Current Diabetes Reports (2019) 19: 102
https://doi.org/10.1007/s11892-019-1211-9

OBESITY (KM GADDE, SECTION EDITOR)

# Pharmacological Modulation of Ghrelin to Induce Weight Loss: Successes and Challenges

Martha A. Schalla[1] · Andreas Stengel[1,2]

Published online: 10 September 2019


## Abstract

**Purpose of Review** Obesity is affecting over 600 million adults worldwide and has numerous negative effects on health. Since ghrelin positively regulates food intake and body weight, targeting its signaling to induce weight loss under conditions of obesity seems promising. Thus, the present work reviews and discusses different possibilities to alter ghrelin signaling.

**Recent Findings** Ghrelin signaling can be altered by RNA Spiegelmers, GHSR/Fc, ghrelin-*O*-acyltransferase inhibitors as well as antagonists, and inverse agonists of the ghrelin receptor. PF-05190457 is the first inverse agonist of the ghrelin receptor tested in humans shown to inhibit growth hormone secretion, gastric emptying, and reduce postprandial glucose levels. Effects on body weight were not examined.

**Summary** Although various highly promising agents targeting ghrelin signaling exist, so far, they were mostly only tested in vitro or in animal models. Further research in humans is thus needed to further assess the effects of ghrelin antagonism on body weight especially under conditions of obesity.

**Keywords** Antagonist · Ghrelin-*O*-acyl transferase · GOAT · Growth hormone · Inverse agonist · Obesity

**Abbreviations**

| | |
|---|---|
| ACTH | Adrenocorticotropic hormone |
| AZ-GHS-22 | Non-CNS penetrant inverse agonist 22 |
| AZ-GHS-38 | CNS penetrant inverse agonist 38 |
| BMI | Body mass index |
| CpdB | Compound B |
| CpdD | Compound D |
| DIO | Diet-induced obesity |
| GH | Growth hormone |
| GHRP-2 | Growth hormone–releasing peptide-2 |
| GHRP-6 | Growth hormone–releasing peptide 6 |
| GHSR | Growth hormone secretagogue receptor |
| GOAT | Ghrelin-*O*-acyltransferase |
| GRLN-R | Ghrelin receptor |
| icv | Intracerebroventricular |
| POMC | Proopiomelanocortin |
| sc | Subcutaneous |
| SPM | RNA Spiegelmer |
| WHO | World Health Organization. |

This article is part of the Topical Collection on *Obesity*

✉ Andreas Stengel
andreas.stengel@med.uni-tuebingen.de

Martha A. Schalla
Martha.schalla@charite.de

[1] Charité Center for Internal Medicine and Dermatology, Charité Center for Internal Medicine and Dermatology, Department for Psychosomatic Medicine; Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[2] Department of Psychosomatic Medicine and Psychotherapy, Medical University Hospital Tübingen, Tübingen, Germany

## Introduction

As stated by the World Health Organization (WHO), between 1975 and today, worldwide prevalence of obesity, defined as a body mass index (BMI) greater than or equal to 30 kg/m$^2$, has nearly tripled [1]. Obesity is a risk factor for type 2 diabetes, high blood pressure, heart disease and strokes, certain types of cancer, sleep apnea, osteoarthritis, fatty liver disease, kidney disease, and also psychiatric diseases [1]. Body weight is regulated among others by peripheral adiposity signal hormones such as leptin and ghrelin, which mediate the peripheral energy state to central regulatory centers in the hypothalamus [2].

Ghrelin was identified in 1999 in the rat stomach as an endogenous ligand of the growth hormone secretagogue receptor (GHSR) [3], which was later renamed to ghrelin receptor (GRLN-R) [4]. Thus, ghrelin was early on implicated in the release of growth hormone (GH) both in vitro and in vivo [3]. Ghrelin is predominantly secreted from X/A-like cells of the stomach [5]. Moreover, it is expressed in pancreatic alpha cells [6] and—in lower amounts—in the kidney, liver, spleen, heart, lung, adipose tissue, skin [7], and testis [8]. Ghrelin consists of 28 amino acids with an *n*-octanoylated serine 3 residue responsible for GHSR binding [3] and its GH-releasing activity [5]. The transfer of *n*-octanoic acid to serine 3 of ghrelin is catalyzed by the enzyme ghrelin-*O*-acyltransferase (GOAT) [9]. The distribution of GOAT is very similar to ghrelin with expression in gastrointestinal organs, especially the stomach, as well as in testis and pituitary [10–13]. Prepro-ghrelin, a 117-amino acid peptide which is cleaved to pro-ghrelin, is encoded by the ghrelin gene [3]. Pro-ghrelin cleavage provides two different forms of ghrelin: if not acylated by GOAT instead of ghrelin, desacyl ghrelin is secreted [3]. Noteworthy, desacyl ghrelin seems to exert effects opposite to acylated ghrelin. While the latter stimulates food intake, gastrointestinal motility, lipogenesis, and glycemia and decreases energy expenditure and insulin secretion/sensitivity, desacyl ghrelin was shown to suppress food intake, gastrointestinal motility, and glycemia and stimulate insulin secretion via a so far unknown receptor [14].

The GRLN-R is a 7 transmembrane G protein coupled receptor [3] and found in the central nervous system such as hypothalamic neurons and brainstem [15] as well as in various peripheral regions including vagal afferents, pancreatic cells, spleen, cardiac muscle, bone, adipose, thyroid, adrenal glands, and on immune cells [7].

A publication in 2000 stated that "ghrelin induces adiposity in rodents" due to the observation that ghrelin administered subcutaneously (sc) once daily decreased fat utilization in mice and rats resulting in weight gain and intracerebroventricularly (icv)-injected ghrelin dose-dependently increased food intake and body weight [16]. Subsequent studies showed that chronic icv infusions of ghrelin elevate mRNA expression of fat-sparing enzymes such as fatty acid synthase, acetyl-CoA carboxylase α, stearoyl-CoA desaturase-1, and lipoprotein lipase while decreasing mRNA expression of carnitine palmitoyltransferase-1α [17]. Ghrelin was thus supposed to reduce fat utilization and lipid mobilization [17, 18]. As a result, lipid deposition in human visceral adipose tissue is increased by ghrelin [19].

In animal studies, diet-induced obesity (DIO) led to decreased acylated and total plasma ghrelin levels, *ghrelin* and *GOAT* mRNA expression in the stomach, and expression of hypothalamic *GRLN-R* mRNA [20]. Additionally, reduced responsiveness of NPY/AgRP to plasma ghrelin was observed under conditions of DIO likely blunting ghrelin's effects in obese subjects [20]. Consequently, administration of ghrelin did not increase food intake in high-fat DIO mice [21]. Besides central ghrelin resistance, also peripheral resistance is likely associated with obesity-induced inflammation impairing ghrelin signaling via the nodose ganglion [21]. Impaired ghrelin signaling due to obesity is also expected in humans since obese individuals showed decreased fasting ghrelin levels and a strongly reduced postprandial ghrelin suppression [22]. Similarly, obese children displayed reduced ghrelin levels and a significant negative correlation between BMI and fasting ghrelin levels [23].

Intravenous ghrelin (iv 5.0 pmol/kg/min) in nine healthy volunteers increased calories consumed when exposed to a free-choice buffet and using visual analog scores for appetite [24]. However, here, it had no effect on gastric emptying [24]. Investigation of various ghrelin analogs and ghrelin receptor agonists showed similar results: Ipamorelin, derived from met-enkephalin and acting as a selective GH secretagogue, thus as agonist of the GRLN-R, significantly increased food intake and body weight gain in female rats when repetitively iv administered [25]. NN703 is derived from ipamorelin and acts as orally active GH secretagogue, stimulating GH release from rat pituitary cells. In vivo in rats, body weight gain was significantly increased after oral long-term NN703 treatment [26].

Growth hormone–releasing peptide-2 (GHRP-2), also known as KP-102, is also derived from met-enkephalin. Icv infusion of GHPR-2 significantly elevated food intake and body weight for 6 days in rodents [27]. GHRP-2 sc infused to lean, healthy males increased food intake accompanied by elevated serum GH levels, without altering the macronutrient composition of consumed food [28]. Additionally, in children with GH deficiency, GHRP-2 (900 μg/kg) administered orally twice daily for 12 months significantly increased appetite during the first 6 months in 7 out of 10 children [29].

Anamorelin, another GRLN-R agonist derived from met-enkephalin, had a stimulating dose-related effect on body weight which correlated with changes in IGF-1 levels in healthy subjects [30]. Likewise, in cachectic cancer patients, anamorelin significantly increased body weight without altering food intake [31]. In another study, healthy subjects receiving anamorelin orally (also called RC-1291) had dose-related weight gain after 6 days [32]. In addition, dogs treated with orally administered capromorelin, another GRLN-R ligand derived from small-molecule libraries, had increased food consumption and body weight [33]. Lastly, ulimorelin or Tranzyme Pharma (TZP)-101, a small-molecule agonist derived from peptidomimetic libraries and acting as GRLN-R agonist, stimulated food intake without inducing significant GH release after icv administration [34].

The super-agonist BIM-28131, a GRLN-R ligand derived from ghrelin, stimulated c-Fos activity similarly to ghrelin especially in the arcuate nucleus and showed even higher orexigenic properties than ghrelin [35].

Since ghrelin is considered to be a major stimulator of food intake and likely also involved in the development of obesity—as also suggested by the pharmacological use of GRLN-R ligands—blockade of ghrelin signaling drew a lot of attention in order to induce weight loss. The current review presents and discusses these efforts, successes, and challenges.

## Modulation of Ghrelin Signaling

### Ghrelin Blocking/Binding

The polyethylene glycol–modified l-RNA oligonucleotide with in vivo stability, NOX-B11, has been used as a ghrelin-blocking agent, also called RNA Spiegelmer (SPM). Consequently, SPM was shown in vitro to bind ghrelin with low-nanomolar affinity and block the ghrelin-induced activation of the GRLN-R [36]. l-NOX-B11 interacts with the N-terminal five amino acids of ghrelin. However, 1-NOX-B11 is highly specific for acylated ghrelin and does not bind to the desacylated form [37]. NOX-B11-3, the latest generation of SPM, was shown to inhibit the excitatory effect of ghrelin on single cells of the rat medial arcuate nucleus in vitro [38]. In vivo, 15 mg/kg intraperitoneally (ip) injected NOX-B11-3 also suppressed the ghrelin-induced c-Fos expression in the arcuate nucleus [38]. Investigation of the acyl ghrelin–binding compound of NOX-B11, NOX-B11-2, showed that a dose of 66 mg/kg (sc) NOX-B11-2 acutely administered inhibited ghrelin-induced food intake but not nonpeptide GRLN-R agonist–induced feeding [36]. Chronic application of NOX-B11-2 (33 mg/kg/d, sc) decreased food intake and body weight gain—mostly via a reduction of fat mass—in DIO mice, an effect absent in ghrelin-deficient mice [36]. This blockade of ghrelin-induced food intake occurred rapidly and was observed during the first 30 min after ghrelin administration [39]. NOX-B1-2 also impaired the recovery of body weight after food restriction in male Wistar rats accompanied by decelerated regeneration of glycogen levels [40]. In addition, the ghrelin-induced increase in food hoarding was also suppressed by NOX-B11-2 during the first 2 days after injection, an effect paralleled by reduced hypothalamic c-Fos-immunoreactivity [41]. Taken together, SPM seems to be a potent agent preventing ghrelin's effects on food intake and body weight as shown in vitro and in animal studies. Investigations in humans should follow.

In order to deplete circulating ghrelin levels, a mammalian expression plasmid vector was constructed which encodes the ligand-binding domains of the GHS-R1a fused with a human IgG constant region (Fc), forming GHSR/Fc. Intramuscular injection of GHSR/Fc in fact reduced circulating levels of acylated ghrelin and decreased weight gain in mice fed a high-fat diet accompanied by decreased fat accumulation in the peritoneum [42]. Increased fat utilization was indicated by increased PPARγ and hormone-sensitive lipase transcript levels in adipose tissue [42]. GHSR/Fc plasmid also improved glucose clearance and insulin sensitivity in vivo [42]. However, further research evaluating ghrelin binding and effects in humans is needed. To improve the usability of these ghrelin-binding substances, oral formulations should be introduced.

In 2006, it was shown that the active vaccination of mature rats with ghrelin immunoconjugates had a significant effect on body weight gain. Two of the active vaccines based on the 28-aa residue sequence of ghrelin, Ghr1 or Ghr3, selectively bound to acylated ghrelin, resulting in attenuated body weight gain, by reduction of body fat in rats [43]. Similarly, in pigs actively immunized against ghrelin, food intake was reduced by more than 15% so that immunized pigs weighed 10% less than control animals at the end of the trial [44]. Immunization in mice with a chemical conjugation of ghrelin with a virus-like particle increased titers of anti-ghrelin antibodies, leading to decreased cumulative food intake and increased energy expenditure. However, no significant effect on body weight could be detected [45]. In the first human randomized, double-blind, placebo-controlled trial using anti-ghrelin vaccination administered in 4 injections of 300 μg at weeks 0, 4, 8, and 16, in 87 obese patients aged 18–55 years with a body mass index between 30 and 35, no additional weight loss was observed in comparison with the control group despite a significant increase in ghrelin antibodies [46].

### GOAT Inhibition

In 2008, it was shown that the enzyme-acylating ghrelin, ghrelin-*O*-acyl transferase (GOAT), can be inhibited by synthetic octanoylated ghrelin pentapeptides [47]. Another specific GOAT inhibitor developed in 2010, GO-CoA-Tat, blocked GOAT assessed radioactively in cultured cells in vitro as well as in mice [48]. In mice, glucose tolerance and weight loss were enhanced by ip administered GO-CoA-Tat, an effect absent in ghrelin-deficient mice [48]. The GOAT inhibitor induced a dose-dependent decrease of food intake with a maximum effect observed after 96 mg/kg (−27%) due to a reduction of meal frequency but not meal size [49]. GO-CoA-Tat also reduced circulating acyl ghrelin levels and weight gain in mice fed a high-fat diet [41]. Siberian hamsters, known for increased hoarding instead of food consumption after food restriction, receiving GO-CoA-Tat injections (ip, 11 μmol/kg) every 6 h (representing the duration of its effective inhibition) during food deprivation showed reduced foraging, food intake, and food hoarding during refeeding [41].

Another study showed that GO-CoA-Tat did not affect plasma levels of desacyl and acyl ghrelin, but reduced plasma adrenocorticotropic hormone (ACTH), aldosterone, and corticosterone concentrations 60 min after injection, while hypothalamic *CRF* mRNA levels and pituitary *proopiomelanocortin (POMC)* mRNA levels were increased [50]. Similarly, no effect was observed on *GOAT* mRNA levels in the hypothalamus, pituitary, adrenal, and stomach fundus following administration of GO-CoA-Tat [50].

Regarding liver metabolism, inhibition of GOAT by GO-CoA-Tat in human cells reduced IL-6 and TNF-α concentrations, ALT and AST in serum as well as blood glucose, total cholesterol, and triglycerides, effects accompanied by—and possibly secondary to—significantly decreased body weight [51•]. Despite the fact that GO-CoA-Tat showed promising effects in different animal models, data in humans are lacking so far.

In the last decade, several other GOAT inhibitors have been identified: In 2011, the first non-peptide small-molecule antagonist of GOAT was discovered, displaying inhibition of the enzyme at the micromolar level [52]. In 2015, it was found that the incorporation of triazole linkage in GOAT inhibitors creates a biostable isosteric replacement for the ester bond in ghrelin and amide bonds, thereby optimizing the inhibitor of the human isoform of GOAT [53]. Through screening of a small-molecule library, the GOAT inhibitory activity of a class of synthetic triterpenoids was identified in 2017. These behave as covalent reversible inhibitors of human but not mouse GOAT, further underlining the importance of human studies [54]. In 2018, the discovery of a novel series of GOAT inhibitors was reported. Using a novel high-throughput assay system compound B (4-chloro-6-{[2-methyl-6-(trifluoromethyl)pyridin-3-yl]methoxy}-1-benzothiophen-3-yl) acetic acid) was shown to behave as potent GOAT inhibitor with oral bioavailability by reducing acyl ghrelin production in the stomach of mice [55]. However, although chemically interesting and pharmacologically promising, the effects on body weight of these inhibitors are yet to be established.

## Ghrelin Receptor Antagonism

Since ghrelin is known to transmit its orexigenic effect via the GRLN-R [56] and Ghsr(−/−) mice displayed decreased fat and a healthier lipid profile associated with increased energy expenditure and resting metabolic rate [57], various GRLN-R antagonists were developed in order to identify approaches to induce weight loss.

In 1992, the examination of met-enkephalin-derived GH-releasing peptides led to the observation that [D-Lys3]-growth hormone–releasing peptide 6 (GHRP-6), consisting of the amino acid sequence His-D-Trp-D-Lys-Trp-D-Phe-Lys-$NH_2$ is able to block GHRP binding to the anterior pituitary and hypothalamic membranes [58]. While the hexapeptide GHRP-6 stimulated food intake in rats [59] and GH secretion in children with short stature [60], [D-Lys3]-GHRP-6 was shown to act as a partial antagonist with a strong bias towards GHSR-1a-β-arrestin signaling [61]. In lean mice, in mice with DIO, and in leptin-deficient *ob/ob* obese mice, ip-injected [D-Lys-3]-GHRP-6 reduced food intake [62]. Subsequent studies showed that long-term treatment with [D-Lys3]-GHRP-6 in mice not only decreased GH secretion but also body fat [63]. Strikingly, food intake was not reduced but unexpectedly increased, caused by reduced POMC gene expression and GH-releasing hormone (GHRH) gene expression in the hypothalamus [63]. [D-Lys3]-GHRP-6 additionally reduced plasma insulin and C-peptide levels resulting in increased blood glucose and insulin intolerance [63]. These findings were accompanied by a reduction of insulin-positive cells and an increase in somatostatin-positive cells in pancreatic tissue as detected by immunofluorescent staining [63]. This leads to the conclusion that [D-Lys3]-GHRP-6—mainly due to its side effects—might not be a promising candidate for inhibition of ghrelin signaling.

In contrast, another met-enkephalin-derived antagonist His-D2NaI-DLys-Trp-DPhe-Lys$NH_2$ successfully reduced the GHRP-2-induced GH release and food intake in rats after iv and icv injection [64]. Several evaluations of met-enkephalin-derived GRLN-R antagonists with a trisubstituted 1,2,4-triazole structure demonstrated that JMV2810 (icv, sc) is a partial antagonist in vitro while being a full antagonist in vivo [65]. Sc injection of JMV2810 inhibited the hexarelin-stimulated food intake with no effect on GH release in rats [65]. Similarly, JMV2844 (sc) suppressed food intake [65]. Also JMV2959 (icv, sc, ip) acted as a full unbiased GRLN-R antagonist [61] and a potent in vivo antagonist of hexarelin-stimulated food intake but did not stimulate GH secretion in rats [66]. Interestingly, although it decreased food intake 4 h post injection, it had no effect on body weight [67]. JMV3002 (icv) decreased the food consumed during the first hour of food exposure after a 16-h fast in rats and suppressed ghrelin-induced changes in electric activity of arcuate neurons in vitro [68]. Lastly, JMV3021 (sc) significantly inhibited hexarelin-induced effects on feeding behavior in adolescent rats [69].

Besides met-enkephalin-derived antagonists, ghrelin-derived ligands such as BIM-28163 and peptide G5-1 were tested. BIM-28163 is an analog of full-length human ghrelin binding to the GRLN-R without activating the receptor [70]. As a consequence, BIM-28163 administered iv inhibited ghrelin-induced GH secretion in vivo and reduced ghrelin-induced c-Fos immunoreactivity in the medial arcuate nucleus [70]. However, regarding body weight, BIM-28163 acted as a GRLN-R agonist in male Sprague Dawley rats [70]. Similarly, in the dorsal medial hypothalamus, BIM-28163 behaved as an agonist and upregulated c-Fos immunoreactivity [70]. BIM-28163 was less potent than ghrelin in inducing c-Fos

immunoreactivity in arcuate neuropeptide Y neurons as well as in the area postrema but similarly potent in the nucleus of the solitary tract [35]. These observations led to the assumption that the body weight–stimulating effect of ghrelin might not be transmitted via the GRLN-R or that the different effects are mediated through different states of activity of the receptor. A study in 2012 using cDNA display led to the identification of an antagonistic GRLN-R ligand derived from ghrelin, namely peptide G5-1 [71]. G5-1 was shown to reduce food intake and ghrelin-induced gastric contractions after iv application in mice, likely by suppressing the ghrelin-induced intracellular calcium increase [71].

The effects of small-molecule GRLN-R antagonists seem highly promising. Oral application of the small-molecule GRLN-R antagonists YIL-781 and YIL-870 increased glucose tolerance resulting from increased insulin secretion in rats [72]. Additionally, in DIO mice, these GRLN-R antagonists decreased body weight and fat mass resulting from reduced food consumption [72]. Delayed gastric emptying was only observed at the highest dose tested (10 mg/kg); thus, it was concluded to be not responsible for the weight loss observed since lower doses without an effect on gastric emptying still induced body weight loss [72]. Small-molecule GRLN-R antagonist compound D (CpdD) induced a transient decrease in food intake while compound B (CpdB) exerted a sustained body weight decrease due to a reduction of white adipose tissue associated with improved glucose disposal, insulin sensitivity, and liver function preventing hepatic steatosis in mice fed a high-fat diet [73]. Most importantly, no systemic toxicity was detected in mice treated with small-molecule GRLN-R antagonists compared with that in controls [73].

Another small-molecule group antagonizing the GRLN-R is piperidine-substituted quinazolinone derivatives. These were derived from an agonist with poor oral bioavailability, but modulation resulted in an orally bioavailable antagonist [74]. Suppression of food intake and body weight along with glucose-lowering effects mediated by glucose-dependent insulin secretion in rodents showed that this antagonist, compound 26, is potent and selective for the GRLN-R [74].

Ulimorelin, as mentioned already in the introduction, is a macrocyclic peptide acting as an GRLN-R agonist. A search in peptidomimetic libraries showed that novel conformationally defined macrocyclic compounds exist that antagonize the GRLN-R. Compound 1505, orally applied at 30 mg/kg, significantly decreased cumulative food intake and body weight over 7 days as well as blood glucose at days 3 and 7 by increased insulin sensitivity in obese Zucker rats [75]. Compound 1505 also reduced free fatty acids and total cholesterol [75] further pointing towards a healthier phenotype.

Taken together, although various GRLN-R antagonists exist, [D-Lys3]-GHRP-6 seems not to exert the expected positive effect on food intake, body weight, or glucose metabolism, probably due to incomplete antagonism of the receptor. However, other promising compounds showing positive effects on weight loss such as YIL 781 and 870 as well as compound B, compound 1505, and compound 26 need to be evaluated further in human studies. Noteworthy, oral application is a great advantage of small-molecule GRLN-R antagonists and macrocyclic compounds.

## Inverse Ghrelin Receptor Agonists

Since the GRLN-R exhibits a high constitutive activity accounting for 50% of its maximal activity [76], inverse agonists should reduce the constitutive activity of the GRLN-R reflecting a suppression. The first inverse agonist of the GRLN-R to be identified was the substance P derivate [D-Arg(1), D-Phe(5), D-Trp(7,9), Leu(11)]-substance P [62]. In lean, DIO and *ob/ob* mice, this substance P derivate reduced food intake [62]. Overall, substance P analogs showed properties of an inverse agonist–enhancing G protein–dependent signaling at high concentrations and attenuating β-arrestin-dependent signaling at lower concentrations [61]. Its receptor binding core motif was shown to be D-Trp-Phe-D-Trp-Leu-Leu; thus, its form of modification determined the efficacy of the peptide: addition of positively charged amino acids induced full inverse agonism but attaching alanin created a partial agonist [77]. Additionally, in [D-Arg1,D-Phe5,D-Trp7,9,Leu11]-substance P, the C-terminal carboxyamidated pentapeptide wFwLX was described as the central component displaying only low inverse agonistic properties [78]. Introduction of β-(3-benzothienyl)-D-alanine (D-Bth), 3,3-diphenyl-D-alanine (D-Dip) and 1-naphthyl-D-alanine (D-1-Nal) at position 2 of the carboxyamidated wFwLL peptide created a highly potent and efficient inverse agonist [78]. As a result, the inverse agonist K-(D-1-Nal)-FwLL-NH(2) displayed high affinity to the ghrelin receptor. This so-called peptide 3, when administered icv into rats, was shown to reduce food intake [79, 80]. It is assumed that the inverse agonists insert deeply into the receptor across the main ligand-binding pocket, thereby blocking the configuration of the receptor resulting in the inhibition of spontaneous receptor activation [78]. These studies show how small changes have a major impact on the biological activity and characteristics of ligands and receptor.

Liver-expressed antimicrobial peptide 2 (LEAP-2) is an endogenous full antagonist of the GRLN-R [81•]. In vivo, LEAP-2 inhibited ghrelin's major effects on food intake, GH release, and regulation of stable glucose levels during starvation. Consequently, LEAP-2-neutralizing antibodies enhanced ghrelin's actions [81•]. Especially, LEAP-2's N-terminal sequence acts not only as an inverse agonist of the GRLN-R but also competitively antagonizes inositol phosphate production and calcium mobilization induced by ghrelin [82]. In mice, sc injection of the N-terminal region of LEAP-2 suppressed ghrelin-induced food intake 2 h after injection [82].

Therefore, decreasing LEAP-2 degradation could be another possible method to antagonize ghrelin's effect.

From a series of 2-alkylamino nicotinamide analogs, orally active compound 33 behaved as an inverse agonist decreasing weight gain in rats [83]. The core structure of 2-aminoalkyl nicotinamide derivatives responsible for in vitro potency as a GRLN-R inverse agonist is the 5-position of the pyridine ring. Attachment of a diazabicyclo ring induced potent inverse agonist activity reducing food intake in both normal and obese mice [84]. However, this compound 20 iv applicated was only peripherally acting and displayed only low brain permeability. Also, oral bioavailability was insufficient [84].

High-throughput screening in 2014 led to the identification of an acylurea series as modulators of ghrelin [85]. Here again, small sub-structural changes switched partial agonistic activity to inverse agonistic activity, resulting in the detection of the non-CNS penetrant inverse agonist 22 (AZ-GHS-22) and the CNS penetrant inverse agonist 38 (AZ-GHS-38) [85]. In vivo data of these components should be obtained in the future.

The search in peptidomimetic libraries for conformationally defined macrocyclic compounds showed that compound 1505 is a GRLN-R agonist, while other compounds also had inverse agonistic properties. Compound 1712 sc and orally applicated reduced cumulative food consumption over a 2-h period in fasted *ob/ob* mice [75]. Similarly, at a dose of 75 mg/kg, *ob/ob* mice treated with compound 1848 showed reduced cumulative food intake over a time period of 14 days [75]. Blood glucose, insulin, glucagon, and free fatty acids were also decreased, while insulin sensitivity was increased by compound 1848 [75]. Noteworthy, compound 1848 acted both as an inverse agonist and also as an antagonist of the GRLN-R [75].

In 2017, it was shown that acute application of two different artificial small molecules functioning as GRLN-R inverse agonists—GHSR-IA1 and 2—decreased food intake in mice [86]. GHSR-IA1, chronically orally administered, also suppressed food intake but enhanced metabolic rate and oral glucose tolerance as well, decelerating the progression of islet hyperplasia to fibrosis in Zucker diabetic fatty rats and suppressing hepatic steatosis in DIO mice [86]. Oral GHSR-IA2 also decreased food intake, fasting and stimulated glucose levels, blood lipids, and body weight in DIO mice and was shown to be more effective since it additionally reduced blood triglyceride levels [86].

Another small-molecule GRLN-R inverse agonist is 2-(2-methylimidazo[2,1-b][1,3]thiazol-6-yl)-1-{2-[(1R)-5-(6-methylpyrimidin-4-yl)-2,3-dihydro-1 H-inden-1-yl]-2,7-diazaspiro[3.5]non-7-yl}ethanone), also known as PF-05190457 [87]. PF-05190457 is derived from the modification of a spiro-azetidino piperidine analog and showed a Kd of 3 nM across different species [88]. It increased intracellular calcium within dispersed islets and vagal afferent firing ex vivo in pancreatic tissue of rats [89]. PF-05190457 was the first GRLN-R inverse agonist tested in humans [90••]. In vivo in healthy human subjects, oral PF-05190457, which was absorbed quickly, inhibited ghrelin-induced GH and delayed gastric emptying, resulting in reduced postprandial glucose [90••].

In summary, small changes in the structure of ghrelin analogs or GRLN-R agonists can improve the antagonistic potency of agents. Although positive effects on body weight were shown in animals, only few human data were reported so far. Moreover, long-term investigations are lacking. Similar to the GRLN-R antagonists, small-molecule GRLN-R inverse agonists and macrocyclic compounds can be applied orally.

Notably, although adenosine and adenosine analogs were assumed to be inverse agonists of the GRLN-R, binding studies illustrated that ghrelin could not be displaced by adenosine or 2-chloroadenosine in vitro [91]. However, adenosine stimulated calcium mobilization in GHSR positive cells, but not via IP(3) production per se. Adenosine induced $A_{2B}R$ activation, leading to cAMP production. Inhibition of the GRLN-R did not affect cAMP levels, indicating that adenosine and ghrelin signal via different pathways and adenosine is not a direct GRLN-R agonist [92].

## Conclusions

The present review illustrates that a great effort has been made to develop pharmacological agents suppressing ghrelin activity (Fig. 1). Direct inhibition of the peptide was shown to be induced by the polyethylene glycol-modified l-RNA oligonucleotide RNA Spiegelmer [36–42] and a GHS-R1a-fusion construct of GHSR/Fc [42]. Both are able to bind ghrelin and thus prevent ghrelin's effects. Although the RNA Spiegelmer was examined in various studies, both ghrelin-binding agents have not been tested in humans so far. Anti-ghrelin immunization, in contrast to the findings in animal studies, had no effect on body weight in humans [46].

Ghrelin's effects can also be prevented by blocking the enzyme catalyzing its activation, GOAT, e.g., by octanoylated ghrelin pentapeptide [47]. Additionally, a well-established inhibitor, GO-CoA-Tat, successfully inhibits ghrelin-induced food intake and weight gain in animals [41, 48–50, 51•]. More recently detected GOAT inhibitors were also tested only in vitro or in rodents [52–55].

Even before ghrelin's discovery, a ghrelin receptor antagonist was detected: [D-Lys3]-growth hormone–releasing peptide 6 (GHRP-6), whose ghrelin-inhibiting effect is not complete, rather pointing towards a partial antagonistic activity [62, 63]. Other met-enkephalin-derived GHS-R1a receptor antagonists such as His-D2NaI-DLys-Trp-DPhe-Lys$NH_2$ (JMV2810), JMV2844 [65], JMV2959 [61, 66, 67], JMV3002 [68], and

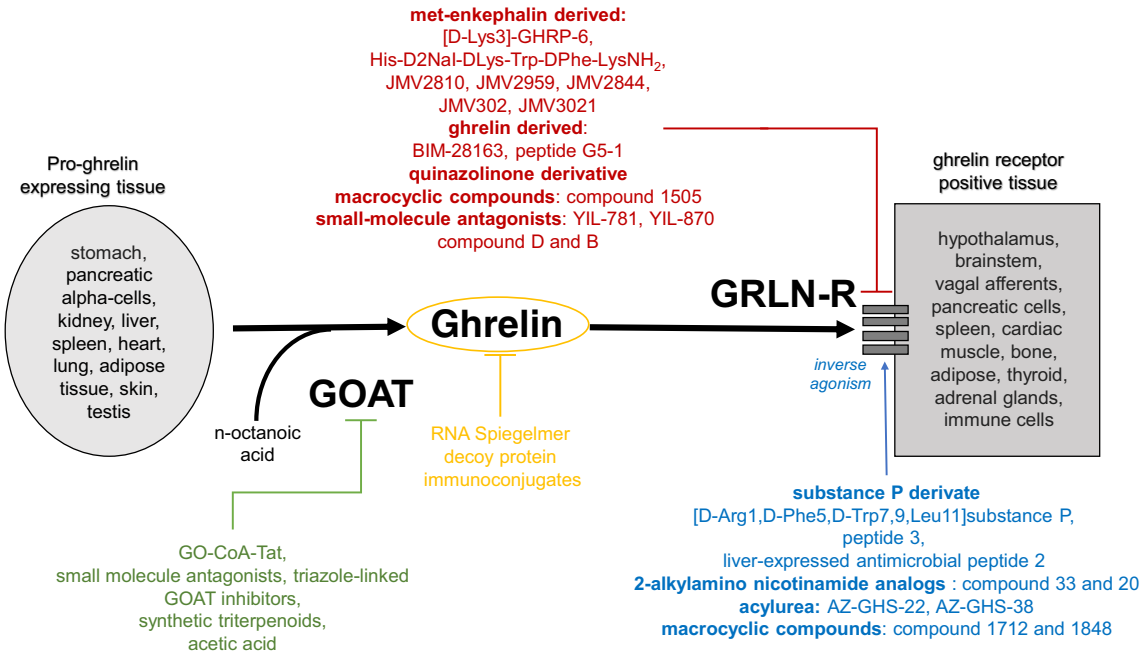


**Fig. 1** Modulation of ghrelin signaling to induce weight loss

JMV3021 [69] behaved as more potent antagonists and reduced food intake. The ghrelin-derived receptor antagonist BIM-28163 which acts as an agonist regarding body weight [35, 70] and peptide G5–1 reducing food intake and gastric contractions [71] were tested only in animals so far.

The data obtained from several studies examining small-molecule GRLN-R antagonists, including YIL-781 and YIL-870 [72], compound D (CpdD) and compound B (CpdB) [73] as well as piperidine-substituted quinazolinone derivatives [74] seem highly promising due to the oral availability and absence of toxicity in rodents. Macrocyclic compound 1505 is also orally available like compounds 1848 and 1712; noteworthy, the first two act as receptor antagonists, while the latter two as inverse agonists [75]. The first ghrelin receptor inverse agonist was a substance P derivate, [D-Arg(1), D-Phe(5), D-Trp(7,9), Leu(11)]-substance P [61, 62, 77]. Several studies evaluating the effects of substance P modulation led to the identification of compounds with more potent inverse agonistic properties such as peptide 3 [78–80].

In 2018, it was observed that liver-expressed antimicrobial peptide 2 (LEAP-2) is an endogenous full antagonist of the ghrelin receptor [81•], thus suppressing ghrelin-induced food intake [82]. 2-Alkylamino nicotinamide analogs [83, 84] and acylurea analogs [85] were also shown to act as inverse agonists to the ghrelin receptor. Additionally, artificial small molecules including GHSR-IA1 and 2 [86] and oral PF-05190457 [87–89] showed a potent ghrelin-suppressing effect on the animal model. Most importantly, PF-05190457 administered in human subjects showed effects on GH, gastric emptying, and glucose; data on body weight were not reported [90••]. While PF-01590457-associated increased heart rate and attenuated growth hormone secretion and postprandial glucose were subject to tachyphylaxis after 14 days, somnolence persisted [90••]. Interestingly, PF-05190457 development for weight loss in humans was terminated; the only ongoing human study tests PF-05190457's effects on alcoholism. However, the termination of the most recent study in patients with diabetes type 2 was due to strategy and not safety reasons [93].

Taken together, data from in vitro and animal studies appear highly promising. Notably, with the exception of two (PF-05190457 and immunoconjugates) agent, none of the ghrelin-inhibiting substances was tested in humans so far, underlining the need for further studies. Especially the effect of ghrelin's/GRLN-R's inhibition on food consumption and body weight in humans is unknown. This gap in knowledge should be filled in the near future.

**Author Contributions** M.S. wrote the first draft of the paper, and A.S. thoroughly reviewed the manuscript; both authors finalized the manuscript.

**Funding Information** This work was supported by funding of the German Research Foundation (STE 1765/3-2) and Charité University Funding (UFF 89/441-176, A.S.).

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance


1. World Health Organization. Obesity and Overweigth. 2018. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed 1 June 2019.
2. Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev. 2007;8(1):21–34. https://doi.org/10.1111/j.1467-789X.2006.00270.x.
3. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402(6762):656–60. https://doi.org/10.1038/45230.
4. Davenport AP, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, et al. LVI. Ghrelin receptor nomenclature, distribution, and function. Pharmacol Rev. 2005;57(4):541–6. https://doi.org/10.1124/pr.57.4.1.
5. Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab. 2001;86(10):4753–8. https://doi.org/10.1210/jcem.86.10.7885.
6. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, et al. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. Diabetes. 2002;51(1):124–9.
7. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab. 2002;87(6):2988. https://doi.org/10.1210/jcem.87.6.8739.
8. Barreiro ML, Gaytán F, Caminos JE, Pinilla L, Casanueva FF, Aguilar E, et al. Cellular location and hormonal regulation of ghrelin expression in rat testis. Biol Reprod. 2002;67(6):1768–76. https://doi.org/10.1095/biolreprod.102.006965.
9. Kojima M, Hamamoto A, Sato T. Ghrelin O-acyltransferase (GOAT), a specific enzyme that modifies ghrelin with a medium-chain fatty acid. J Biochem. 2016;160(4):189–94. https://doi.org/10.1093/jb/mvw046.
10. Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, et al. Ghrelin octanoylation mediated by an orphan lipid transferase. Proc Natl Acad Sci U S A. 2008;105(17):6320–5. https://doi.org/10.1073/pnas.0800708105.
11. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell. 2008;132(3):387–96. https://doi.org/10.1016/j.cell.2008.01.017.
12. Sakata I, Yang J, Lee CE, Osborne-Lawrence S, Rovinsky SA, Elmquist JK, et al. Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells. Am J Physiol Endocrinol Metab. 2009;297(1):E134–41. https://doi.org/10.1152/ajpendo.90859.2008.
13. Stengel A, Goebel M, Wang L, Tache Y, Sachs G, Lambrecht NW. Differential distribution of ghrelin-O-acyltransferase (GOAT) immunoreactive cells in the mouse and rat gastric oxyntic mucosa. Biochem Biophys Res Commun. 2010;392(1):67–71. https://doi.org/10.1016/j.bbrc.2009.12.169.
14. Weibert E, Stengel A. The X/A-like cell revisited - spotlight on the peripheral effects of NUCB2/nesfatin-1 and ghrelin. J Physiol Pharmacol. 2017;68(4):497–520.
15. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, et al. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res. 1997;48(1):23–9.
16. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature. 2000;407(6806):908–13. https://doi.org/10.1038/35038090.
17. Theander-Carrillo C, Wiedmer P, Cettour-Rose P, Nogueiras R, Perez-Tilve D, Pfluger P, et al. Ghrelin action in the brain controls adipocyte metabolism. J Clin Invest. 2006;116(7):1983–93. https://doi.org/10.1172/jci25811.
18. Davies JS, Kotokorpi P, Eccles SR, Barnes SK, Tokarczuk PF, Allen SK, et al. Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. Mol Endocrinol (Baltimore, Md). 2009;23(6):914–24. https://doi.org/10.1210/me.2008-0432.
19. Rodriguez A, Gomez-Ambrosi J, Catalan V, Gil MJ, Becerril S, Sainz N, et al. Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes. Int J Obes (2005). 2009;33(5):541–52. https://doi.org/10.1038/ijo.2009.40.
20. Briggs DI, Enriori PJ, Lemus MB, Cowley MA, Andrews ZB. Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons. Endocrinology. 2010;151(10):4745–55. https://doi.org/10.1210/en.2010-0556.
21. Naznin F, Toshinai K, Waise TMZ, NamKoong C, Md Moin AS, Sakoda H, et al. Diet-induced obesity causes peripheral and central ghrelin resistance by promoting inflammation. J Endocrinol. 2015;226(1):81–92. https://doi.org/10.1530/JOE-15-0139.
22. le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. J Clin Endocrinol Metab. 2005;90(2):1068–71. https://doi.org/10.1210/jc.2004-1216.
23. Onnerfalt J, Erlanson-Albertsson C, Montelius C, Thorngren-Jerneck K. Obese children aged 4–6 displayed decreased fasting and postprandial ghrelin levels in response to a test meal. Acta Paediatrica (1992). 2018;107(3):523–8. https://doi.org/10.1111/apa.14165.
24. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab. 2001;86(12):5992. https://doi.org/10.1210/jcem.86.12.8111.
25. Venkova K, Mann W, Nelson R, Greenwood-Van MB. Efficacy of ipamorelin, a novel ghrelin mimetic, in a rodent model of postoperative ileus. J Pharmacol Exp Ther. 2009;329(3):1110–6. https://doi.org/10.1124/jpet.108.149211.
26. Hansen BS, Raun K, Nielsen KK, Johansen PB, Hansen TK, Peschke B, et al. Pharmacological characterisation of a new oral GH secretagogue, NN703. Eur J Endocrinol. 1999;141(2):180–9.
27. Kuriyama H, Hotta M, Wakabayashi I, Shibasaki T. A 6-day intracerebroventricular infusion of the growth hormone-releasing peptide KP-102 stimulates food intake in both non-stressed and intermittently-stressed rats. Neurosci Lett. 2000;282(1–2):109–12.
28. Laferrere B, Abraham C, Russell CD, Bowers CY. Growth hormone releasing peptide-2 (GHRP-2), like ghrelin, increases food intake in healthy men. J Clin Endocrinol Metab. 2005;90(2):611–4. https://doi.org/10.1210/jc.2004-1719.
29. Mericq V, Cassorla F, Bowers CY, Avila A, Gonen B, Merriam GR. Changes in appetite and body weight in response to long-term oral administration of the ghrelin agonist GHRP-2 in growth hormone deficient children. J Ped Endocrinol Metab. 2003;16(7):981–5.
30. Garcia JM, Polvino WJ. Pharmacodynamic hormonal effects of anamorelin, a novel oral ghrelin mimetic and growth hormone secretagogue in healthy volunteers. Growth Hormon IGF Res. 2009;19(3):267–73. https://doi.org/10.1016/j.ghir.2008.12.003.
31. Garcia JM, Friend J, Allen S. Therapeutic potential of anamorelin, a novel, oral ghrelin mimetic, in patients with cancer-related cachexia: a multicenter, randomized, double-blind, crossover, pilot study.

Supp Care Cancer. 2013;21(1):129–37. https://doi.org/10.1007/s00520-012-1500-1.
32. Garcia JM, Polvino WJ. 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. 2007;12(5):594–600. https://doi.org/10.1634/theoncologist.12-5-594.
33. Zollers B, Rhodes L, Heinen E. Capromorelin oral solution (ENTYCE(R)) increases food consumption and body weight when administered for 4 consecutive days to healthy adult Beagle dogs in a randomized, masked, placebo controlled study. BMC Vet Res. 2017;13(1):10. https://doi.org/10.1186/s12917-016-0925-z.
34. Fraser GL, Hoveyda HR, Tannenbaum GS. Pharmacological demarcation of the growth hormone, gut motility and feeding effects of ghrelin using a novel ghrelin receptor agonist. Endocrinology. 2008;149(12):6280–8. https://doi.org/10.1210/en.2008-0804.
35. Hassouna R, Labarthe A, Zizzari P, Videau C, Culler M, Epelbaum J, et al. Actions of agonists and antagonists of the ghrelin/GHS-R pathway on GH secretion, appetite, and cFos activity. Front Endocrinol. 2013;4:25. https://doi.org/10.3389/fendo.2013.00025.
36. Shearman LP, Wang SP, Helmling S, Stribling DS, Mazur P, Ge L, et al. Ghrelin neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice. Endocrinology. 2006;147(3):1517–26. https://doi.org/10.1210/en.2005-0993.
37. Helmling S, Maasch C, Eulberg D, Buchner K, Schroder W, Lange C, et al. Inhibition of ghrelin action in vitro and in vivo by an RNA-Spiegelmer. Proc Natl Acad Sci U S A. 2004;101(36):13174–9. https://doi.org/10.1073/pnas.0404175101.
38. Becskei C, Bilik KU, Klussmann S, Jarosch F, Lutz TA, Riediger T. The anti-ghrelin Spiegelmer NOX-B11-3 blocks ghrelin- but not fasting-induced neuronal activation in the hypothalamic arcuate nucleus. J Neuroendocrinol. 2008;20(1):85–92. https://doi.org/10.1111/j.1365-2826.2007.01619.x.
39. Kobelt P, Helmling S, Stengel A, Wlotzka B, Andresen V, Klapp BF, et al. Anti-ghrelin Spiegelmer NOX-B11 inhibits neurostimulatory and orexigenic effects of peripheral ghrelin in rats. Gut. 2006;55(6):788–92. https://doi.org/10.1136/gut.2004.061010.
40. Sangiao-Alvarellos S, Helmling S, Vazquez MJ, Klussmann S, Cordido F. Ghrelin neutralization during fasting-refeeding cycle impairs the recuperation of body weight and alters hepatic energy metabolism. Mol Cell Endocrinol. 2011;335(2):177–88. https://doi.org/10.1016/j.mce.2011.01.010.
41. Teubner BJ, Bartness TJ. Anti-ghrelin Spiegelmer inhibits exogenous ghrelin-induced increases in food intake, hoarding, and neural activation, but not food deprivation-induced increases. Am J Physiol Regul Integr Comp Physiol. 2013;305(4):R323–33. https://doi.org/10.1152/ajpregu.00097.2013.
42. Gagnon J, Zhu L, Anini Y, Wang Q. Neutralizing circulating ghrelin by expressing a growth hormone secretagogue receptor-based protein protects against high-fat diet-induced obesity in mice. Gene Ther. 2015;22(9):750–7. https://doi.org/10.1038/gt.2015.38.
43. Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, et al. Vaccination against weight gain. Proc Natl Acad Sci U S A. 2006;103(35):13226–31. https://doi.org/10.1073/pnas.0605376103.
44. Vizcarra JA, Kirby JD, Kim SK, Galyean ML. Active immunization against ghrelin decreases weight gain and alters plasma concentrations of growth hormone in growing pigs. Dom Anim Endocrinol. 2007;33(2):176–89. https://doi.org/10.1016/j.domaniend.2006.05.005.
45. Andrade S, Pinho F, Ribeiro AM, Carreira M, Casanueva FF, Roy P, et al. Immunization against active ghrelin using virus-like particles for obesity treatment. Curr Pharm Des. 2013;19(36):6551–8. https://doi.org/10.2174/13816128113199990506.
46. Biotechnology C. Phase I/IIa clinical trial with obese individuals shows no effect of CYT009-GhrQb on weight loss. Cytos Biotechnology Press release November 2006;7.
47. Yang J, Zhao TJ, Goldstein JL, Brown MS. Inhibition of ghrelin O-acyltransferase (GOAT) by octanoylated pentapeptides. Proc Natl Acad Sci U S A. 2008;105(31):10750–5. https://doi.org/10.1073/pnas.0805353105.
48. Barnett BP, Hwang Y, Taylor MS, Kirchner H, Pfluger PT, Bernard V, et al. Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. Science. 2010;330(6011):1689–92. https://doi.org/10.1126/science.1196154.
49. Teuffel P, Wang L, Prinz P, Goebel-Stengel M, Scharner S, Kobelt P, et al. Treatment with the ghrelin-O-acyltransferase (GOAT) inhibitor GO-CoA-Tat reduces food intake by reducing meal frequency in rats. J Physiol Pharmacol. 2015;66(4):493–503.
50. Rucinski M, Ziolkowska A, Szyszka M, Hochol A, Malendowicz LK. Evidence suggesting that ghrelin O-acyl transferase inhibitor acts at the hypothalamus to inhibit hypothalamo-pituitary-adrenocortical axis function in the rat. Peptides. 2012;35(2):149–59. https://doi.org/10.1016/j.peptides.2012.04.007.
51.• Zhang S, Mao Y, Fan X. Inhibition of ghrelin o-acyltransferase attenuated lipotoxicity by inducing autophagy via AMPK-mTOR pathway. Drug Des Dev Ther. 2018, 873;12:–85. https://doi.org/10.2147/dddt.s158985. **A very recent study identifying the molecular changes induced by GO-CoA-Tat administation**.
52. Garner AL, Janda KD. A small molecule antagonist of ghrelin O-acyltransferase (GOAT). Chem Commun. 2011;47(26):7512–4. https://doi.org/10.1039/c1cc11817j.
53. Zhao Y, Ma X, Wang Q, Zhou Y, Zhang Y, Wu L, et al. Nesfatin-1 correlates with hypertension in overweight or obese Han Chinese population. Clin Exp Hypertens(1993). 2015;37(1):51–6. https://doi.org/10.3109/10641963.2014.897722.
54. McGovern-Gooch KR, Mahajani NS, Garagozzo A, Schramm AJ, Hannah LG, Sieburg MA, et al. Synthetic triterpenoid inhibition of human ghrelin-O-acyltransferase: the involvement of a functionally required cysteine provides mechanistic insight into ghrelin acylation. Biochemistry. 2017;56(7):919–31. https://doi.org/10.1021/acs.biochem.6b01008.
55. Yoneyama-Hirozane M, Deguchi K, Hirakawa T, Ishii T, Odani T, Matsui J, et al. Identification and characterization of a new series of ghrelin O-acyl transferase inhibitors. SLAS Discov. 2018;23(2):154–63. https://doi.org/10.1177/2472555217727097.
56. Howick K, Griffin BT, Cryan JF, Schellekens H. From belly to brain: targeting the ghrelin receptor in appetite and food intake regulation. Int J Mol Sci. 2017;18(2). https://doi.org/10.3390/ijms18020273.
57. Lin L, Saha PK, Ma X, Henshaw IO, Shao L, Chang BH, et al. Ablation of ghrelin receptor reduces adiposity and improves insulin sensitivity during aging by regulating fat metabolism in white and brown adipose tissues. Aging Cell. 2011;10(6):996–1010. https://doi.org/10.1111/j.1474-9726.2011.00740.x.
58. Veeraragavan K, Sethumadhavan K, Bowers CY. Growth hormone-releasing peptide (GHRP) binding to porcine anterior pituitary and hypothalamic membranes. Life Sci. 1992;50(16):1149–55.
59. Lawrence CB, Snape AC, Baudoin FM, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology. 2002;143(1):155–62. https://doi.org/10.1210/endo.143.1.8561.
60. Bellone J, Ghizzoni L, Aimaretti G, Volta C, Boghen MF, Bernasconi S, et al. Growth hormone-releasing effect of oral growth hormone-releasing peptide 6 (GHRP-6) administration in children with short stature. Eur J Endocrinol. 1995;133(4):425–9.
61. Ramirez VT, van Oeffelen W, Torres-Fuentes C, Chruscicka B, Druelle C, Golubeva AV, et al. Differential functional selectivity and downstream signaling bias of ghrelin receptor antagonists and inverse agonists. FASEB J. 2019;33(1):518–31. https://doi.org/10.1096/fj.201800655R.
62. Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, et al. Antagonism of ghrelin receptor reduces food intake and body

weight gain in mice. Gut. 2003;52(7):947–52. https://doi.org/10.1136/gut.52.7.947.

63. Mosa R, Huang L, Li H, Grist M, LeRoith D, Chen C. Long-term treatment with the ghrelin receptor antagonist [d-Lys3]-GHRP-6 does not improve glucose homeostasis in nonobese diabetic MKR mice. Am J Physiol Regul Integr Comp Physiol. 2018;314(1):R71–r83. https://doi.org/10.1152/ajpregu.00157.2017.
64. Bowers CY, Tannenbau GS, Coy DH, Hocart SJ. Ghrelin/growth hormone releasing peptide/growth hormone secretatogue receptor antagonists and uses thereof. US Patent Application No. 010389 2007. https://patents.google.com/patent/WO2007127457A2/nl.
65. Demange L, Boeglin D, Moulin A, Mousseaux D, Ryan J, Berge G, et al. Synthesis and pharmacological in vitro and in vivo evaluations of novel triazole derivatives as ligands of the ghrelin receptor. J Med Chem. 2007;50(8):1939–57. https://doi.org/10.1021/jm070024h.
66. Moulin A, Demange L, Berge G, Gagne D, Ryan J, Mousseaux D, et al. Toward potent ghrelin receptor ligands based on trisubstituted 1,2,4-triazole structure. 2. Synthesis and pharmacological in vitro and in vivo evaluations. J Med Chem. 2007;50(23):5790–806. https://doi.org/10.1021/jm0704550.
67. Gomez JL, Ryabinin AE. The effects of ghrelin antagonists [D-Lys(3) ]-GHRP-6 or JMV2959 on ethanol, water, and food intake in C57BL/6J mice. Alcohol Clin Exp Res. 2014;38(9):2436–44. https://doi.org/10.1111/acer.12499.
68. Salome N, Haage D, Perrissoud D, Moulin A, Demange L, Egecioglu E, et al. Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats. Eur J Pharmacol. 2009;612(1–3):167–73. https://doi.org/10.1016/j.ejphar.2009.03.066.
69. Torsello A, Bresciani E, Tamiazzo L, Bulgarelli I, Caporali S, Moulin A, et al. Novel potent and selective non-peptide ligands of ghrelin receptor: characterization of endocrine and extraendocrine actions. Endocr Abstr. 2008;16:P575.
70. Halem HA, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, et al. A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. Neuroendocrinology. 2005;81(5):339–49. https://doi.org/10.1159/000088796.
71. Ueno S, Yoshida S, Mondal A, Nishina K, Koyama M, Sakata I, et al. In vitro selection of a peptide antagonist of growth hormone secretagogue receptor using cDNA display. Proc Nat Sci U S A. 2012;109(28):11121–6. https://doi.org/10.1073/pnas.1203561109.
72. Esler WP, Rudolph J, Claus TH, Tang W, Barucci N, Brown SE, et al. Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss. Endocrinology. 2007;148(11):5175–85. https://doi.org/10.1210/en.2007-0239.
73. Longo KA, Govek EK, Nolan A, McDonagh T, Charoenthongtrakul S, Giuliana DJ, et al. Pharmacologic inhibition of ghrelin receptor signaling is insulin sparing and promotes insulin sensitivity. J Pharmacol Exp Ther. 2011;339(1):115–24. https://doi.org/10.1124/jpet.111.183764.
74. Rudolph J, Esler WP, O'Connor S, Coish PD, Wickens PL, Brands M, et al. Quinazolinone derivatives as orally available ghrelin receptor antagonists for the treatment of diabetes and obesity. J Med Chem. 2007;50(21):5202–16. https://doi.org/10.1021/jm070071+.
75. Hoveyda H, Marsault È, Thomas H, Fraser G, Beaubien S, Mathieu A et al. Macrocyclic ghrelin receptor antagonists and inverse agonists and methods of using the same. US Patent Application No. 0105389 2011 www.google.com/patents/US20110105389.
76. Els S, Beck-Sickinger AG, Chollet C. Ghrelin receptor: high constitutive activity and methods for developing inverse agonists. Methods Enzymol. 2010;485:103–21. https://doi.org/10.1016/b978-0-12-381296-4.00006-3.
77. Mokrosinski J, Holst B. Modulation of the constitutive activity of the ghrelin receptor by use of pharmacological tools and mutagenesis. Methods Enzymol. 2010;484:53–73. https://doi.org/10.1016/b978-0-12-381298-8.00003-4.
78. Holst B, Lang M, Brandt E, Bach A, Howard A, Frimurer TM, et al. Ghrelin receptor inverse agonists: identification of an active peptide core and its interaction epitopes on the receptor. Mol Pharmacol. 2006;70(3):936–46. https://doi.org/10.1124/mol.106.024422.
79. Els S, Schild E, Petersen PS, Kilian TM, Mokrosinski J, Frimurer TM, et al. An aromatic region to induce a switch between agonism and inverse agonism at the ghrelin receptor. J Med Chem. 2012;55(17):7437–49. https://doi.org/10.1021/jm300414b.
80. Holst B, Mokrosinski J, Lang M, Brandt E, Nygaard R, Frimurer TM, et al. Identification of an efficacy switch region in the ghrelin receptor responsible for interchange between agonism and inverse agonism. J Biol Chem. 2007;282(21):15799–811. https://doi.org/10.1074/jbc.M609796200.
81.• Ge X, Yang H, Bednarek MA, Galon-Tilleman H, Chen P, Chen M, et al. LEAP2 is an endogenous antagonist of the ghrelin receptor. Cell Metab. 2018;27(2):461–9.e6. https://doi.org/10.1016/j.cmet.2017.10.016. **This recent study reports the effects of an endogenous GHSR antagonist.**
82. M'Kadmi C, Cabral A, Barrile F, Giribaldi J, Cantel S, Damian M, et al. N-terminal liver-expressed antimicrobial peptide 2 (LEAP2) region exhibits inverse agonist activity toward the ghrelin receptor. J Med Chem. 2019;62(2):965–73. https://doi.org/10.1021/acs.jmedchem.8b01644.
83. Takahashi B, Funami H, Iwaki T, Maruoka H, Shibata M, Koyama M, et al. Orally active ghrelin receptor inverse agonists and their actions on a rat obesity model. Bioorg Med Chem. 2015;23(15):4792–803. https://doi.org/10.1016/j.bmc.2015.05.047.
84. Takahashi B, Funami H, Iwaki T, Maruoka H, Nagahira A, Koyama M, et al. 2-Aminoalkyl nicotinamide derivatives as pure inverse agonists of the ghrelin receptor. Bioorg Med Chem Lett. 2015;25(13):2707–12. https://doi.org/10.1016/j.bmcl.2015.04.040.
85. McCoull W, Barton P, Brown AJ, Bowker SS, Cameron J, Clarke DS, et al. Identification, optimization, and pharmacology of acylurea GHS-R1a inverse agonists. J Med Chem. 2014;57(14):6128–40. https://doi.org/10.1021/jm500610n.
86. Abegg K, Bernasconi L, Hutter M, Whiting L, Pietra C, Giuliano C, et al. Ghrelin receptor inverse agonists as a novel therapeutic approach against obesity-related metabolic disease. Diabetes Obes Metab. 2017;19(12):1740–50. https://doi.org/10.1111/dom.13020.
87. Bhattacharya SK, Andrews K, Beveridge R, Cameron KO, Chen C, Dunn M, et al. Discovery of PF-5190457, a potent, selective, and orally bioavailable ghrelin receptor inverse agonist clinical candidate. ACS Med Chem Lett. 2014;5(5):474–9. https://doi.org/10.1021/ml400473x.
88. Cameron KO, Bhattacharya SK, Loomis AK. Small molecule ghrelin receptor inverse agonists and antagonists. J Med Chem. 2014;57(21):8671–91. https://doi.org/10.1021/jm5003183.
89. Kong J, Chuddy J, Stock IA, Loria PM, Straub SV, Vage C, et al. Pharmacological characterization of the first in class clinical candidate PF-05190457: a selective ghrelin receptor competitive antagonist with inverse agonism that increases vagal afferent firing and glucose-dependent insulin secretion ex vivo. Br J Pharmacol. 2016;173(9):1452–64. https://doi.org/10.1111/bph.13439.
90.•• Denney WS, Sonnenberg GE, Carvajal-Gonzalez S, Tuthill T, Jackson VM. Pharmacokinetics and pharmacodynamics of PF-05190457: The first oral ghrelin receptor inverse agonist to be profiled in healthy subjects. Br J Clin Pharmacol. 2017;83(2):326–38. https://doi.org/10.1111/bcp.13127. **This is the first study testing an inverse agonist of the ghrelin receptor in humans showing inhibitory actions on growth hormone secretion, gastric emptying and postprandial glucose levels as well as safety and torability.**

91. Johansson S, Fredholm BB, Hjort C, Morein T, Kull B, Hu PS. Evidence against adenosine analogues being agonists at the growth hormone secretagogue receptor. Biochem Pharmacol. 2005;70(4): 598–605. https://doi.org/10.1016/j.bcp.2005.05.023.
92. Hermansson NO, Morgan DG, Drmota T, Larsson N. Adenosine is not a direct GHSR agonist–artificial cross-talk between GHSR and adenosine receptor pathways. Acta Physiologica (Oxford, England). 2007;190(1):77–86. https://doi.org/10.1111/j.1365-201X.2007.01691.x.
93. NIH U. S National Library of Medicine ClinicalTrails.gov. https://clinicaltrials.gov/ct2/results?term=PF-05190457 Accessed 3 August 2019.