MECHANISMS IN ENDOCRINOLOGY

Regulation of glucose metabolism by the ghrelin system: multiple players and multiple actions

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Abstract
Ghrelin is a 28-amino acid peptide secreted mainly from the X/A-like cells of the stomach. Ghrelin is found in circulation in both des-acyl (dAG) and acyl forms (AG). Acylation is catalyzed by the enzyme ghrelin O-acyltransferase (GOAT). AG acts on the GH secretagogue receptor (GHSR) in the CNS to promote feeding and adiposity and also acts on GHSR in the pancreas to inhibit glucose-stimulated insulin secretion. These well-described actions of AG have made it a popular target for obesity and type 2 diabetes mellitus pharmacotherapies. However, despite the lack of a cognate receptor, dAG appears to have gluco-regulatory action, which adds an additional layer of complexity to ghrelin’s regulation of glucose metabolism. This review discusses the current literature on the gluco-regulatory action of the ghrelin system (dAG, AG, GHSR, and GOAT) with specific emphasis aimed toward distinguishing AG vs dAG action.

Introduction
Ghrelin is a 28-amino acid peptide that was discovered as the endogenous ligand for the growth hormone secretagogue receptor 1a (GHSR) (1). Ghrelin possesses a unique post-translational modification where an n-octanoic acid is esterified to the serine3 residue of the peptide molecule. The presence of a fatty acid (FA) side chain attached to the ghrelin peptide is required for the full agonism of GHSR (1). Acylation of ghrelin occurs before secretion and is catalyzed by the enzyme, ghrelin O-acyltransferase (GOAT) (2, 3). Ghrelin was initially noted for its GH-releasing property and was later found to regulate energy metabolism. Ghrelin has potent orexigenic and adipogenic effects that are mediated through GHSR located in the CNS (4, 5). These findings highlighted ghrelin as a key component of the gut–brain axis in regulating energy metabolism. In addition to being expressed in areas that regulate energy homeostasis, ghrelin and its receptor are both expressed in pancreatic islet cells, suggesting that ghrelin may have paracrine or autocrine action in the pancreas (6). Therefore, much effort was dedicated to exploring the role of ghrelin in regulating glucose homeostasis. The biological role of des-acyl ghrelin (dAG) has been questioned due to the lack of a known cognate receptor. However, increasing...
evidence suggests that both dAG and AG have glucose-regulatory action in different species (7, 8, 9). The recent discovery of the ghrelin-acylating enzyme, GOAT, has allowed for a more thorough dissection of AG vs dAG action. This review focuses on the regulation of glucose metabolism mediated by the ghrelin system, which consists of dAG, AG, GHSR, and GOAT.

**In vitro action of AG on pancreatic hormone secretion**

Both ghrelin and GHSR1A are expressed in human, rat, and mouse islets from early gestation to adulthood (10, 11, 12, 13, 14). Ghrelin is expressed in both α- and β-cells (15, 16) as well as in a novel, developmentally regulated endocrine islet cell type that shares lineage with glucagon-secreting cells (17, 18). While the functional role of ghrelin in the embryonic islet is not fully known, the ghrelin-expressing epsilon lineage has a significant impact on the final cell type composition of mature islets (19). It has been proposed that the presence of higher number of ghrelin-expressing epsilon cells in the embryonic islet may modulate other islet hormone secretion (i.e. insulin) to prevent hypoglycemia in the fetal period (17). Moreover, the presence of GOAT expression in the pancreas suggests that ghrelin acylation can occur within pancreatic cells (2, 20). A large number of reports demonstrate that AG acts on isolated islets and pancreatic β-cells to inhibit glucose-stimulated insulin secretion (GSIS) (11, 18, 21, 22). However, this effect seems to be dependent on dose and experimental condition and a stimulatory effect of AG on insulin secretion has also been reported (13, 16, 21, 23). Application of a GHSR antagonist to isolated rat islets or perfused pancreas enhances glucose-induced insulin release (24), suggesting that the constitutive activity of the receptor (25, 26, 27) alone may regulate islet hormone secretion. However, other groups have reported that a GHSR antagonist itself has no effect on GSIS in isolated rat islets, but does act to block the inhibitory action of exogenous AG on GSIS (28).

The inhibitory action of ghrelin on insulin secretion initially seemed paradoxical because GHSR couples to a Gαq/11 signaling pathway as first described in pituitary cells (29). Activation of the Gαq/11 pathway stimulates insulin secretion rather than inhibition (30, 31). In the pancreas, AG attenuates cAMP and [Ca²⁺] mobilization. These data indicate that conditions that alter circulating ghrelin levels (such as fasting and feeding) can modulate the effect of ghrelin on insulin secretion (either inhibitory or stimulatory) through the interaction of ghrelin and SST signaling. Collectively, the in vitro data in the literature indicate that AG action on insulin secretion is influenced by the cell type used and the presence of other G-protein-coupled receptors, as well as the concentration of ghrelin present. Therefore, differences in these factors may account for discrepancies in the literature, which demonstrate that AG can have both stimulatory and inhibitory actions on GSIS.

In addition to regulating insulin secretion, AG acts on isolated islet cells and cultured α-cells to regulate glucagon secretion (34, 35). AG increases glucagon secretion from both isolated islets and cultured α-cells (16, 34, 36). By contrast, islets isolated from Ghsr<sup>−/−</sup> mice show no changes in glucagon secretion in response to AG, indicating a GHSR-mediated action (34). This effect is mediated, in part, through an AG-induced rise in intracellular calcium as well as AG-induced ERK-phosphorylation in α-cells (34).

Taken together, these data demonstrate that AG acts directly on pancreatic islets to regulate insulin and glucagon release.

**In vitro action of dAG on pancreatic hormone secretion**

The des-acyl form of ghrelin was initially characterized as an inactive by-product of ghrelin secretion and degradation because it does not act as a full GHSR agonist (1). Despite this initial characterization, several studies investigated GHSR-independent actions of dAG on islet cell function. In INS-1E rat insulinoma cells, dAG promotes insulin secretion in the presence or absence of a GHSR antagonist (37). Furthermore, dAG promotes...
insulin secretion from HIT-T15 cells that lack the expression of GHSR further supporting a GHSR-independent mechanism of action (13). However, other groups have found no effect of dAG on GSIS from isolated rat islets even at high doses (1000 nM) (28). There is also evidence that dAG alone does not regulate islet cell function, but rather acts to antagonize the inhibitory action of AG on insulin secretion (21). The receptor that mediates these actions of dAG is still unknown, and although it is largely accepted that dAG does not bind and activate GHSR (1), studies on CHO-K1 cells transfected with human GHSR demonstrate that dAG can act as a full agonist of GHSR in the high nanomolar to micromolar range (38). Similarly, high nanomolar concentrations of dAG activate GHSR in HEK-293 cells transfected with the ghrelin receptor (9). Collectively, dAG action on pancreatic islet cell function is not fully understood, but most studies support a GHSR-independent action. Few studies demonstrate that dAG activates GHSR in cell types other than pancreatic islets. Further investigation of both GHSR-dependent and -independent actions of dAG is necessary to fully understand the action of dAG on islet cell function.

Gluco-regulatory action of AG in rodents

Pharmacological treatment as well as genetic manipulation of ghrelin in rodents indicates that AG regulates GSIS. Although some data suggest that systemic administration of AG enhances insulin secretion in rats (39), most rodent data support that acute systemic administration of AG has an inhibitory effect on insulin secretion. AG administered i.v. to rats or mice attenuates GSIS (40, 41). This effect is likely to be independent of AG-induced GH secretion, as decreased GSIS and impaired glucose tolerance after administration of AG are also observed in GH-deficient mice (11). Pharmacological inhibition of AG synthesis with a GOAT inhibitor (42) or antagonizing GHSR (28) significantly increases GSIS and improves glucose tolerance. Together, these data indicate that antagonizing AG or AG signaling enhances glucose-stimulated insulin release and improves glucose tolerance in rodents.

Consistent with pharmacological studies, genetic ablation of either ghrelin (Ghrl) or Goat (Mboat4) leads to improved glucose tolerance and increased GSIS in 16-h fasted mice fed on a standard chow diet (43, 44). However, other groups have found no differences in glucose tolerance in Goat−/− or Ghrl−/− mice when a shorter fasting paradigm (6 h) was implemented (45, 46). The different fasting durations used in these studies may account for the differences in results. Fasting increases circulating ghrelin levels (47, 48) and it has been recently demonstrated that the (ghrelin):(SST) ratio determines whether ghrelin will be stimulatory or inhibitory on insulin secretion (33). Under fasting conditions, a high (ghrelin):(SST) ratio promotes GHSR:SST5 heteromer formation and GHSR then couples to Ga_i/o to inhibit insulin secretion. However, when the (ghrelin):(SST) ratio is low as seen in fed conditions, the GHSR:SST5 heteromer destabilizes and GHSR no longer couples to Ga_i/o to inhibit insulin secretion. This could explain why the glucose-tolerant phenotype in Goat−/− and Ghrl−/− mice is only apparent under prolonged fasting conditions. However, further investigation is required to test this hypothesis.

Mice lacking Ghsr have similar glucose tolerance but decreased GSIS compared with WT mice suggesting that GHSR ablation leads to improved insulin sensitivity (49, 50). This is supported by studies that implement hyperinsulinemic–euglycemic clamps in Ghsr−/− mice. When compared with WT controls, Ghsr−/− mice have an increased glucose infusion rate (GIR), increased glucose disposal (R_d), and decreased endogenous glucose production (EGP) indicating that peripheral and hepatic insulin sensitivities are both enhanced in the absence of GHSR signaling (50, 51). Similar to Ghsr−/− mice, improved hepatic and peripheral insulin sensitivities measured by hyperinsulinemic–euglycemic clamps have also been reported in ghrelin-deficient mice (43). Subjecting WT and Goat-deficient mice to an i.p. insulin tolerance test led to a similar decrease in blood glucose levels in both groups suggesting similar insulin sensitivity (52). However, a more sensitive measure, such as a hyperinsulinemic–euglycemic clamp, will be needed to more accurately determine whether Goat−/− mice have altered insulin sensitivity compared with WT animals. Together, pharmacological inhibition or genetic ablation of AG or AG signaling improves glucose metabolism by enhancing insulin secretion and insulin sensitivity.

Gluco-regulatory action of dAG in rodents

Although the receptor-mediated actions of dAG have not been established, dAG has been shown to regulate glucose metabolism in vivo. During an intravenous glucose tolerance test (iGTt), dAG increased GSIS in rats (53). This effect was blocked by co-administration of AG (53). Chronic peripheral infusion of dAG does not alter glucose metabolism in mice fed on a standard chow diet (9, 54). However, chronic peripheral dAG treatment prevented high-fat diet-induced glucose intolerance and insulin resistance (54). Transgenic mice that express the
preproghrelin gene under control of the mouse Fabp4 promoter have elevated levels of dAG and display improved glucose tolerance and insulin sensitivity (55). These findings suggest that dAG analogs might be a viable option for the treatment of type 2 diabetes mellitus (T2DM). However, a better understanding of dAG physiology, pharmacology, and receptor-mediated actions is required before a safe and effective therapy can be developed.

**Gluco-regulatory action of AG in humans**

The effect of AG on fasting plasma insulin and glucose levels in humans is still a matter of debate. Some groups have found no changes (56, 57, 58), whereas others have found that AG increased fasting blood glucose and decreased plasma insulin following administration of ghrelin (59, 60, 61). Infusion of supraphysiological doses of AG to healthy humans decreased insulin secretion during an ivGTT, which led to an impairment in glucose tolerance (56). Moreover, infusion of physiological concentration of AG also attenuated GSIS without altering insulin sensitivity (57). These findings highlight a pharmacological and physiological role of AG in regulating GSIS in humans.

Under hyperinsulinemic–euglycemic clamp conditions, ghrelin has also been reported to regulate insulin action in peripheral tissues. AG infusion decreases GIR and glucose disposal, while having no effect on EGP in healthy subjects (62), GH-deficient patients (63), and those who underwent gastrectomy (64). These data are consistent with an acute effect of ghrelin to induce peripheral insulin resistance while sparing insulin sensitivity in the liver through a GH-independent mechanism. AG infusion into the femoral artery of healthy subjects increased free FA (FFA) levels suggesting that ghrelin can act directly on muscle to increase lipolysis which in turn leads to the development of insulin resistance (65). This concept was challenged by the finding that AG infusion directly into the muscle resulted in decreased interstitial blood glucose concentrations, which indicates improved muscle insulin sensitivity (66). Furthermore, no alteration in classical insulin signaling pathways was evident in muscle biopsy samples taken during ghrelin infusion in another study (63). Further studies are needed to understand the tissue-specific actions of AG in regulating insulin sensitivity. As a whole, data in the literature demonstrate that acute administration of physiological and pharmacological doses of AG inhibit GSIS, whereas supraphysiological doses are required to decrease peripheral insulin sensitivity and glucose tolerance in humans.

The long-term effects of AG on glucose metabolism are not well defined. In a healthy elderly population, an oral ghrelin mimetic taken daily for 1 year caused an increase in fasting blood glucose levels and a decline in insulin sensitivity as estimated by the Quicki Index (67). Although ghrelin mimetics are currently being developed for the treatment of cancer cachexia, heart failure, and conditions related to GH deficiency, a better understanding of the long-term effects of these ghrelin-related pharmaceutical agents on glucose metabolism is necessary to avoid unwanted side effects.

**Gluco-regulatory action of dAG in humans**

The effects of dAG on glucose metabolism have not been extensively studied in humans (summarized in Table 1) and the findings have been inconsistent. During i.v. infusion in humans, AG is deacylated to dAG and therefore it is important to understand the possible actions of dAG. It is also important to note that dAG has a slower rate of clearance when compared with AG (68). An acute i.v. bolus of dAG in healthy subjects did not alter fasting GH, insulin, or glucose levels (59), whereas co-administration of dAG with AG acted to abolish AG’s inhibitory action on fasting insulin in a similar study population (61). Similarly, dAG given for 4 days as an i.v. bolus once daily to obese non-diabetic patients had no effect on fasting or postprandial serum insulin, glucose, or FFA levels (69). We have recently shown that a 210-min continuous infusion of AG or co-infusion of AG and dAG to healthy subjects yielded a decreased acute insulin response to glucose and i.v. glucose tolerance, whereas dAG infusion alone did not alter these parameters (70). However, the lack of effect of dAG on glucose metabolism is not universally observed. An overnight infusion of dAG to healthy humans decreased overall glucose and FFA levels but did not have a significant impact on overall insulin concentration during the 16-h infusion period when compared with saline (71). In obese patients with T2DM, the same duration of dAG infusion decreases postprandial glucose levels as well as fasting and postprandial AG levels without altering postprandial insulin. Insulin sensitivity, as estimated by the M-index during a hyperinsulinemic–euglycemic clamp, was improved when compared with saline infusion (72). Taken as a whole, the literature on dAG action in humans is very inconsistent and may be a result of different doses, subject populations, and length of infusion. Furthermore, the method employed to stabilize AG (and, therefore, dAG) in blood samples varies from study to study and this may also account for the discrepancies between studies. The long-term effects of dAG and how it interacts with AG to regulate glucose metabolism still warrant further investigation.

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Table 1  Effects of dAG on glucose homeostasis in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample size</th>
<th>Dose of dAG</th>
<th>Measurements</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tong et al. (2014) (69)</td>
<td>17 healthy subjects</td>
<td>210 min of continuous infusion at (4 μg/kg per h)</td>
<td>Fasting glucose and insulin</td>
<td>≫ Fasting insulin</td>
</tr>
<tr>
<td>Ozcan et al. (2013) (71)</td>
<td>Eight overweight T2DM patients</td>
<td>Overnight (16 h) infusion (3 or 10 μg/kg per h)</td>
<td>Glucose and insulin response to a standardized meal. Insulin sensitivity (M-Index calculated from H–E clamp)</td>
<td>↓ Postprandial glucose</td>
</tr>
<tr>
<td>Benso et al. (2012) (70)</td>
<td>Eight healthy subjects</td>
<td>Overnight (16 h) infusion (1 μg/kg per h)</td>
<td>Meal-related (dinner and breakfast) and inter-meal glucose, insulin, and FFA</td>
<td>↓ Overall glucose AUC</td>
</tr>
<tr>
<td>Kiewiet et al. (2009) (68)</td>
<td>Eight morbidly obese non-diabetic patients</td>
<td>Once daily i.v. bolus (200 μg/day) for 4 consecutive days</td>
<td>Fasting and postprandial insulin, glucose, and FFA</td>
<td>↑ 1 h post-meal insulin AUC</td>
</tr>
<tr>
<td>Gauna et al. (2004) (113)</td>
<td>Eight adult-onset GH-deficient patients</td>
<td>i.v. bolus (1 μg/kg)</td>
<td>Fasting and postprandial glucose, insulin, and FFA</td>
<td>↑ Overall FFA AUC</td>
</tr>
<tr>
<td>Broglio et al. (2004) (60)</td>
<td>Six healthy subjects</td>
<td>i.v. bolus (1 μg/kg)</td>
<td>Fasting plasma glucose and insulin</td>
<td>Fasting or postprandial insulin</td>
</tr>
<tr>
<td>Broglio et al. (2003) (58)</td>
<td>Seven healthy subjects</td>
<td>i.v. bolus (1 μg/kg)</td>
<td>Fasting plasma glucose and insulin</td>
<td>Fasting or postprandial plasma glucose</td>
</tr>
</tbody>
</table>

Summary of the current literature of dAG action on glucose metabolism in humans. ↑, increase; ↓, decrease; ≫, no change; T2DM, type 2 diabetes mellitus; dAG, des-acyl ghrelin; H–E clamp, hyperinsulinemic–euglycemic clamp; FSIGT, frequently sampled i.v. glucose tolerance test; kg, i.v. glucose tolerance; AIRg, acute insulin response to glucose; FFA, free fatty acid; AUC, area under the curve.

Ghrelin regulation of glucose homeostasis during calorie restriction

Endogenous ghrelin levels rise during fasting or calorie restriction (CR) (4, 47, 48). Furthermore, exogenous ghrelin stimulates the secretion of all four counter-regulatory hormones (GH, cortisol, epinephrine, and glucagon), and therefore it has been implicated in maintaining blood glucose in states of negative energy balance. Ghrl<sup>−/−</sup> and Ghsl<sup>−/−</sup> mice placed on a 50% calorie-restricted diet have significantly lower blood glucose levels when compared with WT controls (73). Placing animals on a 60% calorie-restricted diet depletes fat mass stores, which then leads to severe hypoglycemia in both Ghrl<sup>−/−</sup> and Goat<sup>−/−</sup> mice (44, 74). Hypoglycemia in these animals is attributed to the lack of AG-induced GH secretion as AG or GH treatment rescues the hypoglycemic phenotype in calorie-restricted Goat<sup>−/−</sup> mice (44). However, the hypoglycemic and the relative GH-deficient phenotypes were not universally observed (75, 76). Adult-onset isolated GH-deficient (AOIGHD) mice placed on a 60% calorie-restricted diet lose significantly less fat mass, but maintain similar blood glucose levels as controls, (75) indicating that GH may regulate adiposity during CR, but is not essential to maintain glycemia. Furthermore, mice lacking Ghrl, Ghsr, or Goat expression maintain similar blood glucose levels as WT controls when placed on a 60% calorie-restricted diet even when fat mass stores were completely depleted (76). The reason for the discrepancies among these data is unclear, but differences in the age of the mice could be one of the contributing factors. Zhao et al. (44) and Li et al. (74) used young 8-week-old male mice that are still in the growing phase, whereas Yi et al. (76) and Gahete et al. (75) used mature (7–13 months) adult mice. In rodents, pancreatic ghrelin expression is highest before birth and slowly declines after birth (16), while gastric ghrelin gene expression increases rapidly after birth and then slowly declines with age (77). These data may suggest that ghrelin in the pancreas plays a more predominant role in regulating glucose metabolism at early stages of life. The role of ghrelin in glucose counter-regulation during acute or chronic CR needs to be more clearly defined.
CNS regulation of glucose metabolism

A large body of literature provides clear evidence that the CNS is involved in regulating peripheral glucose metabolism (78, 79). Islet cell function is influenced by the CNS through the autonomic nervous system (80). Both parasympathetic and sympathetic neurons synapse on β- and α-cells (81) to activate or inhibit insulin secretion (82). GHSR is found in parasympathetic preganglionic neurons (83) and the brainstem, where ghrelin activates pathways controlling sympathetic and parasympathetic nerve activity (84, 85, 86). These data raise the possibility that in addition to direct effects, AG may suppress insulin secretion indirectly via neural signaling. For example, AG inhibited GSIS when infused into the portal but not the femoral vein, and hepatic vagotomy or intraportal atropine diminished this inhibitory effect (41, 87). In humans, administration of AG increased plasma epinephrine (88) and decreased heart rate variability (89), suggesting that AG mediates a sympathetic response that could affect islet secretion. An intact vagus nerve is required for many of the physiological effects of AG in mice and humans (10, 89, 90), but no studies have directly tested the hypothesis that AG controls insulin via the autonomic nervous system. I.c.v. administration of AG to rodents has shown a stimulatory rather than an inhibitory action on plasma insulin levels. Chronic i.c.v. administration of AG to rats increases fasting insulin levels that are independent of the hyperphagia induced by AG (91). Similarly, chronic i.c.v. administration of AG increases GSIS in mice (9). However, the central mechanisms that mediate the stimulatory action of AG on GSIS have not been identified. Furthermore, it is unclear as to why AG has an inhibitory action on GSIS in the periphery, whereas it appears to be stimulatory when administered centrally.

Limited data are available on the central effects of dAG on glucose homeostasis. One study demonstrated that i.c.v. administration of dAG to mice increases GSIS through a GHSR-dependent mechanism (9). Whether this is a pharmacological effect or a physiological action of dAG requires further investigation.

Ghrelin interaction with glucagon-like peptide 1

The postprandial release of glucagon-like peptide 1 (GLP1) stimulates GSIS by enhancing cAMP signaling and increasing [Ca^{2+}]_{i} in pancreatic β-cells (92). AG inhibits insulin secretion by suppressing glucose stimulated [Ca^{2+}]_{i} in β-cells (11), and therefore, it was hypothesized that AG can counteract the stimulatory action of GLP1 on insulin secretion (93). Indeed, the effect of GLP1 on [Ca^{2+}]_{i} levels in isolated β-cells was attenuated by administration of AG (93). Isolated rat pancreatic islets treated with both GLP1 and a GHSR antagonist had increased insulin release when compared with treatment with GLP1 alone (93). The clinical relevance of these findings was tested in a study of Prader–Willi syndrome (PWS), a congenital disease that is associated with hyperphagia, T2DM, and elevated ghrelin levels. A patient with PWS treated with liraglutide, a GLP1 analog, for 12 months (94) lost 5.7 kg of weight, and had improved glycemic control and lower plasma ghrelin levels. The metabolic benefits observed with liraglutide treatment may be in part due to GLP1 suppression of ghrelin secretion, but the mechanisms involved require further clarification. However, a recent study using GLP1 infusion at a rate of 1 pmol/kg per min failed to observe any changes in ghrelin levels in either obese T2DM patients or healthy lean subjects (95). Conversely, administration of ghrelin has been reported to accelerate gastric emptying and increase GLP1 secretion following a test meal in healthy subjects (96). Collectively, these data indicate that GLP1 and ghrelin interact with each other at the level of the pancreas to regulate islet cell function. Further studies are needed to address the interaction of ghrelin and GLP1 in other tissues that regulate glucose metabolism such as the brain.

Metabolic benefits of bariatric surgery: is ghrelin a key player?

Bariatric surgery is the most effective way to reduce body weight and improve glucose metabolism in obese subjects (97). Studies comparing the effects of the two most common bariatric procedures, Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG), in obese T2DM patients demonstrate that body weight reduction and remission of T2DM are comparable in patients who undergo one vs the other operation (98). Furthermore, patients receiving either RYGB or VSG had similar improvements in oral glucose tolerance 6 months after surgery (99) as well as improved insulin sensitivity as measured by the homeostasis model assessment index (100). Studies in rodents and humans have demonstrated that both surgical procedures result in the development of improved glycemic control, which interestingly occurs before a significant reduction in body weight and adiposity suggesting that other factors besides weight loss are involved (101, 102, 103, 104). RYGB and VSG produce similar metabolic benefits through a very
different rearrangement of the gastrointestinal anatomy (reviewed in [105]). A common characteristic of the two types of surgeries is that they both prevent nutrients from contacting ghrelin-producing cells in the stomach. A number of studies indicate that AG has detrimental effects on glucose metabolism, and therefore, many groups aimed to determine whether circulating ghrelin levels are lower following bariatric surgery. Indeed, ghrelin levels are lower in subjects who underwent RYGB when compared with normal weight controls as well as subjects who experienced diet-induced weight loss [106]. However, these results have been inconsistent and some groups have found no changes in fasting or postprandial ghrelin levels after RYGB, whereas other groups found decreased fasting and postprandial ghrelin levels (reviewed in [107]). Ghrelin measurements following VSG have been more consistent, and most groups find reduced fasting and postprandial ghrelin levels in VSG-operated patients [108, 109, 110, 111]. Most studies find that VSG causes a 20–30% reduction in fasting ghrelin levels (108, 109, 110, 112), while some show ~60% reduction from presurgical levels 6 months following surgery (111). The relevance that these changes in circulating ghrelin levels have to the metabolic benefits of bariatric surgery has recently been challenged by a study in which DIO Ghr1−/− mice were subjected to VSG. Interestingly, VSG-operated Ghr1−/− mice achieved a similar improvement in oral glucose tolerance when compared with their VSG-operated WT controls [113]. In addition to improvements in glucose metabolism, a reduction in body weight and adiposity is also seen in the VSG Ghr1−/− animals indicating that ghrelin is not the critical hormone that determines the metabolic benefits of VSG [113]. However, these genetically modified animals may undergo compensation by other metabolic signaling systems during development, which is a factor that cannot be ignored using this gene deletion approach. Therefore, the relevance of postoperative ghrelin changes to the overall metabolic benefits of bariatric surgery remains to be elucidated.

![Figure 1](image)

**Figure 1**

AG regulation of islet cell function. Similar to the schematic depicted by Park et al. [33], high AG concentrations activate GHSR on α-cells to promote glucagon secretion and activate GHSR on β-cells to inhibit insulin secretion. GHSR signaling in β-cells may be regulated by heterodimerization with other GPCRs. These combined actions will lead to an overall increase in circulating glucose levels. High AG concentrations occur during CR, weight loss, in PWS, and after pharmacological administration of AG analogs. When AG levels are low, AG does not act to regulate blood glucose through modulation of islet cell function. Low AG concentrations occur in obese and insulin-resistant subjects, following pharmacological inhibition of GOAT, and following gastrectomy or gastric sleeve surgery. AG, acyl ghrelin; GOAT, ghrelin O-acyltransferase; CR, calorie restriction; GHSR, growth hormone secretagogue receptor; SST5, somatostatin receptor subtype 5; GPCR, G-protein-coupled receptor.
Summary

The gastrointestinal hormone ghrelin has received much attention for its ability to regulate glucose metabolism. The two major ghrelin isoforms found in circulation, AG and dAG, appear to have distinct actions. From in vitro studies to clinical studies in humans, the majority of reports demonstrate that AG has an inhibitory effect on GSIS and tissue glucose uptake when administered peripherally. Conversely, blocking AG synthesis improves glucose tolerance and enhances insulin secretion in rodents (42). Therefore, pharmaceutical agents that aim to antagonize AG or AG signaling could be potential therapeutics for treating T2DM. A summary of AG action in pancreatic islets is provided in Fig. 1. The effects of dAG on β-cell function and insulin action are less clear. Some studies suggest that dAG has no effect, whereas others indicate that dAG can stimulate insulin secretion and improve glucose tolerance, and still others show that dAG acts to antagonize AG action. Before dAG analogs can be used for therapeutic purposes, it is essential to clearly define the physiological function of dAG and to uncover possible dAG receptor-mediated actions. The rise and fall of AG and dAG correspond to the duration of fasting (47). It is likely that the ghrelin action on the pancreas and/or the CNS is linked to the metabolic states of the species in order to maintain glucose homeostasis.

Collectively, the major effects of ghrelin are linked as a protective mechanism against starvation: orexigenic actions to promote food intake, stimulation of GH secretion to promote lipolysis and restrict peripheral glucose uptake, and restraint of insulin secretion to prevent hypoglycemia. Much research is still needed to learn about the contribution of each of the components of the ghrelin system (dAG, AG, GOAT, and GHSR) to the regulation of these functions. Lastly, identifying the tissue-specific actions of each of these components through the use of advanced genetic technology and pharmacology will help to pinpoint the underlying mechanisms involved in ghrelin system’s regulation of glucose and energy homeostasis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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