Review Article
Recent Advances in Potential Clinical Application of Ghrelin in Obesity

Christine Delporte

Laboratory of Biological Chemistry and Nutrition, Faculty of Medicine, Université libre de Bruxelles, 1070 Brussels, Belgium

Correspondence should be addressed to Christine Delporte, cdelport@ulb.ac.be

Received 17 June 2011; Accepted 4 December 2011

1. Introduction
Ghrelin, the natural ligand of the growth hormone secretagogue receptor (GHS-R1a) [1], is a potent stimulator of growth hormone secretion [2, 3]. Moreover, ghrelin is also an appetite-stimulating hormone inducing food intake and weight gain in human [4–6], and promoting gastric emptying [7]. Ghrelin is a 28 amino acid peptide predominantly produced and secreted into the blood stream by the endocrine stomach mucosal cells named “X/A like” in rat [8, 9] and P/D1 cells in humans [10]. Ghrelin has the particularity to be acylated on the serine in position 3 [1]. During the processing of preproghrelin, both ghrelin 1-28 and ghrelin 1-27 can result and then are subjected to the acylation of the hydroxyl group of Ser3 [11]. Acylation, a unique peptidic modification, is catalyzed by ghrelin O-acyltransferase, a member of the membrane-bound O-acyltransferase family, during the processing of the peptide [2, 3]. The most frequently acylation is with an octanoyl group (C8:0), and more rarely with a decanoyl (C10:0) or a decenoyl (C10:1) group [11]. Acylation of ghrelin can be increased by ingestion of either medium-chain fatty acids or medium-chain triacylglycerides [12].

Des-acyl ghrelin represents more than 90% of human plasma ghrelin immunoreactivity [13]. It remains presently uncertain if both ghrelin and des-acyl ghrelin, present in the stomach, are both secreted into the bloodstream via similar or different regulated pathway(s). In the rat stomach, ghrelin is deacylated by lysophospholipase I [14, 15], and degraded by N-terminal proteolysis [14, 16]. Shorter half-life of ghrelin compared to des-acyl ghrelin [17] and plasma ghrelin deacylation [16, 18] could account for the vast predominance of des-acyl ghrelin in the circulation. Human butyrylcholinesterase and other esterase(s), such as platelet-activating factor acetylhydrolase, and rat carboxylesterase are responsible for ghrelin desoctanoylation in these species [16, 19]. Interestingly, butyrylcholinesterase knockout mice fed with a normal standard 5% fat diet had normal body weight while mice fed with high-fat diet (11% fat) became obese. Butyrylcholinesterase was suggested to play a role in fat catabolism as the obese phenotype could not be explained by increased ghrelin, caloric intake, or decreased exercise [20]. The suggested participation of human paraoxonase in ghrelin deacylation [21] remains controversial [16]. Due to ghrelin degradation by serum, it is difficult to accurately...
determine the ghrelin level and consequently its physiological and pathophysiological roles. In the circulation, des-acyl ghrelin is mostly present as a free peptide while the vast majority of acyl ghrelin is bound to larger molecules and in particular to lipoproteins [19, 21]. The presence of the acyl group is necessary for ghrelin interaction with triglyceride-rich lipoproteins and low-density lipoprotein but not high-density lipoproteins and very high-density lipoproteins. Besides, ghrelin interacts via its N- and C-terminal parts with high-density lipoproteins and very high-density lipoproteins. These data support the transport of acylated ghrelin by triglyceride-rich lipoproteins and of both ghrelin and des-acyl ghrelin by high-density lipoproteins and very high-density lipoproteins. These data support the transport of acylated ghrelin by high-density lipoproteins and very high-density lipoproteins.

Administration of ghrelin to rats leads to stimulation of food intake and decrease of energy expenditure, accounting for body weight increase [6, 22–25]. Intravenous ghrelin administration in humans also increases appetite and stimulates food intake [6]. Plasma ghrelin levels are negatively correlated with BMI and fluctuate in a compensatory manner to body weight variations [26]. Indeed, plasma ghrelin level is increased in anorexia nervosa and cachexia, and decreased in obesity [27]. Ghrelin levels decrease with weight gain resulting from overfeeding [28], pregnancy [29], olanzapine treatment [30], or high fat diet [31]. Central ghrelin administration to rats submitted to high fat diet does not result in greater food intake, while increased adiposity in white adipose tissue occurs [32]. In white adipose tissue, ghrelin stimulates the gene expression of lipogenic enzymes such as stearoyl CoA desaturase, acetyl CoA carboxylase, and fatty acid synthase. These data suggest that central ghrelin simultaneously regulates food intake and adipose tissue metabolism through distinct mechanisms [32].

Acute feeding response appears to be mediated by GHS-R1a [33]. Chronic weight gain effect of ghrelin may be modulated by both GHS-R1a [34] as well as an as yet unidentified receptor for ghrelin as both ghrelin and a ghrelin antagonist induced body weight gain [35]. Nevertheless, further studies would be required to clarify this issue.

Des-acyl ghrelin has been recently taken into consideration as a modulator of food intake that could act through an as yet unidentified receptor [36, 37]. However, des-acyl ghrelin appears to have controversial effects on food intake [36, 37]. Indeed, GOAT knockout mice displayed reduced fat mass despite increased des-acyl ghrelin levels [38]. The identification of speculated des-acyl ghrelin receptors could deeply increase our knowledge on the mechanisms and actions sites of this peptide.

High plasma ghrelin levels have been reported in patients with Prader-Willi syndrome (PWS), a genetic disorder characterized by mental retardation and hyperphagia leading to severe obesity [39, 40]. In this disorder, ghrelin may be responsible, at least partially, for the inattentive appetite and the obesity of the patients.

From the molecular biological point of view, it is interesting to note that both ghrelin and its receptor (GHSR) genes are located on chromosome 3 in regions that have been linked to obesity [41, 42]. Polymorphisms of both ghrelin and its receptor GHSR1a have been studied in obesity [43–52]. However, further studies are required to assess unambiguously the functional significance of these mutations in the pathogenesis of obesity.

Due to the observed association between plasma ghrelin levels and insulin levels as well as insulin resistance [53–56], it was suggested that inhibition of ghrelin secretion and/or of GHS-R1a could be a useful treatment and/or prevention for type 2 diabetes. In this respect, data from numerous studies evaluating the therapeutic implications of ghrelin on glucose-insulin homeostasis have been recently reviewed [57, 58].

The involvement of ghrelin in obesity led to the development of several ghrelin-related pharmacological tools for the treatment of obesity. The present review focuses on the recent advances made in potential clinical applications of ghrelin in obesity.

2. Ghrelin O-Acyltransferase: A Pharmacological Target to Decrease Acylated Ghrelin Levels

Ghrelin O-acetyltransferase (GOAT), identified as being an orphan membrane-bound O-acyltransferase (MBOAT), catalyzes the addition of an octanoyl group on the serine in position 3 [2, 3] (Figure 1). Ghrelin octanoylation is essential for its recognition by GHS-R1a. Two distinct scientific approaches led to the identification of GOAT. The first approach was based on gene-silencing experiments targeting a candidate gene encoding an uncharacterized protein containing structural motifs reminiscent of the MBOAT acyltransferase family in the human medullary thyroid carcinoma cells expressing octanoylated ghrelin [2]. The authenticity of the human gene identified was verified using RT-PCR and 5′ RACE reactions, and the predicted protein encoded by the gene was named GOAT. The functional activity of GOAT, the octanoylation of ghrelin, was demonstrated by transient transfections of the GOAT cDNA. GOAT was also shown to acylate ghrelin with fatty acid ranging from C7 to C12. Furthermore, additional experiments revealed the importance of the conserved MBOAT-histidine residue in position 338 of GOAT to its acylation activity [2]. The second approach was based on the transfection of rat insulinoma Ins-1 cells with ghrelin cDNA and subsequently of one at a time, of the sixteen MBOAT cDNAs [3]. Only one MBOAT cDNA led to acylated ghrelin production. Furthermore, mutation of either serine in position 3 of ghrelin or of the conserved MBOAT-histidine residue in position 338 of GOAT abolished ghrelin acylation. GOAT exhibits some specificity for medium chain fatty acids like octanoate and proceeds to ghrelin octanoylation before its translocation to the Golgi where it is cleaved by prohormone convertase 1/3 to form mature ghrelin. This suggested that GOAT is located in the endoplasmic reticulum [3].

GOAT is a highly hydrophobic protein with eight postulated membrane-spanning helices presenting a high degree of sequence conservation across vertebrates. GOAT is coexpressed with acyl ghrelin in ghrelin-expressing tissues [59].
GOAT displays a preference for hexanoyl-CoA over octanoyl-CoA as an acyl donor [60]. However, the precise mechanism leading to the entry of acyls-CoA into the endoplasmic reticulum lumen remains unknown. One hypothesis is that GOAT could possibly bind acyl-CoA and, due to its hydrophobic properties, allow the acylation of ghrelin in the endoplasmic reticulum lumen.

An in vitro biochemical assay for GOAT activity [3] revealed the importance of proper recognition of several amino acids in proghrelin (glycine-1, serine-3, and phenylalanine-4) for GOAT activity [61].

Fasting and satiation could modulate the activity of GOAT as ghrelin levels rise before meals [4, 62] and decrease with food intake [5]. Moreover, long-term fasting inhibits ghrelin acylation but not total ghrelin secretion whereas feeding suppresses both acyl and des-acyl ghrelin [63]. However, the effect of fasting and feeding on GOAT mRNA levels remain unclear [38, 64]. Experimental evidences showed that GOAT is a leptin-regulated gene [38]. Increased GOAT mRNA levels in response to long-term chronic malnutrition [64] could represent the underlying mechanism responsible for increased acylated ghrelin levels in anorexia nervosa [26].

Dietary lipids are critical for the activation of GOAT, and consequently ghrelin acylation. Indeed, GOAT knock-out mice submitted to a diet containing 10% medium-chain triglyceride exhibited lower body weight that can be explained by lower fat mass compared to wild-type mice [38]. In addition, GOAT transgenic mice only fed with a medium-chain triglycerides supplementation produced large amounts of acyl ghrelin [38].

An essential function of ghrelin could be the maintenance of viability during periods of famine. This hypothesis is supported by the data showing that wild-type and GOAT knock-out mice submitted to 60% calorie-restricted diet displayed 30% and 75% body weight loss, respectively [65].

Much work remains to be done to fully understand how GOAT fits into the control of energy homeostasis. However, measurement of both GOAT protein levels and GOAT activity will be crucial to determine its gene expression and functional regulation. Indeed, GOAT knock-out mice represent

---

**Figure 1**: Synthesis, processing, and release of ghrelin by the human endocrine stomach P/D1 cells. Synthesis of ghrelin and acylation by GOAT occur in the endoplasmic reticulum (ER). Both acyl and des-acyl ghrelin are then secreted into the blood stream by fusion of secretory vesicles with the plasma membrane. Ghrelin secretion and/or acylation are upregulated following T2R activation. After secretion of acyl and/or des-acyl ghrelin, acyl ghrelin binds to specific GHS-R1a receptors. Pharmacological tools tried or currently developed are indicated and targeted GOAT, T2R, ghrelin, and GHS-R1a.
a valuable tool to determine the physiological consequences of a specific deficiency in acylated ghrelin.

Recently, genetic variation of GOAT was suggested to be involved in the etiology of anorexia nervosa [66]. It would be interesting to determine if genetic variation of GOAT might also be linked to obesity. If this proves to be the case, personalized medicine targeting GOAT could be envisioned as a novel therapeutic approach for the treatment of obesity.

Pharmacological tools have been developed to target the inhibition of GOAT (Figure 1). Indeed, a pentapeptide, corresponding to the first five N-terminal amino acids of ghrelin with its C-terminal end amidated competitively inhibited GOAT activity through an end-product inhibition mechanism. The inhibition of GOAT is better achieved when pentapeptides contain an octanoyl group linked to serine-3 by an amide linkage [3]. Moreover, GOAT was also inhibited by peptide-based bisubstrate analog, GO-CoA-Tat, in cultured cells, as well as in mice [67]. The design of this bisubstrate analog was based on the theory that GOAT could use a ternary complex mechanism to proceed to the linkage of octanoyl-CoA to ghrelin. The intraperitoneal administration led to reduced weight gain and improved glucose tolerance in wild-type mice but not in ghrelin knock-out mice [67]. Even though GO-CoA-Tat presents some limitations as a peptide-based drug, it is likely that future synthetic derivatizations will maximize its pharmacological properties.

In conclusion, GOAT represents an extremely promising candidate for the development of antiobesity and/or antidiabetes drugs. Indeed, it is the unique enzyme responsible for ghrelin acylation and its modulation would only affect the physiological process of ghrelin acylation.

3. Neutralization of Ghrelin

Vaccination against ghrelin represents a strategy to block the effects of ghrelin (Figure 1). Rats immunized with ghrelin hapten immunoconjugates led to the production of antibodies specifically directed against acylated ghrelin, and reduced body weight gain with preferential reduction of fat mass concomitant to decreased feeding efficiency [68]. The human relevance of using vaccination against ghrelin remains uncertain. Indeed, phase I/II a trial using CYT 009-Ghr Qb vaccine, from Cytos Biotechnology AG, demonstrated no weight-loss effect in obese humans despite efficient antibody response.

High-affinity antiacyl ghrelin specific monoclonal antibodies specifically bind acyl ghrelin, dose-dependently inhibits GHS-R1a activation in vitro, and block ghrelin-induced food intake in mice in vivo [69].

Neutralization of ghrelin was also achieved using spiegelmers, antisense polyethylene glycol-modified L-oligonucleotides capable of specifically binding a target molecule (Figure 1). The spiegelmer NOX-B11-2 decreased food intake and body weight in diet-induced obese mice [70–72]. Another spiegelmer, NOX-B11-3 exerted a long-lasting action on the inhibition of ghrelin-induced GH release in rats [73], but did not block the fasting-induced neuronal activation in the hypothalamic arcuate nucleus [74]. The neutralization of circulating ghrelin by spiegelmers may be useful to treat diseases associated with high ghrelin levels such as PWS characterized by severe obesity. Pfizer Inc. has taken over further development of the NOX-B11 spiegelmers originally developed by NOXXON Pharma AG.

In conclusion, the therapeutic usefulness of vaccination against ghrelin and the use of ghrelin spiegelmers in the treatment of obesity remain to be proven.

4. GHS-R1a: A Pharmacological Target to Antagonize Ghrelin-Induced Responses

4.1. GHS-R1a Antagonists. The inhibition of ghrelin signaling represents an attractive target for pharmacological treatment of type 2 diabetes, obesity, particularly PWS, and metabolic syndrome. Consequently, several classes of GHS-R1a antagonists have been developed (Figure 1).

[D-Lys-3]GHRP-6, a peptide GHS-R1a antagonist, decreased food intake in lean and obese mice, and reduced weight gain [70, 75].

Piperidine-substituted quinazolinone derivatives were identified as a novel class of small GHS-R1a antagonists molecules [76]. Phenyl or phenoxy groups are optimal substituents at position 6 of the quinazolinone core, and the replacement of phenyl groups in position 2 by small alkyl substituents were proven to be beneficial [76]. YIL-781, a piperidine-substituted quinazolinone derivative acting as a potent GHS-R1a antagonist, improved glucose-stimulated insulin secretion and reduced food intake and weight loss in diet-induced obese mice [77].

Some GHS analogs carrying a trisubstituted 1,2,4-triazole structure, such as JMV2866 and JMV2844, behaved as GHS-R1a antagonists [78, 79]. Recently, additional new GHS-R1a antagonists of global similar structure have been identified using homogenous time-resolved fluorescence-based assay screening [80].

Optimization of piperazine-bisamide analogs synthesis led to potent GHS-R1a antagonists. One of these analogs featured especially high potency as well as other interesting pharmacological properties, and inhibited GH release ex vivo [81].

Several carbohydrazide derivatives were identified as being potent and selective GHS-R1a antagonists [82]. Among these compounds, GSK1614343 was shown to be a potent competitive antagonist of rat GHS-R1a [83]. Unexpectedly, GSK1614343 produced an increase in food intake and body weight in both rats and dogs [84].

BIM-28163 was identified as a ghrelin antagonist blocking ghrelin-induced GH secretion [85]. However, chronic administration of GHS-R1a antagonist unexpectedly induced body weight gain [85].

Other GHS-R1a analogs developed to treat weight disorders, including obesity, are also still considered as preclinical compounds (TZP-301, from Tranzyme Pharma, and EX-1350, from Elixir Pharmaceuticals) [86].

In conclusion, several classes of GHS-R1a antagonists have been identified and could represent an interesting pharmacological tools for the treatment of obesity as well as type 2 diabetes and metabolic syndrome. However, long-term animal and human studies still remain necessary to
appropriately evaluate the beneficial properties of ghrelin antagonists in the context of obesity.

4.2. GHS-R1a Inverse Agonists. The high constitutive activity of GHS-R1a suggested that inverse GHS-R1a agonists, decreasing its constitutive activity, may be useful for the treatment of obesity [87, 88]. Long fasting induced, in the hypothalamus, increased GHS-R1a expression and concomitant signaling causing higher appetite and decreased energy expenditure. Therefore, reduction of the GHS-R1a constitutive activity by an inverse agonist could increase the sensitivity to anorexigenic hormones like leptin or PYY, and prevent food intake between meals [89].

[D-Arg1, D-Phe3, D-Trp7,9, Leu11] substance P was identified as an inverse agonist on GHS-R1a [90].

In conclusion, GHR-R1a inverse agonists represent interesting pharmacological tool to inhibit GHS-R1a activity (Figure 1). However, additional studies evaluating the long-term use of the compounds in animal models are necessary to elucidate their usefulness in the treatment of obesity and related diseases in humans.

5. New Potential Pharmacological Target to Decrease Ghrelin Secretion

Very recently, gavage of bitter taste receptor (T2R) agonists was shown to increase plasma acyl ghrelin in mice through the stimulation of α-gustducin, the α-subunit of a trimeric G-protein complex involved in taste signal transduction [91]. Immunofluorescence studies revealed that the stomach endocrine cells expressing ghrelin displayed up to 90–95% colocalization with α-gustducin. Furthermore, gavage of T2R-agonists increased food intake in wild-type mice but not in α-gustducin or GHS-R1a knock out mice [91]. It is presently unclear if the transduction pathways induced following T2R activation could affect ghrelin acylation by GOAT and/or ghrelin release.

In conclusion, T2R could represent a new interesting pharmacological target to modulate ghrelin secretion (Figure 1). Furthermore, the potential use of T2R antagonists for the treatment of obesity remains to be evaluated.

6. General Conclusions

The involvement of ghrelin in obesity and the better understanding of ghrelin biology have led to the identification of pharmacological targets and the development of pharmacological compounds for the treatment of obesity and related diseases. So far, pharmacological compounds have been designed to target GOAT, ghrelin, and GHS-R1a. Very recently, it has been suggested that T2R could also represent an interesting target in the context of ghrelin and the treatment of obesity.

Acknowledgments

This paper was supported by Grant 3.4510.03 and 3.4561.07 from the Fund for Medical Scientific Research (FRSM, Belgium). The author would like to thank Dr. Jason Perret for his useful discussions, support and critical reading during the preparation of the paper.

References

[1] M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, and K. Kangawa, “Ghrelin is a growth-hormone-releasing acylated peptide from stomach,” Nature, vol. 402, no. 6762, pp. 656–660, 1999.
[2] J. A. Gutierrez, P. J. Soelenberg, D. R. Perkins et al., “Ghrelin octanoylation mediated by an orphan lipid transferase,” Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 17, pp. 6320–6325, 2008.
[3] J. Yang, M. S. Brown, G. Liang, N. V. Grishin, and J. L. Goldstein, “Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone,” Cell, vol. 132, no. 3, pp. 387–396, 2008.
[4] D. E. Cummings, J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse, and D. S. Weigle, “A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans,” Diabetes, vol. 50, no. 8, pp. 1714–1719, 2001.
[5] M. Tschöp, M. R. Wartaw, R. L. Riepl et al., “Post-prandial decrease of circulating human ghrelin levels,” Journal of Endocrinological Investigation, vol. 24, no. 6, pp. RC19–RC21, 2001.
[6] A. M. Wren, L. J. Seal, M. A. Cohen et al., “Ghrelin enhances appetite and increases food intake in humans,” Journal of Clinical Endocrinology and Metabolism, vol. 86, no. 12, pp. 5992–5995, 2001.
[7] L. Trudel, C. Tomasetto, M. C. Rio et al., “Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric post-operative ileus in rat,” American Journal of Physiology, vol. 282, no. 6, pp. G948–G952, 2002.
[8] Y. Date, M. Kojima, H. Hosoda et al., “Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans,” Endocrinology, vol. 141, no. 11, pp. 4255–4261, 2000.
[9] G. Rindi, V. Necchi, A. Savio et al., “Characterisation of gastric ghrelin cells in man and other mammals: studies in adult and fetal tissues,” Histochemistry and Cell Biology, vol. 117, no. 6, pp. 511–519, 2002.
[10] I. Sakata, K. Nakamura, M. Yamazaki et al., “Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract,” Peptides, vol. 23, no. 3, pp. 531–536, 2002.
[11] H. Hosoda, M. Kojima, T. Mizushima, S. Shimizu, and K. Kangawa, “Structural divergence of human ghrelin: identification of multiple ghrelin-derived molecules produced by post-translational processing,” Journal of Biological Chemistry, vol. 278, no. 1, pp. 64–70, 2003.
[12] Y. Nishi, H. Hiejima, H. Hosoda et al., “Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin,” Endocrinology, vol. 146, no. 5, pp. 2255–2264, 2005.
[13] M. Patterson, K. G. Murphy, C. W. Le Roux, M. A. Ghatei, and S. R. Bloom, “Characterization of ghrelin-like immunoreactivity in human plasma,” Journal of Clinical Endocrinology and Metabolism, vol. 90, no. 4, pp. 2205–2211, 2005.
[14] Y. Shanado, M. Kometani, H. Uchiyama, S. Koizumi, and N. Teno, “Lysophospholipase I identified as a ghrelin deacylation enzyme in rat stomach,” Biochemical and Biophysical Research Communications, vol. 325, no. 4, pp. 1487–1494, 2004.
[46] O. Ukkola, E. Ravussin, P. Jacobson et al., “Mutations in the preproghrelin/ghrelin gene associated with obesity in humans,” Journal of Clinical Endocrinology and Metabolism, vol. 86, no. 8, pp. 3996–3999, 2001.

[47] D. Vivenza, A. Rapa, N. Castellino et al., “Ghrelin gene polymorphisms and ghrelin, insulin, IGF-I, leptin and anthropometric data in children and adolescents,” European Journal of Endocrinology, vol. 151, no. 1, pp. 127–133, 2004.

[48] H. J. Wang, F. Geller, A. Dempfle et al., “Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature,” Journal of Clinical Endocrinology and Metabolism, vol. 89, no. 1, pp. 157–162, 2004.

[49] M. Korbonits, M. Gueorguiev, E. O’Grady et al., “A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children,” Journal of Clinical Endocrinology and Metabolism, vol. 87, no. 8, pp. 4005–4008, 2002.

[50] G. R. Martin, J. C. Loredo, and G. Sun, “Lack of association of ghrelin precursor gene variants and percentage body fat or serum lipid profiles,” Obesity, vol. 16, no. 4, pp. 908–912, 2008.

[51] D. G. Miraglia, N. Santoro, G. Cirillo et al., “Molecular screening of the ghrelin gene in Italian obese children: the Leu72Met variant is associated with an earlier onset of obesity,” International Journal of Obesity, vol. 28, no. 3, pp. 447–450, 2004.

[52] R. M. Dardenne, P. Zizzari, V. Tolle et al., “Family trios analysis of common polymorphisms in the obestatin/ghrelin, BDNF and AGRP genes in patients with Anorexia nervosa: association with subtype, body–mass index, severity and age of onset,” Psychoneuroendocrinology, vol. 32, no. 2, pp. 106–113, 2007.

[53] M. Tschöp, C. Weyer, P. A. Tataranni, V. Devanarayan, E. Ravussin, and M. L. Heiman, “Circulating ghrelin levels are decreased in human obesity,” Diabetes, vol. 50, no. 4, pp. 707–709, 2001.

[54] A. Ikezaki, H. Hosoda, K. Ito et al., “Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAL-1, but not with leptin, in obese children and adolescents,” Diabetes, vol. 51, no. 12, pp. 3408–3411, 2002.

[55] S. M. Pöykkö, E. Kellokoski, S. Hörkkö, H. Kauma, Y. A. Kesäniemi, and O. Ukkola, “Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes,” Diabetes, vol. 52, no. 10, pp. 2546–2553, 2003.

[56] L. Pacífico, E. Poggiogalle, F. Costantino et al., “Acylated and nonacylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndrome,” European Journal of Endocrinology, vol. 161, no. 6, pp. 861–870, 2009.

[57] P. J. Delhanty and A. J. van der Lely, “Ghrelin and glucose homeostasis,” Peptides, vol. 32, no. 11, pp. 2309–2318, 2011.

[58] S. Sangiao-Alvarellos and F. Cordido, “Effect of ghrelin on glucose-insulin homeostasis: therapeutic implications,” International Journal of Peptides, vol. 2010, Article ID 234709, 25 pages, 2010.

[59] I. Sakata, J. Yang, C. E. Lee et al., “Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells,” American Journal of Physiology, vol. 297, no. 1, pp. E134–E141, 2009.

[60] H. Ohgusu, S. Shiromizu, Y. Nakamura et al., “Ghrelin O-acyltransferase (GOAT) has a preference for n-hexanoyl-CoA over n-octanoyl-CoA as an acyl donor,” Biochemical and Biophysical Research Communications, vol. 386, no. 1, pp. 153–158, 2009.

[61] M. Koijima and K. Kangawa, “Structure and function of Ghrelin,” Results and Problems in Cell Differentiation, vol. 46, pp. 89–115, 2008.

[62] D. E. Cummings, “Ghrelin and the short- and long-term regulation of appetite and body weight,” Physiology and Behavior, vol. 89, no. 1, pp. 71–84, 2006.

[63] J. Liu, C. E. Prudom, R. Nass et al., “Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men,” Journal of Clinical Endocrinology and Metabolism, vol. 93, no. 5, pp. 1980–1987, 2008.

[64] C. R. González, M. J. Vázquez, M. López, and C. Diéguez, “Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa,” Journal of Molecular Endocrinology, vol. 41, no. 5–6, pp. 415–421, 2008.

[65] T. J. Zhao, G. Liang, R. L. Li et al., “Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice,” Proceedings of the National Academy of Sciences of the United States of America, vol. 107, no. 16, pp. 7467–7472, 2010.

[66] T. D. Müller, M. H. Tschöp, J. Jarick et al., “Genetic variation of the ghrelin activator gene ghrelin O-acyltransferase (GOAT) is associated with anorexia nervosa,” Journal of Psychiatric Research, vol. 45, no. 5, pp. 706–711, 2011.

[67] B. P. Barnett, Y. Hwang, M. S. Taylor et al., “Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor,” Science, vol. 330, no. 6011, pp. 1689–1692, 2010.

[68] E. P. Zorrilla, S. Iwasaki, J. A. Moss et al., “Vaccination against weight gain,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 35, pp. 13226–13231, 2006.

[69] S. C. Lu, J. Xu, N. Chinookoswong et al., “An acyl-ghrelin-specific neutralizing antibody inhibits the acute ghrelin-mediated orexigenic effects in mice,” Molecular Pharmacology, vol. 75, no. 4, pp. 901–907, 2009.

[70] A. Asakawa, A. Inui, T. Kaga et al., “Antagonism of ghrelin receptor reduces food intake and body weight gain in mice,” Gut, vol. 52, no. 7, pp. 947–952, 2003.

[71] L. P. Shearman, S. P. Wang, S. Helming et al., “Ghrelin neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice,” Endocrinology, vol. 147, no. 3, pp. 1517–1526, 2006.

[72] P. Kobelt, S. Helming, A. Stengel et al., “Anti-ghrelin Spiegelmer NOX-B11 inhibits neurostimulatory and orexigenic effects of peripheral ghrelin in rats,” Gut, vol. 55, no. 6, pp. 788–792, 2006.

[73] S. Helming, C. Maasch, D. Eulberg et al., “Inhibition of ghrelin action in vitro and in vivo by an RNA-Spiegelmer,” Proceedings of the National Academy of Sciences of the United States of America, vol. 101, no. 36, pp. 13174–13179, 2004.

[74] C. Becskei, K. U. Bilik, S. Klussmann, F. Jarošch, T. A. Lutz, and T. Riediger, “The anti-ghrelin spiegelmer NOX-B11-3 blocks ghrelin- but not fasting-induced neuronal activation in the hypothalamic arcuate nucleus,” Journal of Neuroendocrinology, vol. 20, no. 1, pp. 85–92, 2008.

[75] B. Beck, S. Richy, and A. Stricker-Krongrad, “Feeding response to ghrelin agonist and antagonist in lean and obese Zucker rats,” Life Sciences, vol. 76, no. 4, pp. 473–478, 2004.

[76] J. Rudolph, W. P. Esler, S. O’Connor et al., “Quinazolinone derivatives as orally available ghrelin receptor antagonists for the treatment of diabetes and obesity,” Journal of Medicinal Chemistry, vol. 50, no. 21, pp. 5202–5216, 2007.

[77] W. P. Esler, J. Rudolph, T. H. Claus et al., “Small-molecule Ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss,” Endocrinology, vol. 148, no. 11, pp. 3175–3185, 2007.
[78] L. Demange, D. Boeglin, A. Moulin et al., “Synthesis and pharmacological in vitro and in vivo evaluations of novel triazole derivatives as ligands of the ghrelin receptor. 1,” *Journal of Medicinal Chemistry*, vol. 50, no. 8, pp. 1939–1957, 2007.

[79] A. Moulin, L. Demange, J. Ryan et al., “Trisubstituted 1,2,4-triazoles as ligands for the ghrelin receptor: on the significance of the orientation and substitution at position 3,” *Bioorganic and Medicinal Chemistry Letters*, vol. 18, no. 1, pp. 164–168, 2008.

[80] J. P. Leyris, T. Roux, E. Trinquet et al., "Homogeneous time-resolved fluorescence-based assay to screen for ligands targeting the growth hormone secretagogue receptor type 1a," *Analytical Biochemistry*, vol. 408, no. 2, pp. 253–262, 2011.

[81] M. Yu, M. Lizarzaburu, H. Beckmann et al., "Identification of piperazine-bisamide GHSR antagonists for the treatment of obesity," *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 5, pp. 1758–1762, 2010.

[82] F. M. Sabbatini, R. Di Fabio, M. Corsi et al., "Discovery process and characterization of novel carbohydrazide derivatives as potent and selective GHSR1a antagonists," *ChemMedChem*, vol. 5, no. 9, pp. 1450–1455, 2010.

[83] E. Perdonà, F. Faggioni, A. Buson, F. M. Sabbatini, C. Corti, and M. Corsi, "Pharmacological characterization of the ghrelin receptor antagonist, GSK1614343 in rat RC-4B/C cells natively expressing GHS type 1a receptors," *European Journal of Pharmacology*, vol. 650, no. 1, pp. 178–183, 2011.

[84] V. J. Costantini, E. Vicentini, F. M. Sabbatini et al., "GSK1614343, a novel ghrelin receptor antagonist, produces an unexpected increase of food intake and body weight in rodents and dogs," *Neuroendocrinology*, vol. 94, no. 2, pp. 158–168, 2011.

[85] H. A. Halem, J. E. Taylor, J. Z. Dong et al., "Novel analogs of ghrelin: physiological and clinical implications," *European Journal of Endocrinology*, vol. 151, no. 1, pp. S71–S75, 2004.

[86] I. Depoortere, "Targeting the ghrelin receptor to regulate food intake," *Regulatory Peptides*, vol. 156, no. 1–3, pp. 13–23, 2009.

[87] B. Holst, A. Cygankiewicz, T. H. Jensen, M. Ankersen, and T. W. Schwartz, "High constitutive signaling of the ghrelin receptor—identification of a potent inverse agonist," *Molecular Endocrinology*, vol. 17, no. 11, pp. 2201–2210, 2003.

[88] N. D. Holliday, B. Holst, E. A. Rodionova, T. W. Schwartz, and H. M. Cox, "Importance of constitutive activity and arrestin-independent mechanisms for intracellular trafficking of the ghrelin receptor," *Molecular Endocrinology*, vol. 21, no. 12, pp. 3100–3112, 2007.

[89] B. Holst and T. W. Schwartz, "Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation," *Trends in Pharmacological Sciences*, vol. 25, no. 3, pp. 113–117, 2004.

[90] B. Holst, M. Lang, E. Brandt et al., "Ghrelin receptor inverse agonists: identification of an active peptide core and its interaction epitopes on the receptor," *Molecular Pharmacology*, vol. 70, no. 3, pp. 936–946, 2006.

[91] S. Janssen, J. Laermans, P. J. Verhulst, T. Thijs, J. Tack, and I. Depoortere, "Bitter taste receptors and α-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 5, pp. 2094–2099, 2011.