

Review

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Review

Beyond Hunger: Structure, Signaling and Systemic Roles of Ghrelin

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Abstract

Ghrelin, the endogenous ligand of the growth hormone secretagogue receptor 1a (GHSR1a), has expanded from a “hunger hormone” to a pleiotropic regulator of whole-body physiology. This review synthesizes current advances spanning ghrelin biogenesis, signaling, and systems biology. Physiologically, proghrelin processing and O-acylation by ghrelin O-acyltransferase (GOAT) generate acyl-ghrelin, the high-potency GHSR1a agonist; des-acyl ghrelin predominates in circulation and exerts context-dependent, GHSR1a-independent or low-potency effects, while truncated “mini-ghrelins” can act as competitive antagonists. Emergence of synthetic ligands, agonists, antagonists and reverse-agonists provided tools necessary to decipher of GHSR1a activity. Recent cryo-EM structures of GHSR1a with peptide and small-molecule ligands reveal a bipartite binding pocket and provide a framework for biased signaling, constitutive activity, and receptor partner selectivity. Beyond regulation of feeding and growth hormone release, ghrelin modulates glucose homeostasis, gastric secretion and motility, cardiovascular tone, bone remodeling, renal hemodynamics, and innate immunity. Ghrelin broadly dampens pro-inflammatory responses and promotes reparative macrophage phenotypes. In the emerging field of ghrelin activity in central nervous system, ghrelin influences neuroprotection, stress reactivity, sleep architecture, and is implicated in depression, Alzheimer's disease, and substance abuse disorders. Practical and transitional aspects are also highlighted: approaches for ghrelin stabilization; recent GHSR1a agonists/antagonists and inverse agonists findings; LEAP-2-based strategies; and emerging GOAT inhibitors. Together, structural insights and pathway selectivity position the ghrelin system as a druggable axis for the management of inflammatory diseases, neuropsychiatric and addiction conditions and for obesity treatment in post-GLP-1 receptor agonist era.

Keywords: ghrelin; GHSR1a; G protein-coupled receptor; GOAT

1. Introduction

In 1999, *Kojima M. et al.* utilized a screening method to associate orphan G protein-coupled receptors (GPCRs) with unknown ligands. Their method detected an increase in intracellular calcium levels induced by agonists, leading to the purification of a 28-amino-acid peptide from the gut. This peptide was identified as the natural ligand for the growth hormone secretagogue receptor 1a (GHSR1a) and was named “ghrelin”, derived from the Proto-Indo-European root “ghre”, signifying “grow” [1]. Since then, ghrelin has been discovered in many different organs, where it is involved in a wide range of processes and activities.

Comprehensive RT-PCR studies revealed that ghrelin mRNA is present in various tissues. The highest levels were observed in the stomach, whereas lower levels were detected in the liver, lung, kidney, and skeletal muscle [2]. In turn, at the protein level, ghrelin was detected in the stomach,

small intestine, brain, cerebellum, pituitary, lung, skeletal muscle, pancreas, salivary glands, adrenal gland, ovary, and testis, with concentrations ranging from 0.05 to 1.43 ng/mg of homogenate protein. The lung and brain showed the highest levels. Protein was not found in the heart, liver, and kidney, highlighting a potential discrepancy between gene and protein expression [2]. Recent studies have shown that ghrelin is also expressed in the oral cavity since it was detected in saliva, gingival crevicular fluid (GCF), salivary glands, oral epithelial cells, and oral squamous cell carcinoma lesions. Its concentration is lowest in serum, while in GCF it is ~500-fold higher than in saliva [3,4].

The level of ghrelin-like immunoreactivity in plasma among healthy individuals, as determined by a specialized radioimmunoassay (RIA), stood at 117 ± 37 fmol/mL [5]. Ghrelin levels in the serum rise with age irrespective of sex. They fluctuate considerably over the course of the day, peaking during sleep [6]. Unlike other gut hormones, ghrelin levels in the plasma rise during fasting, and drop after eating [7]. Chronic high-calorie diets and obesity tend to lower plasma ghrelin levels in humans [7,8]. In rodents, long-term high-fat diets result in obesity and reduced stomach ghrelin production and secretion, simultaneously increasing the number of ghrelin-secreting cells [9]. However, the exact impact of increased adiposity on ghrelin production is unclear [10].

Ghrelin is reported to be the orexigenic peptide mainly secreted by the X/A-like cells, a group of unique endocrine cells in the gastrointestinal tract [11]. Norepinephrine mediates the increase in ghrelin levels before a meal, whereas the decrease in ghrelin after a meal is regulated by glucose and insulin, with insulin contributing additively [12,13]. In addition to reduced stomach production of ghrelin in obesity, decreased systemic levels have been observed in conditions such as male hypogonadism, untreated hyperthyroidism, polycystic ovary syndrome, and *H. pylori*-induced gastritis [14–17]. Conversely, elevated ghrelin levels are observed in conditions such as anorexia nervosa, in lean individuals, Prader-Willi syndrome, and following the eradication of *H. pylori* [17].

Two other research groups employed ghrelin-secreting cell lines from transgenic mice and primary cell cultures from mice and rats to demonstrate the mechanism underlying the regulation of ghrelin secretion [18,19]. The data demonstrate that a number of factors, including glucose, glucagon, dopamine, insulin, oxytocin, somatostatin, and long-chain fatty acids, directly act on the ghrelin-producing cells, thereby controlling ghrelin release [12,18–20]. Recently, significant progress in understanding of the ghrelin system was made, reflected by the determination of the ghrelin-bound GHSR1a structure, functional description of the receptor's biased signaling and ongoing progress in structural and functional analysis of the ghrelin O-acyltransferase (GOAT). Together with the increased understanding of ghrelin's role in immune and central nervous systems and an emerging role of ghrelin in dopamine-related signaling and addiction, ghrelin is again found at the forefront of research. This review aims to provide a comprehensive, modern update on ghrelin biology.

2. Processing and Maturation of Ghrelin

To carry out its diverse functions, ghrelin must undergo a series of maturation steps. Ghrelin prepropeptide gene (*GHRL*) (Figure 1) consists of 6 exons and 4 introns, where mature preproghrelin mRNA is translated into preproghrelin, a 117-amino-acid product [21,22]. One noteworthy feature of this construct is the presence of obestatin, a putative proteolytic fragment with activities opposite to those of ghrelin [22,23]. While ghrelin has an appetite-stimulating impact, obestatin, which is made up of 23 amino acids, is mainly linked to enhancing feelings of fullness [23]. Rats given obestatin had lower food intake, inhibited jejunal contractions, and gained less weight [24]. Human studies, however, could not demonstrate any connection between obestatin and anorexia in general, weight loss, or other dietary issues [25].

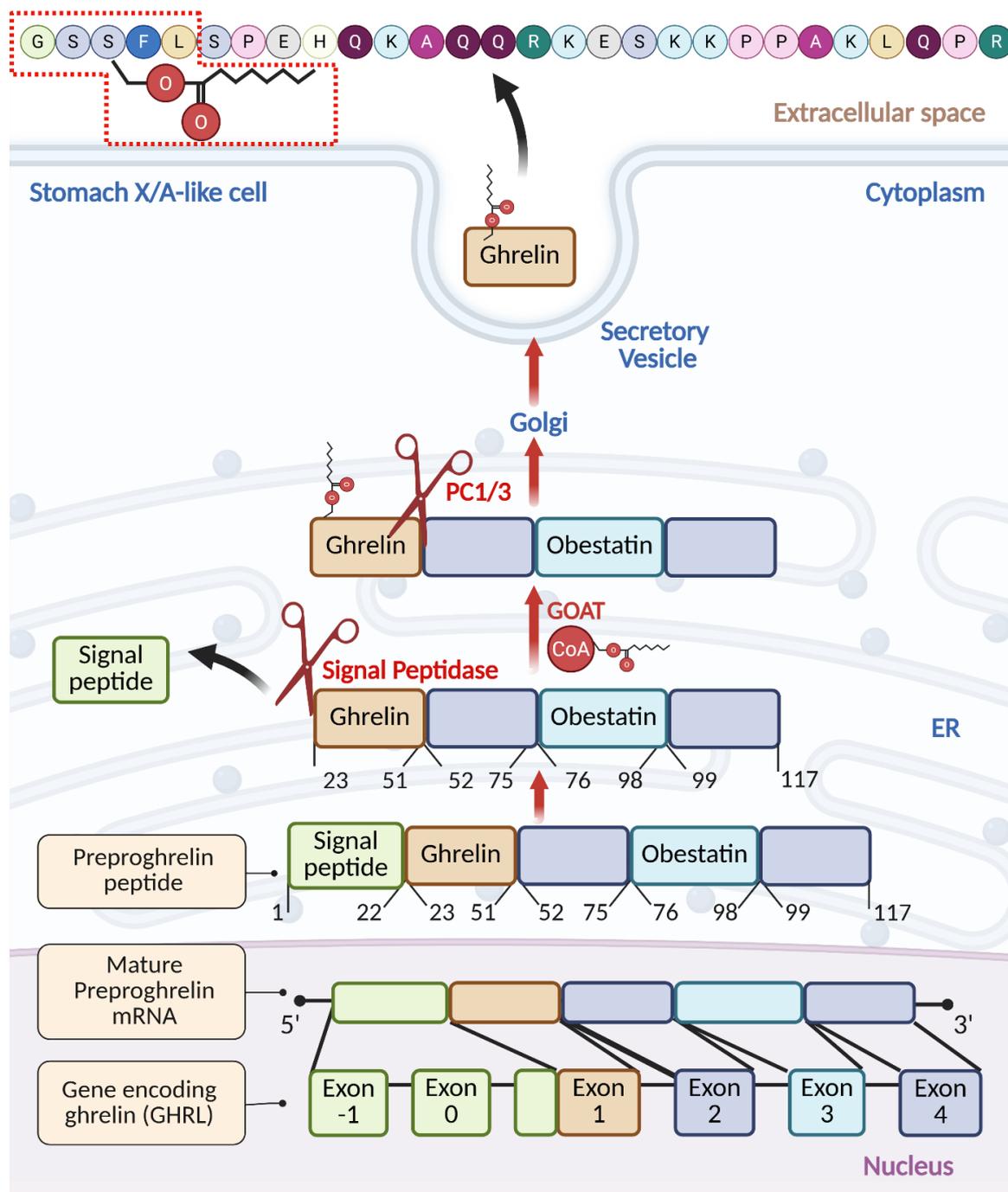


Figure 1. Intracellular processing of ghrelin. The human GHRL gene resides on the short arm of chromosome 3p25-26 and consists of six exons. The ghrelin gene undergoes transcription and splicing to generate mature mRNA. This mRNA is translated into pre-proghrelin, which then undergoes translation to yield a 117 amino acid peptide. Within the ER, this precursor undergoes a systematic cleavage process to produce the mature 28 amino acid form of ghrelin. Before secretion, unmaturing non-acylated ghrelin undergoes acylation by ghrelin-O-acyltransferase (GOAT) in the presence of CoA as an acyl donor, facilitating its binding to the growth hormone secretagogue receptor 1a (GHSR1a). The highlighted box indicates the minimal active n-terminal pentapeptide core of the ghrelin sequence necessary for GHSR1a activation. Created with BioRender.com.

To release mature ghrelin, the 117-amino-acid precursor undergoes systematic proteolytic processing within the endoplasmic reticulum (ER) to form the 28-amino-acid ghrelin. The process begins with signal peptidase-mediated removal of the signal peptide at Arg²³, essential for translocation to the ER [26]. In stomach X/A-like cells, where processing is best described, ghrelin undergoes O-octanoylation at Ser³, catalyzed by ER-localized enzyme ghrelin O-acyltransferase

(GOAT), and is subsequently cleaved by the prohormone convertase PC1/3 at Arg⁵¹ to yield the active 28-amino-acid acylated form of ghrelin (AG) [26,27]. The GOAT enzyme was initially identified to be expressed in the gut and ghrelin *O*-octanoylation is essential for its binding to GHSR1a [27,28]. Recently, however, GOAT was detected in urine and blood of prostate cancer (PC) patients and, in this limited studies, its levels were reported to outperform PSA in predicting aggressive disease [29,30].

Some portion of preproghrelin undergoes C-terminal trimming by a carboxypeptidase-B-like enzyme, resulting in the 27-amino-acid sequence of ghrelin, lacking the C-terminal Arg²⁸ [31]. This type of processing, commonly seen in other peptide hormones (e.g., endorphins [32], cholecystokinin [33]), often involves cleavage at, or near basic residues (Arg or Lys) [32,33]. The 27-residue isoform can also arise from alternative splicing. A splice variant, prepro-des-Gln14-ghrelin (116 residues), produces des-Gln14-ghrelin, a second endogenous GHSR ligand. However, des-Gln¹⁴⁴-ghrelin appears to be less abundant in humans compared to rodents, where the ratio of ghrelin to des-Gln¹⁴-ghrelin precursors varies by species (e.g., ~5:1 in rat, ~6:5 in mouse) [34].

Several studies have demonstrated the existence of truncated, bioactive ghrelin isoforms, collectively termed “mini-ghrelins”, that retain the essential N-terminal octanoyl modification and can modulate ghrelin receptor signaling [35–37]. Nearly a decade ago, *Satou et al.* identified activated protein C (APC), a serine protease found in bovine plasma, as a novel ghrelin endopeptidase. APC selectively cleaves human octanoylated ghrelin between Arg15 and Lys16, generating ghrelin(1–15) as the predominant cleavage product. In vivo, the administration of ProTac, a snake venom-derived pharmacological activator of APC, significantly enhanced this cleavage in mice [35].

Two additional, shorter isoforms of mini-ghrelin have also been identified. Ghrelin(1–14) likely arises via C-terminal trimming of ghrelin(1–15), possibly mediated by plasma carboxypeptidases that remove terminal basic residues. The shortest characterized variant, ghrelin(1–11), is generated by cleavage at the Arg11-Val12 bond in human ghrelin. This specific cleavage does not occur in rodents, where the corresponding residues are Lys-Ala, highlighting differences in protease recognition and substrate specificity [37].

Naturally occurring splice variants of the ghrelin gene (*GHRL*) have been identified across vertebrate species. Comparative genomic analysis of 77 species revealed that exon 2, which encodes part of the ghrelin peptide, is a symmetrical exon, allowing it to be skipped without altering the downstream reading frame. Exon 2 skipping produces a truncated preproghrelin transcript that encodes a 13-amino-acid peptide, followed immediately by the obestatin coding region. This exon 2-deleted isoform retains the overall structural organization of the preprohormone and has been detected in species such as mice and sheep, indicating evolutionary conservation and supporting its potential functional relevance [36].

Despite the preserved ability of mini-ghrelins to bind GHSR1a and inhibit calcium channel activity in vitro, similar to full-length ghrelin, mini-ghrelins do not elicit canonical ghrelin responses in vivo, such as food intake stimulation or hypothalamic c-Fos activation. Instead, these peptides act as competitive antagonists, inhibiting the binding and orexigenic effects of exogenous ghrelin [37].

The amino acid sequences of mammalian ghrelin exhibit significant conservation, especially in the N-termini, where the ten amino acids display complete identity. The structural likeness and the ongoing necessity for acyl-modification of the third residue indicate the pivotal importance of this N-terminal segment in the peptide's function. Also, it's noteworthy that rat and human ghrelin differ by only two amino acid residues and both show comparable potency in activation of the human GHSR1a [38,39].

3. The GOAT – A Single Enzyme for the Single Substrate

GOAT was independently discovered by two labs in 2008 [27,28]. It is a member of membrane-bound-*O*-acyltransferase (MBOAT) family and is an integral membrane protein with 11 transmembrane α -helices. The GOAT structure remains uncharacterized, due to inherent difficulty in structural characterization of integral membrane proteins. Structures of other enzymes from the

same family; DltB, a bacterial alanyltransferase solved by X-ray diffraction [40] and human sterol O-acyltransferase solved by cryo-EM [41] provide some insight into the enzyme mechanism and inhibitor design strategies for GOAT. In recent years, a topology-guided, computational model of GOAT structure was developed by James L. Houglund's group [41] and facilitated identification of the unique internal channel in GOAT structure, enabling modification of the ghrelin present in the ER lumen with cytoplasmic octanoyl-CoA. GOAT forms an ellipsoidal cone embedded in the ER membrane, with a narrow end facing the lumen and the wide side directed toward the cytoplasm. Octanoyl-CoA is recruited from the cytoplasmic side and is not transported across the membrane, but rather the catalytic machinery of GOAT attaches the fatty acid chain to the lumen-delivered ghrelin within the channel. Based on the absolute conservation and its critical position within the channel, His³³⁸ is proposed as the catalytic residue of GOAT.

It has been proposed that the unexpected biological activity of des-acyl ghrelin (DAG) may, at least in part, be mediated by re-acylation. Indeed, this emerging hypothesis was raised by several reports indicating that adipogenic activity of des-acyl ghrelin is mediated by the presence of GHSR1a in rats and suggested extracellular activity of GOAT enzyme [42]. Similarly, the presence and GOAT activity on exogenous des-acyl ghrelin were reported in mouse hippocampus [43]. This is consistent with earlier reports, which indicated that acyl-ghrelin is subjected to unidirectional transport through the blood-brain barrier (BBB) in mice. The mouse acyl-ghrelin transport was effective only in brain-to-blood direction and negligible in the opposing blood-to-brain influx, while des-acyl mouse ghrelin was subjected to passive, membrane diffusion-based transport. As acyl-ghrelin is readily detectable in mouse brain, these findings indirectly suggested the presence of the local, extracellular GOAT activity [44]. Indeed, it was demonstrated recently that GOAT is expressed on the surface of LNCaP and 22Rv1 prostate cancer cell lines and is able to bind and modify exogenous des-acyl ghrelin [45]. The current model of the potential extracellular GOAT activity postulates that GOAT is present within the cell membrane, where it binds and modifies the exogenous des-acyl ghrelin, which is then recognized by the adjacent GHSR1a, enabling signal transduction into the target cell.

Des-acyl ghrelin (DAG), the predominant form of ghrelin in the bloodstream, comprises approximately 90% of total circulating ghrelin [46]. While ghrelin acylation is crucial for its complete activity through GHSR1a [47], research indicates that DAG, despite exhibiting weak yet complete agonism, shows low potency in displacing ligand binding at GHSR1a [38]. Significant differences in potency have been observed: DAG has an EC₅₀ ranging from 1.6 to 2.4 μ M, compared to ghrelin's EC₅₀ of 2–2.6 nM, spanning three orders of magnitude [48]. Another study found an even more significant disparity, with ghrelin having an EC₅₀ of 1–2 nM compared to over 3.5 μ M for DAG, again indicating a $\sim 10^{33}$ -fold lower potency of DAG than acyl-ghrelin at GHSR1a [39]. Circulating levels of ghrelin and DAG range from 0.1 to 0.5 nmol/L, though assays detecting bound peptides show higher levels (3–4 nmol/L). Therefore, DAG levels in the bloodstream are much lower than the concentration needed to activate GHSR1a [49].

Nevertheless, by activating the survival-promoting extracellular signal-regulated kinase 1/2 (ERK1/2) and PI3K/Akt signaling pathways, DAG has been demonstrated to support adipogenesis and have an anti-apoptotic impact on cardiomyocytes [42,50]. DAG was also reported to mediate anxiety-like behavior [51], and shift adipose tissue residual macrophages to M2 [52] in mice. In addition, DAG has been reported to reduce alcohol intake in rats in a dopamine-dependent manner [53]. In contrast to DAG's function, AG appears important in controlling autophagy, a cellular process that breaks down proteins and organelles [54–56]. Des-acyl ghrelin also induces food intake via orexin neurons, independent of GHSR1a in mice and rats [57], although contrasting data were also reported [58]. Acylated ghrelin is swiftly cleared from plasma with a half-life of 9–13 minutes, whereas total ghrelin (including DAG) persists for 27–34 minutes [59]. Early reports indicated that ratio of active ghrelin to total ghrelin was approximately 1:20, as measured in healthy human sera by polyclonal rabbit antibodies raised against N-terminal fragment of ghrelin (active) and C-terminal fragment (total), respectively [60]. Similar findings were reported in rat serum, where early reports indicated 1:5 ratio of active to total ghrelin [61,62]. The presence of des-acyl ghrelin was attributed to

serum-mediated degradation of the active molecule, not to the production and release of the des-acyl molecule. Indeed, after 240 minutes of acyl ghrelin incubation with human serum, nearly 50% of the peptide was converted to des-acyl form and no further processing, including proteolysis, was observed [63]. Some other reports hinted at the possibility of even faster processing of ghrelin in human serum [64,65]. More pronounced effect was observed in rat serum, as already after 30 minutes, ~60% of ghrelin-derived peptide was converted to des-acyl form [63]. This activity was attributed to butyrylcholinesterase and possibly other esterases in human serum, whereas in rat serum, only carboxylesterase was involved [63,64,66]. Proteolytic degradation of ghrelin was observed not in serum, but in stomach, liver and kidney tissue homogenates, which led to the generation of the biologically inactive fragments, indicating that proteases may be involved in ghrelin processing locally, but rather not systemically [63]. In parallel, other activities were identified, including description of Acyl-Protein Thioesterase 1/Lysophospholipase (APT1) as ghrelin deacylating enzyme in rat stomach and in fetal bovine serum [67,68]. Unexpectedly, α_2 -macroglobulin hydrolase activity was identified in rat serum by active-site labeling approach with ghrelin-derived activity-based probe (ABP), potentially accounting for up to 50% of ghrelin deacylase activity [69]. Further, activated protein C (APC) in bovine plasma was described as ghrelin-cleaving protease, leading to the release of biologically active ghrelin₁₋₁₅ octanoylated peptide [35].

These findings led to the investigations on the appropriate conditions of the optimal collection and storage of samples for the subsequent ghrelin detection. Indeed, some level of protection was observed after the addition of PMSF to serum samples [63] or after the addition of EDTA and aprotinin during sample collection [70]. Also, the acidification of the serum samples with HCl to pH 3-4 led to the significant protection of ghrelin, an observation consistent with the inhibition of serum esterases by low pH [70]; however, increased ghrelin deacylation was observed in the samples containing ≥ 100 mM HCl [65]. Further research indicated 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF) as effective compound in the active ghrelin protection in the human serum samples [65]. In recent years alkyl fluorophosphonate inhibitors were proposed as the optimal compounds stabilizing ghrelin octanoylation in biological samples. This is exemplified by active ghrelin protection in the presence of methoxy arachidonyl fluorophosphonate (MAFP) in biological samples, including cell lysates and rat blood, a treatment superior to all previously described [71]. Indeed, addition of MAFP during the collection of the rat blood samples resulted in the unprecedented acyl-ghrelin protection and led to the detection of unexpectedly high active ghrelin levels [71]. It is worth noting that significant levels of ghrelin deacylase APT1 are released from the mouse-derived RAW264.7 macrophage cell line upon LPS stimulation and similar enzymes were identified in fetal bovine serum (FBS) [68], indicating the potential need for ghrelin stabilization even in cell culture-based assays. Indeed, it has been suggested that inherent instability of ghrelin in serum may lead to the significant underestimation of active/total ghrelin ratios, especially in the earlier reports, where no protection procedures were employed [71].

4. Ghrelin: Functionality Profile

Ghrelin has emerged as a pivotal factor in numerous physiological functions (*Figure 2*). The functional scope encompasses the growth hormone (GH) secretion; stimulation of the appetite and food intake; maintenance of glucose homeostasis; modulation of gastric secretion and gastrointestinal motility; improvement of the gut barrier function and circulation, and exerting immunomodulatory effects by downregulation of pro-inflammatory and upregulation of anti-inflammatory cytokines [1,72,73]. In addition, ghrelin enhances neuroprotection, contributes to cardiovascular functions such as lowering blood pressure and enhancing coronary blood flow, influences sleep/wake rhythms, increases expression of anti-apoptotic BCL-2 in lung cells, enhances renal blood flow and promotes the glomerular filtration in the kidney, and stimulates osteoblast proliferation and bone formation [74-79].

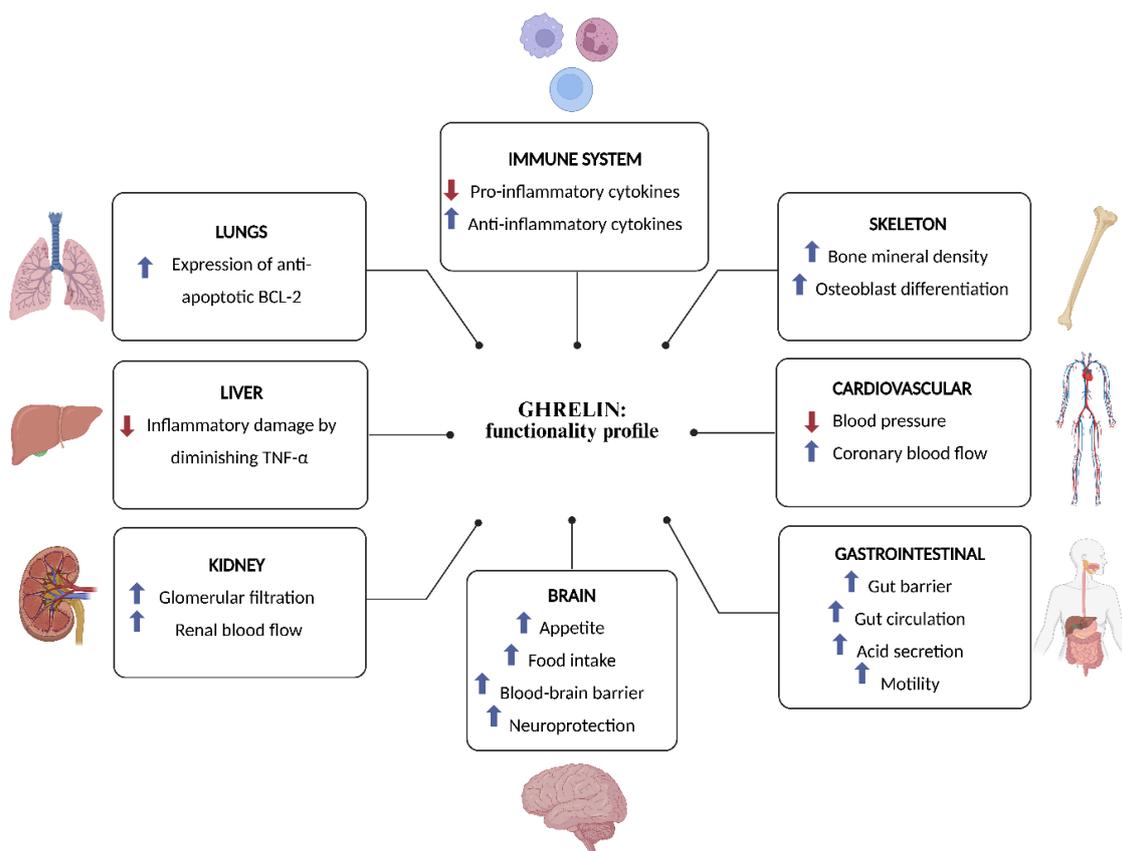


Figure 2. The functionality profile of ghrelin. Schematic illustration of the hormonal actions of ghrelin in different target organs and tissues. The red arrows indicate the decrease, while blue arrows show the increase. Created with BioRender.com.

4.1. Ghrelin Serves as a “Hunger Hormone” Regulating Food Intake and Obesity

Ghrelin earned its nickname as the “hunger hormone” because it stimulates appetite by sending signals to the brain to indicate that it’s time to eat, thereby increasing food intake and promoting fat storage [80]. Ghrelin levels in serum increase before meals and decrease afterward [7,13]. Ghrelin plays a role in short-term food intake regulation and long-term body weight control by reducing fat utilization [81]. This effect on feeding is facilitated through GHSR1a, evidenced by the absence of its orexigenic impact in knockout mice [81].

By regulating appetite ghrelin acts as a key regulator of energy homeostasis integrating signals from peripheral nutritional status to the central nervous system. Specifically, ghrelin promotes increased food consumption and fat storage while potentially slowing overall metabolic rate and reducing the body’s capacity to burn fat, which helps conserve energy during periods of fasting or low nutrient availability [82]. In terms of energy balance, ghrelin contributes to efficient metabolic adaptations by modulating energy expenditure; for instance, it can induce changes that favor energy conservation, such as altering thermogenesis or substrate utilization in tissues like adipose and muscle. It also stimulates the release of growth hormone from the pituitary gland, which indirectly supports metabolic processes like protein synthesis, lipolysis, and glucose regulation [83]. Regarding glucose metabolism, ghrelin influences insulin secretion and sensitivity, often promoting a state that maintains blood sugar levels during fasting, though chronic elevations may contribute to insulin resistance in certain contexts. Additionally, ghrelin exerts control over lipid metabolism by regulating central and peripheral pathways, including the promotion of lipogenesis in the liver and adipose tissue, which aids in energy storage [84]. Emerging research suggests it may also play a protective role in preventing excessive obesity and insulin resistance during growth phases, such as in catch-up

growth scenarios, by balancing energy metabolism with anabolic processes [85]. However, studies on ghrelin knockout models indicate that while it is important, its role in appetite and metabolism may be somewhat redundant, as animals lacking ghrelin do not always show significant reductions in food intake or metabolic disruptions [86]. Overall, ghrelin's metabolic effects are context-dependent, varying with factors like nutritional status, circadian rhythms, and interactions with other hormones such as leptin.

4.2. Ghrelin is a Ligand for the Growth Hormone Secretagogue Receptor 1a (GHSR1a)

Ghrelin conveys its biological activity by binding to homo- and heterodimers of the growth hormone secretagogue receptor 1a (GHSR1a) (AG) and to yet-to-be-identified receptors (DAG) [1,57]. GHSR1a exhibits widespread distribution across tissues and organs, including the lung (especially in alveolar macrophages), kidney, heart, liver, intestine, and adipose tissue [87,88]. Notable locations also include the ventromedial and arcuate nuclei of the hypothalamus that govern feeding and body weight homeostasis [89]. Furthermore, a variety of immune cells, including monocytes, dendritic cells, B and T cells, as well as neutrophils express GHSR1a on their surface [88,90].

Numerous studies indicate that an acyl group on Ser³ is essential for ghrelin's biological activity through GHSR1a. The position of the octanoylated Ser is fundamental: moving the acyl group to Ser² retains partial activity, while C8:0 at Ser⁶ or Ser¹⁸ leads to the activity reduction. The fatty acid chain length also impacts the receptor recognition, as maximal activity is retained by C10:0 Ser³, C12:0 Ser³, and C16:0 Ser³ ghrelin variants, but is decreased in the case of the shorter C4:0 Ser³ or C2:0 Ser³ fatty acid modifications [39,91]. While substituting Ser³ with Trp³ preserves ghrelin activity, replacing it with aliphatic amino acids (Val, Leu, Ile) reduces it. The ester bond at Ser³ can be substituted with thioester or ether without affecting activity, highlighting the flexibility in chemical modifications [39]. The N-terminal positive charge and Phe⁴ are essential for activity and GHSR1a recognition. The minimal active fragment required for activation of GHSR1a is the N-terminal pentapeptide, including C8:0 Ser³ [38]. Amidation of the C-terminus enhances, whereas N-acylation diminishes activity [91,92]. Both acyl and des-acyl ghrelin exhibit a short α -helix conformation when bound to lipids [93]. The minimal core sequence necessary for GHSR1a activation covers the N-terminal ¹GSS_(Octanoyl)FL⁵ sequence with indispensable octanoylated Ser³ in the middle (Figure 1).

5. GHSR1a Signaling Pathways

GHSR1a belongs to the A family of G-protein coupled receptors (GPCRs), characterized by 7-transmembrane (7-TM) regions. Tucked away in cell membranes, these receptors are selective for a wide range of ligands, from sophisticated proteins to small molecule ligands like adrenaline. Classically GHSR1a transduces the signal via GHSR1a-G_{αq}-dependent pathway (Figure 3A), which leads to the increase in intracellular Ca²⁺, mediated by phospholipase C (PLC) activation and release of inositol 1,4,5-trisphosphate (IP3) and downstream activation of protein kinase C (PKC), Ca²⁺/calmodulin-dependent protein kinase-IIa (CamKII), and 5' AMP-activated protein kinase (AMPK) [94–96]. In another G protein-related pathway (Figure 3C), GHSR1a –G_{αi/o} activates phosphoinositide 3-kinases (PI3K) to induce the activation of protein kinase A (PKA), PKC ϵ , and serine/threonine protein kinase (AKT) [97,98]. Alternatively, G-protein independent signaling is based on the signal relay via GSHR1a- β -arrestin complex (Figure 3B), leading to the increase in receptor internalization, accompanied by extracellular signal-regulated kinases (ERK1/2) and AKT activation [99,100]. It was reported that recruitment of G_{αq/11} is required for the regulation of food intake [101], while G_{i2} is necessary for the regulation of insulin release [102].

In recent years, significant progress has been made, and structures of GHSR1a with several ligands have been determined. Recognition of ghrelin and the antagonist Compound 21 involves the binding pocket, which bifurcates into two cavities separated by a salt bridge formed between TM3 E124^{3,33} and TM6 R283^{6,55}, with cavity I created between helices TM6 and TM7 of the receptor dedicated to the binding of the ghrelin N-termini and cavity II created between helices TM4 and TM5 recognizing the octanoylated side chain of Ser³ [103,104]. Further, the recent structure of GHSR1a

with anamorelin, its small-molecule agonist, currently investigated for the cancer-related cachexia and anorexia treatment [105,106] confirmed this mode of binding [107]. Notably, the study combined cryo-EM structural analysis and in-cell functional assays to elucidate biased signaling of the GHSR1a. The repertoire of G-protein families recruited depends on ligand concentration and time; different ligands (agonists/antagonists/reverse agonists) stabilize distinct receptor conformations, producing a characteristic G-protein/ β -arrestin fingerprint [107,108]. These findings provide the structural explanation for the GHSR1a biased signaling and indicate that selected ligand combinations might be chosen for the personalized treatment even in the case of SNP variations in the receptor.

As progress in the clinical development of drugs targeting GHSR1a is relatively slow, new data on the pharmacology of GHSR1a ligands are needed to design new, more selective ligands with predictable biological activity. The pharmacology of GHSR1a is highly complex, involving G protein-dependent and -independent signaling pathways and high constitutive activity. The functional selectivity and signaling bias of many GHSR1a-specific ligands is still not well described. Activity of several peptides was investigated in aspect of constitutive signaling, ligand-directed downstream GHSR1a signaling, functional selectivity, and signaling bias [109]. Biased ligand binding to GHSRs simultaneously stabilizes the receptor in a conformation that is able to selectively activate specific signaling pathways (Figure 3). Ghrelin can stabilize GHSRs in specific conformations that favor G-protein activation ($G_{\alpha q}/G_{\alpha i/o}$) or β -arrestin recruitment [110]. Interestingly, the activity of peptides (inverse agonist KwFwLL and agonist AwFwLL) depends on key residues in transmembrane helices III and IV. Such mutations change the efficacy of KwFwLL from full inverse agonism in the WT receptor to partial agonism in the mutated receptor. In contrast, ghrelin stabilizes a different conformation that is able to recruit $G_{\alpha q}$, $G_{\alpha i/o}$, and β -arrestin but not $G_{\alpha s}$. These findings are in line with knowledge that the GHSR conformation induced by ghrelin in the presence of β -arrestin differs from ghrelin-induced conformation in the presence of $G_{\alpha q}$ [111]. Indeed, GHSR exhibits distinct conformations when activated by different ligands. There is a growing collection of agonists and antagonists for GHSR demonstrating a spectrum of activities. Ramirez et al. examined a group of biologically active substances including: ghrelin, Ibutamoren (MK-0677), L692,585, and [D-Lys3]-growth hormone-releasing peptide-6 (DLys3-GHRP-6), JMV2959, and [D-Arg¹,D-Phe⁵,D-Trp⁷,⁹,Leu¹¹]-substance P (SP-analog) [112]. DLys3-GHRP-6 behaved as a partial antagonist with a strong bias toward GHSR1a- β -arrestin signaling, whereas JMV2959 acted as a full unbiased GHSR1a antagonist. Moreover, the SP-analog at high concentrations behaved as an inverse agonist increasing G-protein-dependent signaling, whereas, at low concentrations, the SP-analog attenuated β -arrestin-dependent signaling (Table 1).

Liver-expressed antimicrobial peptide 2 (LEAP-2) was first described as an antimicrobial peptide expressed in the liver [113]. Subsequent investigation revealed that LEAP-2 is an endogenous antagonist of GHSR1a blocking the action of ghrelin, by competing at the ghrelin-binding site [114]. LEAP-2 increases whereas ghrelin decreases in obesity, and LEAP-2 is implicated in obesity-related disorders including polycystic ovary syndrome and non-alcoholic fatty liver disease [115]. Recent studies have revealed that ghrelin attenuates obesity-induced myocardial injury through various signaling axes [116,117]. It has been shown that knockdown of LEAP-2 relieved hyperlipidemia, inflammation, and myocardial injury in obese mice by polarizing macrophages toward the M2 phenotype [118]. LEAP-2 is being investigated as a potential therapeutic target for obesity and related metabolic diseases, as it can impact food intake and body weight [119]. Recent studies have focused on developing LEAP-2 analogs with potential clinical application [120]. Despite complicated landscape of ghrelin signaling pathways, the understanding of the receptor structure and biased signaling is progressing. As of now only anamorelin, a small-molecule synthetic agonist of GHSR1a has found a limited clinical use in cancer patients cachexia treatment. For the summary on the progress in the clinical trials of ghrelin receptor ligands, please refer to the recent review by Bukhari [121].

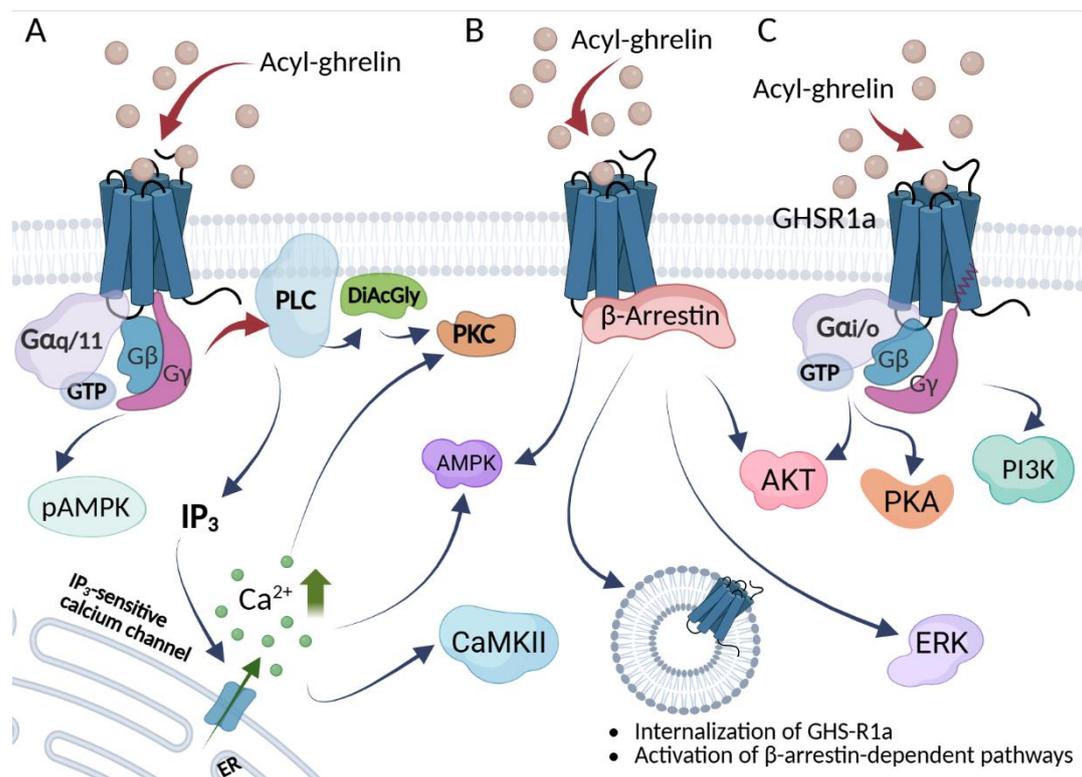


Figure 3. Signaling pathways associated with the ghrelin GHS-R1a receptor. Upon activation by ghrelin, GHSR1a engages *Gαq/11* (A), *Gαi/o* (C) or β -arrestin (B) signaling. Each G-protein/ β -arrestin complex is linked with unique intracellular activation pathway and specific physiological effects. p-: phosphorylated; AMPK: adenosine-monophosphate-activated protein kinase; DiAcGly: diacylglycerol; GTP: guanosine triphosphate; IP₃: inositol 1,4,5-trisphosphate; PKC: Protein Kinase C; CamKII: Ca²⁺/calmodulin-dependent protein kinase-IIa; PLC: phospholipase C; PI3K: phosphoinositide 3-kinase; PKA: protein kinase A; AKT: serine/threonine protein kinase; ERK: extracellular signal-regulated kinases. Created with BioRender.com.

Table 1. Selected ligands of the human growth hormone secretagogue receptor (GHSR1a).

Ligand	Type	Activity	Signaling Pathways				Selected refs
			Ca ²⁺ mobilization	β -arrestin	GHSR1a intern.	ERK Phosph.	
Ghrelin (human, acylated)	Endogenous peptide	Full agonist (canonical)	+	+	+	+	[1,112]
Des--acyl ghrelin (DAG)	Endogenous peptide (des--acyl)	Weak/low--potency agonist in vitro; often functionally GHSR1a--independent in vivo					[38]
Mini-ghrelins (1--15, 1--14, 1--11)	Endogenous peptide fragments	Competitive antagonists					[37,38]
LEAP--2	Endogenous peptide/protein	Competitive antagonist					[114]

Anamorelin	Small--molecule	Potent agonist						[107,122]
Ibutamoren (MK--677)	Small--molecule	Potent, selective, orally active agonist	+	+	+	+		[112,123,124]
L--692,585	Small--molecule	Agonist						[112,125,126]
JMV2959	Small--molecule	Unbiased antagonist; bias-inverse agonist	+/-			Basal -	0	[112,127]
Compound 21 (C21)	Small--molecule	Neutral antagonist						[103]
PF-5190457	Small--molecule	Orally active inverse agonist				Basal -	0	[128,129]
[D--Lys ³]-GHRP--6	Peptide analog	Preferentially β -arrestin pathway blocker; bias-inverse agonist						[112,130,131]
Substance P analog (D--Arg ¹¹ ,D--Phe ⁵⁵ ,D--Trp ^{77,99} ,Leu ¹¹¹ --SP)	Peptide analog	Inverse agonist at higher concentrations; attenuates β -arrestin at low concentrations				Basal -	Basal -	[112,132]
KwFwLL	Peptidomimetic	Inverse agonist						[133]
AwFwLL	Peptidomimetic	Agonist						[133]

GHSR1a intern.: internalization of the receptor; ERK Phosph.: Extracellular signal-regulated kinase phosphorylation; Activity symbols: Basal -: reduction in the constitutive (basal) receptor activity; +: increase in signal; 0: no effect; +/- weak increase in signal.

6. Ghrelin as an Anti-Inflammatory Agent

Ghrelin is recognized for its broad anti-inflammatory effects across multiple physiological systems. In the central nervous system, it reduces neuronal damage following subarachnoid hemorrhage in rats and mitigates neuroinflammation in Alzheimer's disease models [134]. Ghrelin's immunoregulatory role is exemplified by its suppression of pro-inflammatory cytokine production. Notably, in aged septic rats, both ghrelin and GH attenuate immunosuppression via vagus nerve-dependent inhibition of transforming growth factor-beta (TGF- β) production (105).

In the gastrointestinal tract, ghrelin demonstrates therapeutic potential in relieving colitis, while also exerting beneficial effects in metabolic disorders such as type 2 diabetes [135,136]. Beyond these roles, ghrelin influences skeletal muscle, cardiovascular, and respiratory function [137,138].

In a murine model of elastase-induced emphysema, ghrelin treatment attenuated pulmonary inflammation, promoted macrophage polarization toward the M2 phenotype, reduced collagen deposition, and increased elastic fiber content, collectively contributing to improved alveolar architecture and lung function. Additionally, ghrelin improved cardiovascular dysfunction and increased both lean and total body mass [138].

Consistently, ghrelin has been shown to reduce inflammation and disease severity in numerous conditions, including sepsis, inflammatory bowel disease, arthritis, pancreatitis, obesity, autoimmune encephalomyelitis, and diabetic nephropathy [134,139–141]. In turn, this underscores the evident link between ghrelin and chronic diseases. Studies from many laboratories collectively provide robust evidence that ghrelin exerts anti-inflammatory effects in human monocytes and macrophages by reducing pro-inflammatory cytokine production, inhibiting inflammatory signaling pathways, promoting anti-inflammatory macrophage polarization, and decreasing adhesion and oxidative stress [142–145].

Ghrelin hinders the generation of pro-inflammatory cytokines from monocytes, T-cells, and macrophages [142]. It also hampers leptin-triggered expression of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , while boosting the production of anti-inflammatory cytokines like TGF- β and IL-10 [73,143]. HMGB1 (High Mobility Group Box 1) is a nuclear protein produced by the *HMGB1* gene which stabilizes chromosome structure and regulates gene expression [146] and when translocated to the cytoplasm induces autophagy [147]. In immune cells, HMGB1 is directed for secretion [148] and acts as an alarmin, recognized by RAGE and TLR-family receptors [149]. It is secreted by diverse immune cells, including monocytes, macrophages, and dendritic cells, prompting the release of pro-inflammatory cytokines like TNF- α , IL-1, IL-6, and IL-8, thus intensifying inflammation [150,151]. Ghrelin treatment impedes HMGB1 translocation from the nucleus to the cytoplasm, reducing its secretion and thereby dampening inflammation [142]. The delayed release of ghrelin by macrophages when stimulated by substances like LPS, IL-1, and TNF- α may act as a regulatory mechanism to avert excessive inflammation [152].

Remarkably, GHSR deletion mutants (Ghsr^{mutant}) of macrophages and microglia reduce their inflammatory responses to fructose, indicating that GHSR mediates fructose-induced inflammation. Furthermore, GHSR regulates fructose transport and metabolism, and mediates fructose-induced inflammatory activation through CREB–AKT–NF- κ B and p38 MAPK signaling pathways [153]. These findings reveal that the nutrient-sensing receptor GHSR plays a crucial role in fructose-mediated inflammatory activation. Interestingly, at the molecular level, GHSR metabolically programs macrophage polarization through PKA–CREB–IRS2–AKT2 signaling pathway [154], an axis which is not characteristic for other cell types. Insulin signaling is a master regulatory pathway of metabolism, and is involved in macrophage polarization [155]. Insulin receptor deficiency in macrophages protects against inflammation [156–158], and IRS2 deletion promotes IL-4-induced M2 macrophage polarization [159]. Thus, nutrient-sensing ghrelin signaling is linked to immune regulation and meta-inflammation.

Obesity-related chronic inflammation, or meta-inflammation, has been linked to a wide range of metabolic dysfunctions such as insulin resistance [160,161], fatty acid dysregulation [162,163] and non-alcoholic fatty liver disease (NAFLD) [164,165]. Macrophages are among the first responders of inflammation in response to metabolic dysregulation [166]. Meta-inflammation through cytokines and other inflammatory mediators promotes macrophage recruitment and activation in tissues, leading to deleterious effects in, for example, adipose tissue and liver [167–169]. Macrophage diversity is described by the outdated M1/M2 model, created more than 20 years ago, which distinguishes between M1 or “classically” activated macrophages; and M2 or “alternatively” activated macrophages, based on the effect of in vitro macrophage stimulation with type 1 or type 2 cytokines [170]. In the more recent version, the “M1-like” phenotype is usually described as pro-inflammatory and is induced by Toll-like receptor (TLR) ligands and type 1 cytokines, namely IFN- γ and TNF- α ; ‘M2-like’ macrophages, having anti-inflammatory properties, are activated by IL-4 or IL-13 and produce TGF- β . This nomenclature, although oversimplified, is widely used today

[171,172]. Significantly, a considerable heterogeneity in morphology, function and expression of cell surface markers has been observed in resident macrophages from different organs [173]; moreover, co-expression of M1 as well as M2 genes has been described in subgroups of macrophages from most tumor types [174]. Therefore, it is now believed that macrophages form a continuous spectrum of phenotypes during polarization [175,176]. Single-cell RNA sequencing technology (scRNA-seq) confirms the multidimensional diversity of macrophage immunophenotype and function [177]. Ghrelin is known to stimulate appetite and promote obesity/insulin resistance via the G protein-coupled receptor (GPCR) [47,178–181]. GHSR1a expression is very high in macrophages and monocytes [142,182]. In mouse peritoneal macrophages, expression reaches 60% relative to the hypothalamus, the tissue with the highest expression [182]. Global ablation of GHSR1a promotes anti-inflammatory change in peritoneal macrophages and adipose tissue macrophages in aging mice [182]. Global GHSR1a ablation has also been shown to alleviate adipose tissue inflammation and non-alcoholic steatohepatitis (NASH) induced by high fructose corn syrup (HFCS) [183]. Adipose tissue macrophages of such mice have reduced expression of pro-inflammatory markers (M1), including monocyte chemoattractant protein-1 (MCP-1), TNF- α and inducible nitric oxide synthase (iNOS), while the anti-inflammatory markers arginase-1 (Arg-1) and macrophage galactose-type lectin-1 (Mgl-1) were increased [184]. In vitro studies have also shown that antagonist or knock-down (siRNA) of GHSR1a decreases the expression of proinflammatory cytokine genes in the LPS-stimulated macrophage cell line RAW264.7 [182,183]. These observations suggest that GHSR1a has a cell-autonomous effect in macrophages, and that GHSR1a probably plays a key role in macrophage programming. In myeloid-specific GHSR1a knockout mice tissue analysis showed significantly decreased monocyte/macrophage infiltration, pro-inflammatory activation, and lipid accumulation. Ex vivo, GHSR1a-deficient macrophages were protected against palmitic acid (PA)- or LPS-induced pro-inflammatory polarization, showing reduced glycolysis, increased fatty acid oxidation, and decreased NF- κ B nuclear translocation [154]. As a G protein-coupled receptor, GHSR has a high ligand-independent constitutive activity of ~50% of its maximal activity [132,185,186]. Thus, the effect of GHSR1a in macrophages may be mediated by both ligand-dependent and independent (constitutive) mechanisms. Global deletion of GHSR1a does not alter serum ghrelin levels compared to wild-type mice under both fed and fasted conditions [179]. It is likely that the effect of GHSR1a in macrophages is mediated by both ghrelin-dependent and -independent mechanisms. Although evidence in primary human macrophages remains limited, it is speculated that even without endogenous ghrelin, GHSR1a may modulate macrophages by altering downstream signaling pathways. Most evidence comes from animal models (e.g., murine macrophages) or indirect studies in human cell lines (e.g., THP-1 macrophages), which may not reflect primary human macrophage behavior. There is no direct evidence in human macrophages suggesting that GHSR1a's constitutive activity promotes pro-inflammatory responses. Constitutive activity alone does not appear to drive pathways like NF- κ B activation or pro-inflammatory cytokine release in human macrophages [187,188] [187]. Thus, we speculate that the potential inflammatory outcome of GHSR1a's constitutive activity may depend on the macrophage's activation state (e.g., resting, M1, or M2) and the microenvironment. For instance, in resting human macrophages, constitutive activity likely maintains an anti-inflammatory tone, while in highly inflamed states, its impact might be overshadowed by dominant pro-inflammatory signals (e.g., LPS-induced TLR4 activation).

7. The Neuroimmune Connection – Ghrelin's Role in the Nervous System

The hypothalamus is a small brain region located just below the thalamus, responsible for central regulation of appetite and energy homeostasis. Structurally, the hypothalamus consists of several distinct nuclei: the arcuate nucleus (ARC), paraventricular nucleus (PVN), lateral hypothalamic area (LHA), ventromedial nucleus (VMN), and dorsomedial nucleus (DMN). The ARC lies adjacent to the median eminence, a circumventricular organ characterized by fenestrated capillaries and an atypical blood–brain barrier (BBB). This proximity provides ARC neurons with relatively direct access to circulating hormones and nutrients without crossing a typical BBB. Owing to this unique anatomical

feature, the ARC is considered a principal hypothalamic site for sensing peripheral metabolic signals. Two major neuronal populations are present in the ARC: (i) neurons co-expressing orexigenic (appetite-stimulating) neuropeptides, including neuropeptide Y (NPY) and agouti-related peptide (AgRP); and (ii) neurons expressing anorexigenic (appetite-limiting) neuropeptides, including proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). These populations serve as first-order integrators through which peripheral metabolic signals affecting appetite are relayed [189].

As in other organs, the function of ghrelin in the central nervous system (CNS) is mediated by activation of the GHSR1a. Its expression has been identified in the pituitary gland, ARC, VMN, PVN and, to some extent, in the DMN [190–193]. Ghrelin activity in hypothalamic neurons is part of the brain's fatty-acid-sensing mechanism. Binding of ghrelin to GHSR1a activates PLC-IP3/DiAcGly-PKC signaling and increases intracellular calcium levels. This, in turn, activates calcium/calmodulin-dependent protein kinase 2 (CamKII), which phosphorylates AMP-activated protein kinase (AMPK) and promotes formation of a stable AMPK/CaMKK2 complex. This complex increases phosphorylation of acetyl-CoA carboxylase (ACC), lowering malonyl-CoA and thereby disinhibiting carnitine palmitoyltransferase-1 (CPT1A and CPT1C) [194–197]. CPT1A shuttles acylcarnitines, allowing long-chain fatty acids to cross the mitochondrial membrane for subsequent β -oxidation [198,199]. Enhanced fatty-acid metabolism can elevate mitochondrial reactive oxygen species (ROS) and upregulate the free-radical-scavenging uncoupling protein-2 (UCP2), which contributes to activation of NPY/AgRP neurons and stimulation of ghrelin-induced food intake [200]. The CPT1C isoform increases ceramide levels and upregulates NPY and AgRP while downregulating POMC expression, thereby increasing NPY/AgRP and decreasing POMC activity in the ARC, effectively stimulating appetite. In addition, ghrelin modulates NPY-related neurons in the PVN and ARC, increasing GABAergic suppression of POMC neurons, which further promotes appetite [201,202].

Beyond its classical roles in appetite regulation and pituitary signaling, ghrelin exhibits potent anti-inflammatory and neuroprotective properties [143]. Preclinical studies demonstrate that exogenous ghrelin maintains the blood-brain barrier (BBB) integrity, reduces oxidative damage, and limits neuronal apoptosis after traumatic brain injury (TBI) and ischemia-reperfusion injury (IRI) [203–206]. These effects are linked to modulation of fibroblast growth factor signaling [207] and activation of the PI3K/Akt pathway, which promotes neuronal survival via regulation of GSK-3 β and Bcl-2 [208–210].

Ghrelin has been implicated in several mental disorders, including anorexia nervosa and bulimia—via appetite regulation—as well as depression, anxiety, and schizophrenia [211,212]. The ghrelin gene polymorphism Leu72Met has been associated with depression, but not with panic disorder, in patients [213].

Depression is a multifactorial disorder with genetic, neuroendocrine, immune, and metabolic influences [214,215]. Beyond monoamine deficiency [216], hypothalamus-pituitary-adrenal (HPA) axis dysregulation [217], excitatory/inhibitory imbalance [218], and neuroinflammation [219], growing evidence implicates ghrelin in mood regulation.

Ghrelin may influence depression in part through normalization of the hypothalamic-pituitary-adrenal (HPA) axis, which is dysregulated—often hyperactive—in patients with major depressive symptoms [220,221]. It has been reported that ghrelin administration increases cortisol and growth hormone (GH), with only a weak trend toward mood improvement in male patients [222], and that ghrelin can alleviate anxiety after short-term stress by activating the HPA axis [223]. In line with these findings, rat studies using ghrelin O-acyltransferase (GOAT) inhibitors—thus reducing active (acyl-)ghrelin—showed dampened HPA activity, suggesting that active ghrelin modulates HPA tone and may affect depressive phenotypes [224]. In patients, ghrelin administration alters sleep architecture, increasing non-REM and reducing REM sleep [222,225]. In the same vein are the recent results on the efficacy of the novel butyrylcholinesterase inhibitor (BChEI) in the Flinders Sensitive Line rat model of major depressive disorder. Application of BChEI improved behavioral, cognitive and reward-

related parameters of treated animals in a GHSR1a-dependent manner, likely by increased protection of acyl-ghrelin from butyrylcholinesterase in serum [226].

Ghrelin also enhances dopaminergic signaling, and dysregulation of the dopamine system is linked to depressive symptoms [227]. Specifically, ghrelin activates ventral tegmental area (VTA) dopamine neurons, a mechanism also associated with appetite stimulation [228,229]. In mice, depressive-like behavior was reduced by ghrelin, an effect attributed to GHSR1a-dependent increases in dopamine levels and promotion of dopaminergic neuronal responses and synapse formation [230,231]; these effects were at least partially reversed by administration of a ghrelin receptor antagonist [232]. Preclinical evidence suggests that ghrelin inhibits serotonin synthesis via nitric oxide (NO)-dependent suppression of tryptophan hydroxylase [233], a mechanism that may promote depressive phenotypes. Conversely, ghrelin enhances dopaminergic and orexin signaling [234,235], supporting reward processing, stress resilience, and emotional regulation. Recently it was demonstrated that ghrelin-independent, constitutive activity of GHSR1a is required for the reversal (inhibition to excitation) of dopamine D2 receptor signaling in the spinal defecation center [236].

Stress elevates circulating ghrelin, modulating HPA axis activity and glucocorticoid release [237,238], though associations with cortisol in clinical studies remain inconsistent [239,240]. At the neuronal level, ghrelin stimulates hippocampal neurogenesis and synaptic plasticity through PI3K/Akt–MAPK pathways, upregulating brain-derived neurotrophic factor (BDNF) [241,242]. Ligand-free (apo) GHSR1a can heteromerize with dopamine receptor D1 (DRD1), shaping reward-related plasticity [243,244].

Beyond its potential as a therapeutic target, ghrelin has been proposed as a biomarker to monitor antidepressant treatment response [245] and to aid in differential diagnosis between depression and bipolar disorder [246,247].

Disruption of ghrelin signaling is increasingly implicated in Alzheimer's disease (AD). While total plasma ghrelin is often unchanged [248,249], some studies report elevated circulating levels in AD and prodromal AD, inversely correlating with cognitive performance [249,250]. This paradox may reflect reduced local ghrelin availability, as supported by decreased temporal lobe ghrelin mRNA [251]. Central to ghrelin signaling disruption in AD are alterations in ghrelin receptor (GHSR) expression and regulation. GHSR mRNA is reduced in leukocytes and temporal lobes [250,251], yet hippocampal protein levels are increased, where A β directly binds and inhibits GHSR, impairing signaling [252]. GHSR1b, a splice variant that may suppress GHSR function, is also elevated in AD brains [251,253]. Additional modulation may involve liver-expressed antimicrobial peptide 2 (LEAP-2), which could exacerbate ghrelin resistance due to its association with obesity and metabolic syndrome, both recognized AD risk factors [254,255]. In recent years, ghrelin analogs were indeed demonstrated to have neuroprotective properties in vitro and in the AD triple transgenic mouse model in vivo [256,257]. Ghrelin activation may also be altered through ghrelin O-acyltransferase (GOAT). Increased MBOAT4 (GOAT) mRNA has been observed in leukocytes of AD patients [250], though its functional significance outside the gut remains unclear. Evidence for circulating "free" GOAT [30] raises additional questions about peripheral ghrelin activation in AD. Importantly, GOAT activity requires coenzyme A-activated fatty acids, especially medium-chain fatty acids (MCFAs), and dysregulated fatty acid metabolism in AD may further modulate ghrelin activation [258].

Ghrelin signaling plays a central role in alcohol use disorder (AUD) [259]. In the brain, ghrelin affects reward-related regions such as the amygdala [260], Edinger–Westphal nucleus [261], laterodorsal tegmental area, and lateral hypothalamus [262], modulating dopaminergic activity and reinforcing alcohol intake. Notably, chronic alcohol exposure upregulates GHSR expression within these structures [263], while ghrelin enhances alcohol-associated cues and neurochemical responses [264]. Preclinical studies show that ghrelin promotes alcohol intake, reward, and relapse primarily by stimulating mesolimbic dopaminergic VTA–nucleus accumbens (VTA–NAc) circuit [265]. Conversely, genetic deletion or pharmacological inhibition of GHSR (via antagonists or inverse agonists) consistently attenuates alcohol consumption, reduces motivational drive, and relapse-like behaviors across animal models [266,267]. Endogenous ghrelin dynamics appear context-dependent;

acute alcohol administration reduces circulating ghrelin in male rats [268], whereas chronic exposure may lead to elevated levels [259]. Additional modulators of the ghrelin pathway, such as des-acyl ghrelin (DAG) and LEAP-2, may further influence addiction-related behaviors, though their roles remain less defined [269].

Beyond alcohol, ghrelin signaling broadly modulates the reinforcing properties of other addictive substances. In rodents, ghrelin enhances locomotor activity, reward, and conditioned place preference (CPP) associated with psychostimulants (cocaine, amphetamines, nicotine), opioids (morphine, fentanyl, oxycodone), and cannabinoids [270–275]. Conversely, pharmacological inhibition of GHSR attenuates drug-induced dopaminergic transmission, CPP, self-administration, and reinstatement across these substance classes [267]. Complementary human studies indicate associations between GHSR gene polymorphisms and substance dependence, as well as positive correlations between circulating ghrelin levels and craving intensity [129].

8. Conclusions

Ghrelin, an extraordinary molecule with its unique processing system remains a focus of the ongoing research and it is now known that its role extends far beyond its metabolic functions, exerting neuroprotective, anti-inflammatory, and neuromodulatory effects. Disrupted ghrelin signaling contributes to Alzheimer's disease, addiction, and depression, where it regulates neuronal survival, reward pathways, and mood. As ghrelin-GHSR1a structure was recently solved and provided structural foundations for long-reported biased signaling, the precise pharmaceutical targeting of the ghrelin system may finally become feasible in the near future. Together with the functional GOAT model, the development of novel GOAT inhibitors and better understanding of the ghrelin post-translational processing, these findings position ghrelin as a promising therapeutic target not only in the obesity treatment in the post-GLP-1 receptor agonist era, but also in the management of inflammatory diseases, neuropsychiatric disorders and addiction-related therapy.

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Abbreviations

The following abbreviations are used in this manuscript:

GHSR1a	growth hormone secretagogue receptor 1a
GOAT	ghrelin O-acyltransferase
LEAP-2	Liver-expressed antimicrobial peptide 2
GPCR	G protein-coupled receptor
GCF	gingival crevicular fluid
RIA	radioimmunoassay
CoA	Coenzyme A

ER	endoplasmic reticulum
PC1/3	prohormone convertase 1/3
PC	prostate cancer
APC	activated protein
MBOAT	membrane-bound-O-acyltransferase
AG	acyl ghrelin
DAG	des-acyl ghrelin
ERK1/2	extracellular signal-regulated kinase ½
PI3K	Phosphoinositide 3-kinase
AKT	Protein kinase B; serine/threonine protein kinase
APT1	Acyl-Protein Thioesterase 1/Lysophospholipase
PMSF	phenylmethylsulfonyl fluoride
EDTA	Ethylenediaminetetraacetic acid
AEBSEF	4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride
MAFP	methoxy arachidonyl fluorophosphonate
FBS	fetal bovine serum
GH	growth hormone
PLC	phospholipase C
IP3	inositol 1,4,5-trisphosphate
PKC	protein kinase C
DiAcGly	Diacylglycerol
CamKII	Ca ²⁺ /calmodulindependent protein kinase-IIa
AMPK	5' AMP-activated protein kinase
PKA	protein kinase A
PKCε	protein kinase Cε
TM	transmembrane helix
SNP	single-nucleotide polymorphism
DLys3-GHRP-6; D-Lys3	[D-Lys3]-growth hormone-releasing peptide-6
SP-analog	[D-Arg ¹ ,D-Phe ⁵ ,D-Trp ^{7,9} ,Leu ¹¹]-substance P
HMGBl	High Mobility Group Box 1
NAFLD	non-alcoholic fatty liver disease
TLR	Toll-like receptor
scRNA-seq	Single-cell RNA sequencing
NASH	non-alcoholic steatohepatitis
MCP-1	monocyte chemoattractant protein-1
iNOS	inducible nitric oxide synthase
Arg-1	arginase-1
Mgl-1	macrophage galactose-type lectin-1
PA	palmitic acid
ARC	arcuate nucleus
PVN	paraventricular nucleus
LHA	lateral hypothalamic area
VMN	ventromedial nucleus
DMN	dorsomedial nucleus
BBB	blood-brain barrier
NPY	neuropeptide Y
AgRP	agouti-related peptide
POMC	proopiomelanocortin
CART	cocaine- and amphetamine-regulated transcript
CNS	central nervous system
ACC	acetyl-CoA carboxylase
CPT1	carnitine palmitoyltransferase-1
ROS	reactive oxygen species

UCP2	uncoupling protein-2
TBI	traumatic brain injury
IRI	ischemia–reperfusion injury
HPA	hypothalamus-pituitary-adrenal
BChEI	butyrylcholinesterase inhibitor
VTA	ventral tegmental area
NO	nitric oxide
BDNF	brain-derived neurotrophic factor
DRD1	dopamine receptor D1
AD	Alzheimer’s disease
GHSR1b	growth hormone secretagogue receptor 1b
AUD	alcohol use disorder
NAc	nucleus accumbens
CPP	conditioned place preference
GLP-1	Glucagon-like peptide-1

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