo-controlled trial. *J Am Soc Nephrol* **16**:2111–2118.
- Xu Z, Lin S, Wu W, Tan H, Wang Z, Cheng C, Lu L, and Zhang X (2008) Ghrelin prevents doxorubicin-induced cardiotoxicity through TNF-alpha/NF-kappaB pathways and mitochondrial protective mechanisms. *Toxicology* **247**:133–138.
- Yada T, Kaiya H, Mutoh K, Azuma T, Hyodo S, and Kangawa K (2006) Ghrelin stimulates phagocytosis and superoxide production in fish leukocytes. *J Endocrinol* **189**:57–65.
- Yamamoto D, Ikeshita N, Daito R, Herningtyas EH, Toda K, Takahashi K, Iida K, Takahashi Y, Kaji H, Chihara K, et al. (2007) Neither intravenous nor intracerebroventricular administration of obestatin affects the secretion of GH, PRL, TSH and ACTH in rats. *Regul Pept* **138**:141–144.
- Yang J, Brown MS, Liang G, Grishin NV, and Goldstein JL (2008a) Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* **132**:387–396.
- Yang J, Zhao TJ, Goldstein JL, and Brown MS (2008b) Inhibition of ghrelin O-acyltransferase (GOAT) by octanoylated pentapeptides. *Proc Natl Acad Sci U S A* **105**:10750–10755.
- Yang N, Liu X, Ding EL, Xu M, Wu S, Liu L, Sun X, and Hu FB (2009) Impaired ghrelin response after high-fat meals is associated with decreased satiety in obese and lean Chinese young adults. *J Nutr* **139**:1286–1291.
- Yasuda T, Masaki T, Kakuma T, and Yoshimatsu H (2003) Centrally administered ghrelin suppresses sympathetic nerve activity in brown adipose tissue of rats. *Neurosci Lett* **349**:75–78.
- Yeh AH, Jeffery PL, Duncan RP, Herington AC, and Chopin LK (2005) Ghrelin and a novel preproghrelin isoform are highly expressed in prostate cancer and ghrelin activates mitogen-activated protein kinase in prostate cancer. *Clin Cancer Res* **11**:8295–8303.
- Yildiz BO, Suchard MA, Wong ML, McCann SM, and Licinio J (2004) Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci U S A* **101**:10434–10439.
- Yin J and Chen J (2006) Inhibitory effects of gastric electrical stimulation on ghrelin-induced excitatory effects on gastric motility and food intake in dogs. *Scand J Gastroenterol* **41**:903–909.
- Ying BW, Song XB, Fan H, Wang LL, Li YS, Cheng Z, Cheng H, and Wen FQ (2008) Plasma ghrelin levels and weight loss in Chinese Uygur patients with chronic obstructive pulmonary disease. *J Int Med Res* **36**:1371–1377.
- Yoshimoto A, Mori K, Sugawara A, Mukoyama M, Yahata K, Suganami T, Takaya K, Hosoda H, Kojima M, Kangawa K, et al. (2002) Plasma ghrelin and desacyl ghrelin concentrations in renal failure. *J Am Soc Nephrol* **13**:2748–2752.
- Youm YH, Yang H, Sun Y, Smith RG, Manley NR, Vandanmagsar B, and Dixit VD (2009) Deficient ghrelin receptor-mediated signaling compromises thymic stromal cell microenvironment by accelerating thymic adiposity. *J Biol Chem* **284**:7068–7077.
- Zhang JV, Jahr H, Luo CW, Klein C, Van Kolen K, Ver Donck L, De A, Baart E, Li J, Moechars D, et al. (2008a) Obestatin induction of early-response gene expression in gastrointestinal and adipose tissues and the mediatory role of G protein-coupled receptor, GPR39. *Mol Endocrinol* **22**:1464–1475.
- Zhang JV, Ren PG, Avsian-Kretschmer O, Luo CW, Rauch R, Klein C, and Hsueh AJ (2005) Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* **310**:996–999.
- Zhang W, Chai B, Li JY, Wang H, and Mulholland MW (2008b) Effect of des-acyl ghrelin on adiposity and glucose metabolism. *Endocrinology* **149**:4710–4716.
- Zheng J, Ariga H, Taniguchi H, Ludwig K, and Takahashi T (2009a) Ghrelin regulates gastric phase III-like contractions in freely moving conscious mice. *Neurogastroenterol Motil* **21**:78–84.
- Zigman JM, Jones JE, Lee CE, Saper CB, and Elmquist JK (2006) Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol* **494**:528–548.
- Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, et al. (2005) Mice lacking ghrelin receptors resist the development of diet-induced obesity. *J Clin Invest* **115**:3564–3572.
- Zizzari P, Longchamps R, Epelbaum J, and Bluet-Pajot MT (2007) Obestatin partially affects ghrelin stimulation of food intake and growth hormone secretion in rodents. *Endocrinology* **148**:1648–1653.
- Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, Meijler MM, and Janda KD (2006) Vaccination against weight gain. *Proc Natl Acad Sci U S A* **103**:13226–13231.