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REVIEW ARTICLE

Role of ghrelin in pancreatic development and function

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Ghrelin is a gastric peptide with anabolic functions. It acutely stimulates growth hormone (GH) secretion from the anterior pituitary glands and modulates hypothalamic circuits that control food intake and energy expenditure. Besides its central activity, ghrelin is also involved in the regulation of pancreatic development and physiology. Particularly, several studies highlighted the ability of ghrelin to sustain β-cell viability and proliferation. Furthermore, ghrelin seems to exert inhibitory effects on pancreatic acinar and endocrine secretory functions. Due to its pleiotropic activity on energy metabolism, ghrelin has become a topic of great interest for experimental research focused on type II diabetes and obesity. The aim of this review is to illustrate the complex and not fully understood interplay between ghrelin, pancreas and glucose homeostasis.

KEYWORDS

ghrelin, pancreas, glucose homeostasis, insulin, diabetes

1 | INTRODUCTION

Ghrelin is a 28-amino acid peptide that is mainly secreted by the oxyntic glands of the gastric fundus mucosa.[1] It was first identified by Masayasu Kojima and Kenji Kangawa in 1999 as the endogenous ligand of the growth hormone (GH) secretagogue receptor a (GHSR1a).[1] One year later, Mark Heiman and Matthias Tschöp described ghrelin as an orexigenic hormone able to regulate the neuronal circuits that modulate food intake and energy expenditure.[2]

The human *GHRELIN* gene encodes a 117-amino acid precursor peptide, preproghrelin, which is posttranslationally cleaved to generate the mature ghrelin peptide[3] (Figure 1). To activate its only known receptor, GHSR1a, ghrelin requires a unique post-translational modification consisting in the acylation of the hydroxyl group of the serine 3 residue. The attachment of a fatty acid side-chain (mainly 8 carbon- and, less frequently, 10 carbon-fatty acid) is crucial for most of the physiological effects of ghrelin. Ghrelin octanoylation and decanoylation are achieved by the enzyme ghrelin O-acyl-transferase (GOAT)[4,5] (Figure 1). The total absence of octanoyl and decanoyl forms of ghrelin in GOAT-deficient mice shows GOAT enzymatic activity is necessarily required for ghrelin function.[6]

While it was first identified as a potent stimulator of GH secretion, ghrelin became famously known as the "hunger hormone."[3] In both animals and humans, ghrelin is recognized as the only circulating hormone stimulating food intake peripherally.[7] Although secreted in the upper gastrointestinal tract, ghrelin exerts its orexigenic effects by regulating feeding behaviours in the central nervous system (CNS). The CNS integrates the nutrient and hormonal signals from the periphery, thereby reflecting the current *energy status* of the body.[8] Subsequently, this information is translated into appropriate neuronal responses that adapt energy homeostasis to the current needs of the body (Figure 2).

The hypothalamus is the primary brain region that responds to nutritionally relevant signals from peripheral organs to regulate

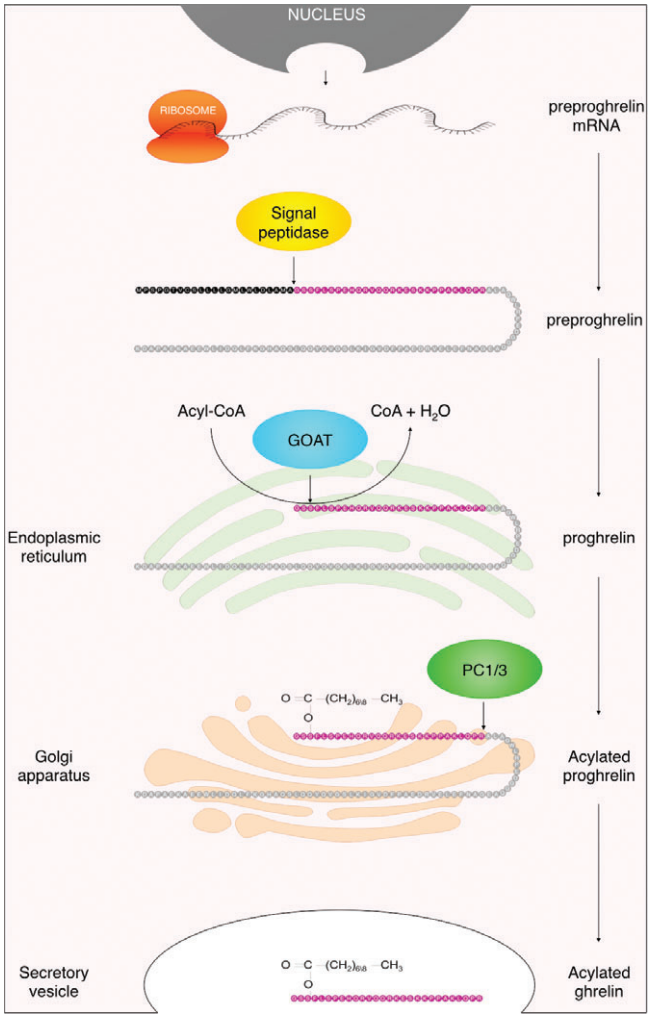

FIGURE 1 Transcription, translation and post-translational modifications of ghrelin. The human *GHRELIN* gene encodes a 117-amino acids precursor protein, preproghrelin. In the endoplasmic reticulum, the signal peptidase cleaves the 23 amino acids signal sequence. Subsequently, ghrelin O-acyl-transferase (GOAT) mediates serine-3 acylation with an eight-carbon (or less frequently 10 carbon) fatty acid, using acyl-CoA as donor. Once the pro-ghrelin precursor reaches the trans-Golgi compartment, it can be processed by the prohormone convertase PC1/3. Finally, the mature 28-amino acid ghrelin is packaged into vesicles, and eventually released in the blood

feeding behaviour.[9] Particularly, the arcuate nucleus of the hypothalamus (ARC nucleus) represents an important site for neuroendocrine integration as it contains a variety of neurons that control food intake and energy expenditure.[10] Neuronal projections from these 2 populations communicate with second-order neurons in hypothalamic areas, generating a circuit that controls energy balance. The microcircuits localized in the ARC contain 2 main neuron populations with antagonistic functions: orexigenic neurons, co-expressing neuropeptide Y and agouti-related peptide (NPY/AgRP)[11] and anorexigenic neurons, synthetizing pro-opiomelanocortin (POMC).[12] One of the most well-established roles of acylated ghrelin is the stimulation of food intake via the activation of NPY/AgRP neurons in the hypothalamus.[13–15] Conversely, leptin secreted by the adipose tissue and insulin released by the pancreas activates POMC neurons in order to suppress feeding behaviour[16] (Figure 2).

> "Ghrelin plays a major role in the hypothalamic regulation of food intake, by activating the NPY/AgRP neurons in the arcuate nucleus."

Beyond its ability in stimulating food intake, ghrelin possesses a vast spectrum of physiological functions, including stimulation of gastric motility,[17] modulation of cardiac functions,[18] thermogenesis,[19] immunity and inflammation.[20] Importantly, a body of evidence shows that both *ghrelin* and its receptor are expressed in the pancreas. For the purpose of the present review, we will focus on the role of ghrelin signalling pathways in regulating pancreatic cell development, function and survival in humans and rodent models.

2 | ROLE OF GHRELIN IN PANCREAS DEVELOPMENT

As previously mentioned, ghrelin is predominantly secreted by the fundus of the stomach. However, 35% to 45% of remaining ghrelin can still be detected in patients after a total gastrectomy.[21,22] Thus, although the stomach is the major source of circulating ghrelin, additional organs clearly contribute. Beside the gastric tissue, *ghrelin* gene expression has been shown in the intestine,[23] the brain,[24] the heart,[25] the lungs,[26] the testis,[27] the immune cells[20] and the pancreas[28] in both humans and rodents at different developmental and adult stages. Importantly, *ghrelin*-expressing cell density and *ghrelin* expression levels are found to be significantly higher in human foetal pancreas when compared to foetal stomach. These observations suggest that, in contrast to the adult, the pancreas may be the main source of circulating ghrelin during in utero development.[29] These data are further supported by similar observations in rodents.[30]

> "In adult rodents and humans, ghrelin is predominantly secreted by the gastric mucosa. However, the pancreas represents the main source of ghrelin expression during perinatal life."

The pancreas is an abdominal gland playing a key role in nutritional homeostasis through the synthesis and secretion of enzymes and hormones. The exocrine tissue accounts for more than 95% of the total organ volume and includes acinar and ductal cells. The pancreatic acinar cells are dedicated to the synthesis, storage and release of a fluid containing ions and a variety of digestive enzymes. This pancreatic juice is collected by a highly branched ductal tree that runs through the entire organ and empties into the duodenum. Scattered throughout the pancreas parenchyma, pancreatic endocrine cells are organized into clusters, termed islets of Langerhans. These cells are classified into the glucagon-producing α-cells, insulin-expressing β-cells, somatostatin-secreting δ-cells and pancreatic polypeptide-producing PP-cells. Insulin and glucagon act co-ordinately to regulate glucose homeostasis, while somatostatin and pancreatic polypeptide modulate the secretory activity of the other pancreatic cells. Using

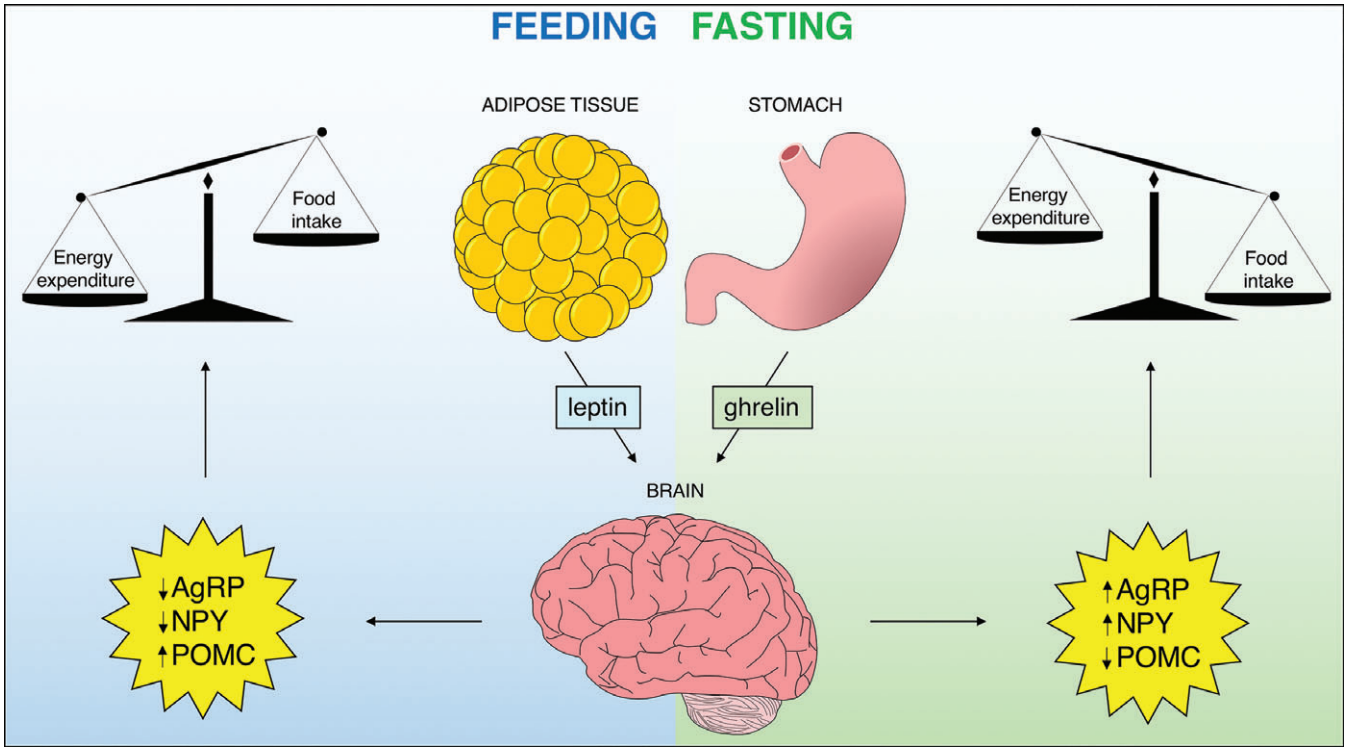

FIGURE 2 The central nervous system integrates metabolic signals from peripheral tissues to maintain energy homeostasis. In the fed state, leptin is released by the adipose tissue and exerts a potent anorexigenic effect by stimulating hypothalamic pro-opiomelanocortin (POMC) neurons. Leptin activity is counteracted by ghrelin, a hormone mainly released by the gastric mucosa during food deprivation and prior to meals. Ghrelin activates neuropeptide Y and agouti-related peptide (NPY/AgRP) neurons to stimulate feeding and weight gain

immunohistochemistry and in situ hybridization on human pancreatic specimens, Wierup et al identified a fifth pancreatic endocrine cell subtype expressing *ghrelin*.[28] These ghrelin-immunoreactive cells were found to be located at the periphery of the islets and consistently devoid of the other islet hormones, therefore constituting a distinct islet cell population. Notably, in human foetal and neonatal pancreatic samples, up to 10% of the endocrine cells express *ghrelin*. This percentage declines to ~1% in adults.

In mice, *ghrelin*-expressing cells are also present in developing islets starting from embryonic day 8.5 (E8.5).[31] Pancreatic *ghrelin* remains permanently expressed throughout embryonic development and rapidly declines *postpartum*. While *ghrelin* is expressed by a discrete cell population in human islets, ghrelin is predominantly produced by a subset of α-cells together with glucagon in mouse islets. However, a small population of ghrelin$^+$ glucagon$^-$ cells has been identified and is referred to as pancreatic ε-cells.[28,32] To date, the knowledge about the origins of the ε-cells is largely derived from studies on rodents in which gene regulation can be widely manipulated to provide information about signalling pathways underlying pancreatic cells development. In mice, all the pancreatic cell lineages derive from progenitor cells expressing the homeodomain transcription factor *Pdx1*.[33–36] Subsequently, a subset of these cells transiently activates the expression of the helix-loop-helix transcription factor *Neurogenin3* (*Neurog3*) and differentiate into endocrine precursors.[37–39] Accordingly, *Neurog3* loss-of-function mutant mice fail to develop any of the endocrine cell subtypes, showing the absolute requirement of Neurog3 activity in the specification of all the islet cell subtypes, including ε-cells.[31,37] Interestingly, an ectopic expression of *ghrelin* has been observed in instances where the inactivation of a transcription factor was preventing the specification of a particular pancreatic cell lineage. For instance, the targeted disruption of *Nkx2.2* in mice results in a complete lack of β-cells and reduced numbers of α- and PP-cells, these cells being replaced by ghrelin$^+$ cells.[32] Similarly, in *Pax4* full knockout (KO) animals, endocrine precursors fail to differentiate into insulin-producing cells and differentiate into *glucagon/ghrelin* co-expressing cells.[40] Furthermore, lineage tracing studies of *ghrelin*-expressing cells during the development showed that these cells do not disappear after birth but might give rise to a broad range of other pancreatic cell types, including α-, PP- and rare β-cells.[41] Therefore, ghrelin might represent a marker of specific pancreatic precursor cells or immature cells that fail to complete their differentiation program during the development.

Little is known about the role of ghrelin within the pancreas, but it has been suggested that this hormone could play a paracrine/autocrine role in the regulation of β-cell survival and function. In this context, ghrelin has recently been reported to promote proliferation and cell growth and to inhibit apoptosis in a pancreatic β-cell line and human islets.[42] This suggests that one of the main functions of ghrelin in the developing pancreas could be the induction of cell growth and maturation. Interestingly, in vitro studies showed that ghrelin could inhibit β-cell apoptosis following exposure to exogenous cytotoxic insults, such as exposition to interferon-r/tumour necrosis factor-a (IFN-r/TNF-a), doxorubicin as well as palmitate acids.[42–44] Additionally, ghrelin has been shown to protect newborn rats treated with streptozotocin against the development of diabetes during adulthood.[45] In this model, the injection of exogenous ghrelin during the

first week of life prevented the onset of diabetes and significantly reduced STZ-induced β-cell loss.

The high expression of *ghrelin* in the pancreas throughout embryogenesis and its ability to regulate β-cells genesis and survival strongly suggest an involvement of this hormone in islet cells development. Thus, it is conceivable that intra-islet ghrelin might promote and sustain β-cells mass expansion during pancreatic ontogenesis. However, *ghrelin* full KO mice showed no alterations of islet cell populations and structure, indicating that ghrelin is dispensable for normal endocrine cell differentiation and maturation during embryogenesis.[46]

3 | GHRELIN REGULATES PANCREATIC SECRETORY ACTIVITY

Several studies clearly showed that *ghrelin* and its receptor are expressed in the pancreas of rodents and humans,[1,47–50] suggesting a role of ghrelin in the regulation of pancreatic physiological activity. Pancreatic biology is exquisitely complex, as it involves both exocrine and endocrine functions and is regulated by parasympathetic neurotransmitters and numerous gut-derived hormones.[51] When the stomach releases the chyme into the duodenum, specialized enteroendocrine cells secrete regulatory peptides that control digestive processes. Among these, cholecystokinin is the major stimulator of pancreatic acinar secretion. Several evidences suggest that ghrelin modulates cholecystokinin stimulatory activities on exocrine cells. Experimentally, both ghrelin and its receptor have been identified in rat acini by evaluation of protein and mRNA expression.[48] Importantly, ghrelin has been shown to inhibit cholecystokinin-stimulated protein secretion in anesthetized rats as well as in dispersed rat acinar cells.[52]

The *ghrelin* and *Ghsr1a* mRNAs and proteins have been identified in both human and rat islets of Langerhans and β-cell lines,[49,53–55] suggesting an additional role for ghrelin in the regulation of endocrine pancreatic activity. Several in vitro studies support a role for ghrelin in the regulation of glucose-stimulated insulin secretion. Ghrelin has been reported to have no effect on insulin biosynthesis and expression nor on basal insulin secretion in mouse insulinoma cells (MIN6 cell line). However, the administration of minimal concentrations (1 nM) of ghrelin significantly inhibited glucose-induced insulin secretion in vivo.[56] These results were confirmed in studies with rat-isolated islets, where ghrelin did not considerably modify basal insulin release but markedly inhibited insulin response to increasing glucose concentrations.[53,57] Importantly, the concomitant incubation with GHSR1a antagonists reversed ghrelin insulinostatic effects, strongly suggesting that the ghrelin-GHSR1a pathway is involved in the regulation of β-cell secretory activity.[53,57] Studies on experimental animals and humans further corroborated these observations. Indeed, to evaluate how ghrelin could influence glucose homeostasis in humans, Broglio et al analysed the consequences of exogenous ghrelin administration on young healthy volunteers. They showed that acute ghrelin treatment induced hyperglycemia and reduced insulin secretion.[58] Additional investigations further confirmed these findings in both humans and rodents.[53,59–64] Accordingly, blockade of endogenous ghrelin by intraperitoneal injection of a GHSR1a antagonist (GHRP-6) lowered fasting blood glucose levels in normal mice. Importantly, the administration of GHRP-6 during an intraperitoneal glucose tolerance test (IP-GTT) attenuated plasma glucose elevation and enhanced insulin response.[53] These results indicate that endogenous ghrelin plays a role in regulating insulin secretion and blood glucose levels.

> "Ghrelin has emerged as an important player of glucose homeostasis, exerting an inhibitory effect on glucose-stimulated insulin secretion."

In order to dissect the role of intra-pancreatic ghrelin in pancreatic endocrine physiology, gastrectomized rats, lacking stomach-derived ghrelin, were treated with GHSR1a antagonists. Intraperitoneal administration of GHRP-6 enhanced plasma insulin concentrations in gastrectomized rats to a similar extent as seen in normal rats.[65] These observations suggest that the effect of GHSR antagonist is primarily attributable to antagonism of locally produced ghrelin in the pancreas rather than circulating ghrelin. To further explore this possibility, the effect of endogenous ghrelin on the pancreatic secretory pattern was investigated in perfused rat pancreas. In this model, GHSR1a antagonist and immunoneutralization of endogenous ghrelin were shown to enhance glucose-stimulated insulin secretion, whereas exogenous ghrelin suppressed it.[65] Consistent with these observations, the analyses of transgenic animals overexpressing *ghrelin* ectopically in the pancreas revealed that pancreatic ghrelin is able to modulate insulin secretion. Indeed, RIP-G transgenic mice, harbouring a cassette containing the *rat insulin II promoter* upstream of the *ghrelin* cDNA sequence, displayed higher pancreatic ghrelin concentrations compared to non-transgenic littermates.[66] Importantly, when these animals were subjected to IP-GTT, plasma insulin concentrations were found significantly lowered in transgenic mice compared to control littermates, despite no significant difference in glucose levels.[66]

The mechanisms of action underlying ghrelin insulinostatic effects are still controversial. Several studies provided evidence to support a direct action of ghrelin on β-cells, where it would attenuate glucose-induced insulin release via $G\alpha_{i2}$-mediated activation of Kv channel. According to this model, the suppression of action potential firings would inhibit intracellular Ca^{2+} increase and suppress β-cell degranulation.[53,67] Additional investigations using INS-1SJ cell line suggested instead a mechanism by which GHSR1a would form a heterodimer with the somatostatin receptor (SST5), this interaction being fundamental for ghrelin-mediated inhibition of insulin release.[68] In a recent work, DiGruccio et al purified α-, β- and δ-cells from mouse islets and generated comprehensive transcriptomes for each islet endocrine cell subtypes.[69] Islet cell transcriptomic analyses revealed that *Ghsr1a* is selectively expressed by pancreatic δ-cells. Of note, the authors failed to detect the expression of *Sst4* or *Sst5* in any of the endocrine cells of the mouse islets. Importantly, they showed that ghrelin promptly induces δ-cells depolarization and promote glucose-stimulated somatostatin secretion. Therefore, the authors hypothesized that ghrelin does not have direct effects on β-cells, but rather engages δ-cells to promote a local inhibitory feedback that inhibits insulin release.[69]

In summary, ghrelin has been widely shown to exert an inhibitory effect on pancreatic secretory activity. Particularly, ghrelin has been showed to reduce pancreatic enzyme output in rats, indicating that ghrelin is involved in the regulation of pancreatic digestive functions. A great deal of evidence also proved that ghrelin suppresses insulin secretion, suggesting that this hormone belongs to the complex network of factors regulating glucose homeostasis.

4 | GHRELIN IN GLUCOSE HOMEOSTASIS AND DIABETES

Despite periods of feeding and starving, normal individuals possess the ability to maintain blood glucose levels within a relatively narrow range. Glucose homeostasis reflects the balance between intestinal carbohydrates absorption, hepatic gluconeogenesis and glucose uptake and metabolism by peripheral tissues. These processes are co-ordinately regulated by a set of metabolic hormones, including ghrelin.

Beside its effects on food intake and modulation of insulin secretion, ghrelin has been shown to play a crucial role in sustaining euglycemia during lengthy caloric restriction. In a recent study, Zhao et al reported that GOAT$^{-/-}$ mice subjected to a 40% caloric restriction regime exhibited a drastic and often fatal drop of blood glucose levels.[6] Importantly, continuous infusion of either ghrelin or GH rescued the lethal hypoglycaemia, showing that when body fat stores decline, ghrelin-stimulated GH secretion is crucial to promote hepatic gluconeogenesis and therefore ensure physiological glycaemia. These observations were corroborated earlier this year by a study which identified Leap2 as an endogenous ghrelin antagonist. Consistent with previous works, mice overexpressing *Leap2* developed severe hypoglycaemia and appeared lethargic and moribund upon chronic exposition to caloric restriction diet.[70]

Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycaemia. While type I diabetes results from the autoimmune destruction of pancreatic β-cells, type II diabetes is caused by the inability of insulin secretory capacity to adequately compensate peripheral insulin resistance. Overweight is a well-established risk factor for type II diabetes and the global pandemic of obesity largely explains the dramatic increase in the incidence and prevalence of type II diabetes over the past 20 years.[71] Ghrelin being a key regulator of food intake and insulin release, one could therefore speculate that this "hunger hormone" might also play a role in the pathogenesis of type II diabetes. Several genetically modified mouse lines have been generated and analysed in order to dissect the role of ghrelin pathways in the development of obesity and/or diabetes. Despite the acute stimulatory effect on appetite elicited by exogenous administration of ghrelin, ablation of *GHSR1a* modestly decreased body weight in mice maintained on standard chow diet. Importantly, insulin concentrations and cumulative food intake of *GHSR1a* full KO animals were found to be similar to that of wild-type littermates.[72] However, several weeks of exposure to high-fat diet (HFD) resulted in significantly less accumulation of both body weight and body fat content in *GHSR*-null mice as compared with littermate controls.[73] Notably, the deletion of ghrelin receptor protected mice against diet-induced obesity (DIO) only when they were fed a HFD immediately after weaning. Conversely, *GHSR1a*-deficient animals raised on chow diet and fed HFD only as adults were not resistant to DIO.[74]

> "Rodent models of disrupted ghrelin signalling display improved glucose homeostasis."

> "Being involved in the regulation of energy homeostasis and insulin release, ghrelin signaling pathway might represent an attractive target for diabetes treatment."

Similar to *Ghsr1a*-null mice, *ghrelin* KO animals exposed to prolonged HFD after weaning showed lower body weight and fat percentage than corresponding controls. Furthermore, *ghrelin*-deficient mice fed a HFD displayed close to normal glucose response and markedly enhanced insulin release compared to wild-type animals.[65] Of note, these changes in glucose and insulin levels between *ghrelin*-lacking mice and controls were masked in animals subjected to HFD late in life or fed with standard chow.[75,76] To further elucidate the role of ghrelin in energy homeostasis, *ghrelin* KO animals were crossed with *leptin*-deficient obese mice (*ob/ob*). The resulting *ghrelin*$^{-/-}$*ob/ob* double mutants became hyperphagic and developed severe obesity. However, *ghrelin* ablation markedly improved glucose homeostasis in *ob/ob* mice and significantly increased serum insulin levels.[77] Altogether, these studies suggest that targeting ghrelin could provide a novel therapeutic avenue to counteract obesity or type II diabetes progression. However, plasma ghrelin concentrations have been shown to be reduced in different pathophysiological conditions, including obesity, type II diabetes and other metabolic disorders.[78–80] Circulating ghrelin levels have been found to be lower in obese Caucasians when compared with lean Caucasians.[81,82] Accordingly, in type II diabetes patients, the fasting ghrelin concentrations are lower in obese than in lean persons.[83] Importantly, while blood ghrelin concentrations increase upon starvation and decrease after a meal in healthy individuals, ghrelin levels do not fluctuate in obese patients with type II diabetes.[84,85] Therefore, in patients with DIO, where plasma ghrelin levels are already relatively low and where ghrelin presumably does not represent the reason for the disease, the efficiency of a putative ghrelin-targeting therapy remains controversial. Importantly, pharmacological therapies targeting ghrelin signalling must take into consideration the physiological roles that ghrelin has in other regions of the body. In patients suffering from type II diabetes, postprandial hyperglycaemia is associated with increased production of reactive oxygen species (ROS) and oxidative stress. The persistence of an oxidative environment is thought to play a crucial role in the development of several diabetic-related complications. Ghrelin is widely regarded as possessing antioxidant properties and exogenous ghrelin administration has been showed to protect skeletal muscle and endothelial cells from ROS imbalance in hind limb ischemia-subjected ob/ob mice.[86] Furthermore, oxidative stress may also lead to neuropathic damage. Intriguingly, ghrelin treatment was shown to increase levels of the antioxidant catalases and superoxide dismutase in the small intestine and protect against peripheral sensory nerve damage in streptozotocin-induced diabetic rats.[87]

In conclusion, the recent literature suggests that ghrelin plays a key role in pancreas development and secretory activities. Strong

evidence indicates that ghrelin acts on the regulation of glucose homeostasis by modulating insulin release. However, whether ghrelin might be involved in the pathogenesis of diabetes and other metabolic diseases remains to be elucidated. Besides its insulinostatic activities, ghrelin is involved in the regulation of food intake, energy expenditure and inflammation. The pleiotropic nature of ghrelin complicates the full understanding of its role in the aetiology of obesity-related dysfunctions. However, gaining further insight into ghrelin signalling pathway remains of high interest in the context of prevention/treatment of diabetes research.

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Conflict of interest

The authors declare no conflict of interests.

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