

Role of ghrelin in glucose homeostasis and diabetes



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Practice Points

- Ghrelin, a hormone released from the stomach, has been shown to inhibit the release of insulin *in vivo* and *in vitro*.
- Obesity and diabetes are associated with decreased plasma ghrelin levels.
- Rodent models of disrupted ghrelin signaling have perturbed glucose homeostasis.
- Ghrelin appears to have a role in maintaining blood glucose levels in starvation.
- Ghrelin may play a paracrine role in the pancreas in the control of insulin release.
- Mutations in ghrelin have been implicated in reduced insulin sensitivity.
- Ghrelin may act to counter the consequences of diabetes induced by oxidative stress.
- Ghrelin may play an antiapoptotic and proliferative role in the pancreatic β -cell.

SUMMARY Ghrelin is an orexigenic hormone released from the stomach that stimulates the release of growth hormone and has been shown to inhibit the release of insulin. Evidence from rodent models of diabetes and perturbed ghrelin signaling suggest that ghrelin plays a role in regulating glucose homeostasis. Ghrelin may act as a paracrine signaling factor within the pancreas in the control of β -cell function. Ghrelin levels are reduced in obesity and diabetes, and insulin resistance has been linked to mutations in the ghrelin precursor molecule. Ghrelin appears to exert antioxidant and antiapoptotic effects upon endothelial and nervous tissue, and may help to protect against diabetes-related disease. The role of ghrelin in the development of diabetes and its potential utility as a treatment for some of the effects of diabetes remain unclear and require further study.

Diabetes mellitus

Diabetes mellitus is typified by an inability to either produce or utilize insulin, resulting in inefficient uptake of glucose into peripheral tissues. The World Health Statistics Report released in May 2012 suggests that approximately one in ten adults worldwide are diabetic [1]. Poor diabetes

management and long-term disease progression may precipitate microvascular and macrovascular complications, increasing morbidity and mortality rates. Determining the underlying factors that contribute to the development and progression of Type 1 and 2 diabetes is key to prevention and treatment. The complexity of the endocrine

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pathways regulating glucose homeostasis, and the cross talk between these pathways, suggest that investigating factors that regulate glucose homeostasis besides insulin may identify novel pharmacological targets. One hormone that has been shown to affect insulin release is ghrelin. Evidence suggests that ghrelin may be implicated in the pathogenesis of diabetes, and that the ghrelin system may be a potential target for novel pharmacotherapies for diabetes.

Ghrelin

Ghrelin is a 28-amino acid peptide hormone cleaved from the precursor molecule prepro-ghrelin [2]. It is produced by and released from the stomach in at least two forms: acylated ghrelin and des-acyl ghrelin [3]. Acylation is vital for ghrelin bioactivity and arises through the addition of an octanoyl group to the serine at the third N-terminal position in a reaction catalyzed by the enzyme GOAT [4]. Acylated ghrelin makes up approximately 20% of the total circulating ghrelin, with the remainder consisting of des-acyl ghrelin (also known as unacylated ghrelin) [5]. Ghrelin is most highly expressed in the stomach [6], which is the source of most circulating ghrelin [7], but lower levels can also be detected in the pancreas, intestine and hypothalamus [2,8,9]. Acylated ghrelin binds to and activates GHS-R with a half maximal effective concentration in the low nanomolar range [10]. Des-acyl ghrelin also binds to GHS-R, but with an efficacy several orders of magnitude lower than acylated ghrelin [11,12]. GHS-R is most highly expressed in the arcuate nucleus of the hypothalamus and in the anterior pituitary [13]. It has been suggested that des-acyl ghrelin can exert some biological effects through an unidentified receptor or via a nonreceptor mediated mechanism [14–16]. Investigations using GHS-R-deficient mice show that central administration of des-acyl, but not acylated ghrelin, induces feeding [17]. This is thought to be mediated through a hypothalamic feeding neuronal circuit distinct from the ghrelin signaling pathway, although other studies have suggested that des-acyl ghrelin lacks biological activity [18]. Other forms of ghrelin also exist [19], but are notably less well studied than acylated and des-acyl ghrelin.

Ghrelin has a number of physiological roles. Ghrelin stimulates growth hormone (GH) release from the pituitary [2], increases food intake and decreases energy expenditure [20–22], has prokinetic effects in the gastrointestinal tract

and is thought to play an anti-inflammatory role [23,24]. In addition, there is convincing evidence that ghrelin plays a role in insulin release and glucose homeostasis. There remains much debate on the effects of ghrelin on insulin signaling, and on its role in diabetes, and these topics are, thus, the focus of this review.

Ghrelin in the pancreas

Ghrelin is expressed in the pancreas [6,25]. The existence of a novel endocrine pancreas cell type, the epsilon cell, which produces and secretes ghrelin, was first suggested a decade ago [26]. Ghrelin is more highly expressed in the developing than in the adult pancreas [27]. Epsilon cells constitute 30% of developing islets at week 23 of gestation, but this decreases to just 1% of pancreatic cell types in the adult pancreas, suggesting that ghrelin may play a role in islet β -cell differentiation and proliferation [28]. Thus, pancreatic ghrelin may act in an autocrine or paracrine fashion to regulate pancreatic function and suppress insulin release. Interestingly, ghrelin attenuates the stimulatory effects of glucose-stimulated insulin secretion that the gut hormone GLP-1 promotes in single β -cell and islet cultures [29]. It may be that ghrelin acts on other hormonal signaling pathways to control glucose homeostasis in the pancreas.

Ghrelin inhibits insulin release

The ghrelin receptor, GHS-R, is expressed in the pancreas [30], where it appears to be present in pancreatic β -cells, and weakly expressed in β -cells [25]. This suggests a role for ghrelin in pancreatic function. Early *in vitro* investigations in isolated rat islet cultures demonstrated that ghrelin at a concentration of 10 nM inhibits, and GHS-R antagonists promote, glucose-induced insulin release [31]. It was also shown in single β -cells that the administration of ghrelin prevented Ca^{2+} induced insulin release. Ghrelin also inhibited glucose-stimulated insulin release from *ex vivo* perfused rat pancreas, and this effect was reversed upon treatment with GHS-R antagonists [32]. Isolated islets from ghrelin null mice had greater glucose-induced insulin release compared with wild-type littermates, but not an increased islet insulin content. Peripheral ghrelin administration to animals and humans has also been shown to reduce circulating insulin levels. Both 10 nmol/kg intraperitoneal and 50 nmol/kg intravenous doses of ghrelin administered to mice decreased circulating insulin

levels, increased fasting glucose and decreased glucose tolerance, suggesting an inhibitory effect on insulin secretion [31,33,34]. In humans, intravenous infusion of 1 µg/kg ghrelin in healthy subjects worsens glucose tolerance by decreasing plasma insulin levels [33,35]. It has been suggested that the effects of ghrelin on glucose homeostasis may be mediated via effects on GH. Certainly, GH replacement therapy administered to GH-deficient humans induces insulin resistance [36], although ghrelin levels remain unaltered in patients both before and after treatment with

GH, suggesting there is no feedback response between manipulating GH and ghrelin signaling [37,38]. To date, the effects of ghrelin administration in diabetics has not been investigated. For an overview of the effects of ghrelin on insulin release *in vitro* and *in vivo* see **Tables 1 & 2**, respectively [9,12,27,29,31–35,39–49].

Physiological role of ghrelin in glucose homeostasis

Ghrelin has been established as a negative regulator of insulin release. In accordance with

Table 1. *In vitro* studies investigating the effects of ghrelin on insulin release.

Study (year)	Species	<i>In vitro</i> system	Ghrelin concentration	Glucose concentration (mmol/l)	Change in insulin concentration	Ref.
Date <i>et al.</i> (2002)	Rat	Isolated islets	1 pmol/l	8.3	~100% increase	[107]
			1 pmol/l	2.8	No change	
Egido <i>et al.</i> (2002)	Rat	Pancreas perfusion	10 nmol/l	5.5	No change	[39]
			10 nmol/l	9	~50% decrease	
Reimer <i>et al.</i> (2003)	Mouse	Isolated islets	10 nmol/l	8.3	~40% decrease	[34]
			10 nmol/l	11.1	~30% decrease	
			10 nmol/l	16.7	~30% decrease	
Dezaki <i>et al.</i> (2004)	Rat	Isolated islets	10 nmol/l	2.8	No change	[31]
			10 nmol/l	8.3	~30% decrease	
			1 pmol/l	2.8	No change	
			1 pmol/l	8.3	No change	
Wierup <i>et al.</i> (2004)	Rat	INS-1 β-cell	100 nmol/l	3	No change	[27]
			100 nmol/l	15	~40% decrease	
Dezaki <i>et al.</i> (2006)	Rat	Pancreas perfusion	10 nmol/l	8.3	~50% decrease	[32]
			10 nmol/l (des-acyl ghrelin)	8.3	No change	
Doi <i>et al.</i> (2006)	Mouse	MIN6 cell	0.1 nmol/l	22.2	~30% decrease	[40]
			1 nmol/l	22.2	~40% decrease	
			10 nmol/l	22.2	~40% decrease	
Gauna <i>et al.</i> (2006)	Rat	INS-1 β-cell	10 nmol/l (rat ghrelin)	20	~300% increase	[41]
			10 nmol/l (human ghrelin)	20	~400% increase	
Granata <i>et al.</i> (2007)	Hamster	HIT-T15	100 nmol/l	1.25	No change	[42]
			100 nmol/l (des-acyl ghrelin)	1.25	~50% increase	
			100 nmol/l	7.5	~30% increase	
			100 nmol/l (des-acyl ghrelin)	7.5	~30% increase	
			100 nmol/l	15	~40% increase	
			100 nmol/l (des-acyl ghrelin)	15	~20% increase	
Qader <i>et al.</i> (2008)	Mouse	Isolated islets	1 pmol/l	12	~50% decrease	[43]
			0.1 nmol/l	12	~30% decrease	
			10 nmol/l	12	~30% increase	
			1 µmol/l	12	~100% increase	
	Rat	Isolated islets	1 pmol/l	8.3	No change	
			10 nmol/l	8.3	~30% decrease	
			1 µmol/l	8.3	~40% decrease	
Wang <i>et al.</i> (2010)	Mouse	MIN6 cell	10 nmol/l	3.3	No change	[44]
			10 nmol/l	22.2	~25% decrease	
Damdindorj <i>et al.</i> (2012)	Rat	Isolated islets	10 nmol/l	8.3 mmol/l + 10 nmol GLP-1	~45% decrease	[29]

Table 2. *In vivo* studies investigating the effects of ghrelin on insulin release.

Study (year)	Species	Conditions	Ghrelin concentration and route of entry	Glucose concentration and route of entry	Change in insulin concentration	Ref.
Broglio <i>et al.</i> (2001)	Human	Overnight fasted, healthy individuals. Bolus injected, measured over 180 min	1 µg/kg iv.	–	~50% decrease	[33]
Reimer <i>et al.</i> (2003)	Mouse	3 h fasted. Bolus injected over 3 s	5 nmol/kg iv. tail vein 50 nmol/kg iv. tail vein 150 nmol/kg iv. tail vein	1 g/kg iv. tail vein 1 g/kg iv. tail vein 1 g/kg iv. tail vein	No change ~40% decrease ~50% decrease	[34]
Akamizu <i>et al.</i> (2004)	Human	Overnight fasted, healthy individuals. Bolus injected, measured over 90 min	1 µg/kg iv. 5 µg/kg iv.	– –	No change ~70% decrease	[45]
Broglio <i>et al.</i> (2004)	Human	Overnight fasted, healthy individuals. Bolus injected, measured over 120 min	1 µg/kg iv. 1 µg/kg iv. (des-acyl ghrelin) 1 µg/kg iv. (ghrelin and des-acyl ghrelin)	– – –	~50% decrease No change No change	[46]
Dezaki <i>et al.</i> (2004)	Mouse	Overnight fasted. Bolus injected, measured over 60 min	1 nmol/kg ip. 10 nmol/kg ip.	1 g/kg ip. 1 g/kg ip.	~50% decrease ~60% decrease	[31]
Gauna <i>et al.</i> (2004)	Human	Overnight fasted, GH-deficient individuals. Bolus injected, measured over 2 h	1 µg/kg iv. 1 µg/kg iv. (des-acyl ghrelin) 1 µg/kg iv. (ghrelin and des-acyl ghrelin)	– – –	No change No change ~50% decrease	[47]
Lucidi <i>et al.</i> (2005)	Human	Overnight fasted, healthy individuals. 120-min infusion	7.5 pmol/kg/min 15 pmol/kg/min	– –	No change No change	[48]
Gauna <i>et al.</i> (2007)	Rat	Overnight fasted. Bolus injected, measured over 50 min	30 nmol/kg iv. jugular vein 30 nmol/kg (des-acyl ghrelin) iv. jugular vein	1 g/kg iv. jugular vein 1 g/kg iv. jugular vein	~20% decrease ~40% increase	[12]
Cui <i>et al.</i> (2008)	Rat	24 h fasted. 10–40-min infusion	1 ng/kg at 1 ml/h iv. femoral vein 1 ng/kg at 1 ml/h iv. portal vein	13.3 mg/kg/min iv. femoral vein 13.3 mg/kg/min iv. femoral vein	No change ~60% decrease	[49]
Tong <i>et al.</i> (2010)	Human	Overnight fasted, healthy individuals. 65-min infusion	0.3 nmol/kg/h iv. 0.9 nmol/kg/h iv. 1.5 nmol/kg/h iv.	11.4 g/m ² body surface area bolus at 55 min iv.	~30% decrease ~35% decrease ~40% decrease	[35]

GH: Growth hormone; ip.: Intraperitoneal; iv.: Intravenous.

this, when blood glucose is high after a meal, and increased insulin release is required, ghrelin levels are decreased [33]. Ghrelin is low in obese individuals, who have higher insulin levels, although it seems likely that the increased insulin is a response to relative insulin insensitivity, rather than an effect of the low ghrelin levels [20,50]. It has been well characterized that ghrelin levels are altered in metabolic disease. However, care must be taken when interpreting the effects of ghrelin on insulin levels in metabolic disease, as changes in insulin may be secondary to changes in body weight, rather than a direct effect of ghrelin signaling. It is,

therefore, currently unclear whether the alterations in insulin levels observed in the obese are due, even in part, to alterations in ghrelin signaling. A recent study in obese individuals with Type 2 diabetes who underwent laparoscopic banding found that their circulating levels of ghrelin increased and insulin decreased after surgery [51]. However, even with these changes, their Type 2 diabetes improved or resolved, likely secondary to their weight loss, and the low insulin levels may well reflect their increased insulin sensitivity rather than a specific effect of the altered ghrelin levels. However, it must be noted that the assays used to measure ghrelin

and sample collection methods vary between papers. Many papers report total ghrelin levels, which refer to the levels of acylated plus des-acyl ghrelin. Commercially available radioimmunoassay kits in which the epitope recognized by the antibody is common to both acylated and des-acyl ghrelin were widely used. Active ghrelin levels constitute acylated ghrelin levels, and are considered more relevant to actual ghrelin activity given the low efficacy of des-acyl ghrelin at GHS-R [12]. Sandwich assays detecting specific epitopes specific for either acylated or des-acyl ghrelin are now more commonly used, allow levels of total and active ghrelin to be specifically reported and are generally more sensitive than traditional competitive radioimmunoassays [52,53]. In addition, circulating levels of ghrelin reported in the literature vary greatly, even when the same form is ostensibly being measured. One study reported a tenfold difference in the total ghrelin levels measured by different assays [54]. Additionally, levels of acyl ghrelin can rapidly alter if blood collection samples are not treated appropriately. Rapid acidification of blood samples has been suggested to be required to stabilize acylated ghrelin, preventing cleavage of the acyl group from the ghrelin peptide by endogenous esterases [55], and specific esterase inhibitors can also be useful [52,56]. Cleavage of the acyl group renders ghrelin inactive, and means that the molecule will be detected as a component of total ghrelin measurements, but not of active ghrelin [52]. However, recent evidence suggests that acidification may not aid plasma ghrelin measurement, and may in fact contribute to the decline of active ghrelin levels in samples [57]. Thus, differences between forms of ghrelin measured and the levels detected with different assays should be considered when interpreting the ghrelin literature.

The stimulatory effects of ghrelin on feeding suggest that the reduction of ghrelin in obesity may represent a feedback loop by which the body is attempting to reduce food intake, rather than modify glucose homeostasis. However, there is evidence that ghrelin is regulated by physiological changes in glucose homeostasis. Recent work suggests that low glucose levels can stimulate, and high glucose levels inhibit, ghrelin release from gastric mucosal cells [58], and it seems possible that ghrelin-releasing cells in other tissues may also be glucose sensitive. However, others have suggested that it is hyperinsulinemia rather than the resulting

low glucose levels that suppress plasma ghrelin, and have found that ghrelin is still suppressed if euglycemia is maintained in the presence of high insulin levels [59]. Other forms of ghrelin may also exert differential effects on glucose homeostasis. The form *N*-decanoyl ghrelin is an alternative form of circulating ghrelin that has a decanoic acid group attached to the serine at position 3, rather than the more common octanoic acid modification. Circulating levels of *N*-decanoyl ghrelin decrease after administration of a 75-g glucose load, but not after ingestion of a 296-kcal meal, whereas *N*-octanoyl ghrelin and total ghrelin both decreased after glucose load and a meal [60]. Given this differential regulation by glucose, it is possible that different forms of ghrelin may play different roles in aspects of glucose homeostasis.

The lack of a profound body weight phenotype of ghrelin-deficient mice originally suggested ghrelin was not critical in the control of energy homeostasis [61]. However, ghrelin-deficient and GHS-R-deficient mice do appear resistant to the development of high-fat diet-induced obesity [62,63], and the same GHS-R-deficient mice were found to have improved insulin sensitivity when fed a high-fat diet compared with wild-type littermates [64]. These same models of perturbed ghrelin signaling also exhibit a differential thermogenic phenotype [65] and genetic ablation of the GHS-R improved insulin sensitivity in older mice, suggesting the GHS-R may have a role in age-related impairment of insulin sensitivity [66].

While the loss of ghrelin signaling may protect against the development of diabetes in these mice, these effects may well be secondary to the lower body weight the ghrelin-deficient mice exhibit on a high-fat diet, rather than resulting from the direct effect of ghrelin on glucose homeostasis. There is evidence that ghrelin can alter insulin sensitivity in addition to regulating insulin release. Another study found an association in humans between the ratio of acylated to unacylated ghrelin and insulin sensitivity [67], and administration of ghrelin has also been suggested to reduce insulin sensitivity in humans [47]. It is also possible that the true physiological role of ghrelin may be masked by the activation of compensatory pathways in these ghrelin signaling-deficient models. Perhaps the most convincing evidence for a specific physiological role for ghrelin in energy homeostasis comes from a recent study in starved mice. Ghrelin is

a potent stimulator of GH release, and it is well known that GH also inhibits insulin release. A recent study demonstrated that active ghrelin-deficient *Goat^{-/-}* mice subjected to a 40% caloric restriction regime develop profound and often fatal hypoglycemia after fasting [68]. Infusion of ghrelin or GH prevented hypoglycemia and death in *Goat^{-/-}* mice [69]. In times of decreased body fat, such as after lengthy calorie restriction, when ghrelin is high, ghrelin-stimulated GH release may be vital in maintaining sufficient glucose levels for life. Thus, it may be that a major physiological role of ghrelin is to maintain blood glucose during starvation, and that this is mediated through the release of GH. However, a subsequent study found that four separate murine models of perturbed ghrelin synthesis and signaling demonstrated no increase in the incidence of hypoglycemia and no significant changes in insulin levels in starvation, and, thus, the physiological role of ghrelin in glucose homeostasis under these conditions remains a point of contention [70]. It is currently unknown whether ghrelin-stimulated GH release is more physiologically important in glucose homeostasis than the direct effects of ghrelin on insulin release. However, the presence of ghrelin-releasing cells in the pancreas suggests that ghrelin may play a paracrine or autocrine role within this organ.

Ghrelin in the development of diabetes

The role of ghrelin in energy homeostasis, and the fact that the obese are more likely to develop Type 2 diabetes, can make it difficult to determine whether associations between ghrelin and altered glucose homeostasis represent a specific role for ghrelin or are secondary to alterations in body weight. Mutations in ghrelin and *GHS-R* have been associated with the development of obesity and Type 2 diabetes [71,72]. Leu72Met is one of the most common polymorphisms in preproghrelin, and has been linked to the development of obesity and Type 2 diabetes [73,74]. Conversely, it has been shown in the Chinese population that there is no association of Leu72Met with the development of Type 2 diabetes [75]. However, using the Homeostasis Model of Assessment – Insulin Resistance (HOMA-IR) test in this population, Leu72Met was associated with insulin resistance. Leu72Met may, thus, contribute to the decline of glucose tolerance, but may not represent a significant risk factor in diabetogenesis.

Prader–Willi syndrome is a genetic disease associated with high circulating levels of ghrelin, hyperphagia, obesity and Type 2 diabetes. It is possible that the elevated levels of ghrelin may contribute to the development of Type 2 diabetes in Prader–Willi sufferers due to both its obesogenic properties and effects on insulin release. Inhibiting ghrelin release using somatostatin analogs does not, however, reduce hyperphagia in these patients, suggesting it is not caused by the high levels of ghrelin alone [76]. The anti-diabetic drug liraglutide, which acts as a GLP-1 analog, has been shown to suppress ghrelin and improve HOMA-IR in a Prader–Willi sufferer; although the improvement in glucose homeostasis may be due to the direct effects of liraglutide, which acts as an incretin [77]. As ghrelin was only measured before and after the 12-month liraglutide treatment, it is not clear whether the decrease in body weight from 2 months post-treatment was as a result of, or preceded, the decrease in ghrelin levels.

Ghrelin as an antioxidant in diabetes-related disease

In addition to putative roles in glucose homeostasis, ghrelin may also be able to modulate or protect against the downstream effects of disrupted glucose homeostasis. The creation of an unfavorable cellular oxidative environment is thought to play a role in the development of diabetic-related complications, including neuropathies and retinopathies. Hyperglycemia directly causes increased production of reactive oxygen species (ROS) through excessive cellular glucose oxidation. These ROS oxidize DNA and activate cellular stress signaling pathways, causing damage and leading eventually to cellular destruction [78]. Ghrelin is widely regarded as possessing antioxidant properties and, thus, may play a role in preventing β -cell decline and the development of diabetes-related complications [79–81]. Obese subjects with Type 2 diabetes have increased plasma levels of lipid peroxidation, a marker of oxidative stress, in addition to the expected hyperleptinemia and hyperinsulinemia. It has been postulated that decreased plasma ghrelin in obese diabetic individuals is secondary to these increased levels of insulin, leptin and lipid peroxidation [82]. Insulin and leptin both inhibit ghrelin, and, thus, ghrelin levels may alter in response to changes in these factors, rather than being a driver towards the development of diabetes.

Oxidative stress may also cause neuropathic damage. Ghrelin treatment increases levels of the antioxidants catalase and superoxide dismutase in the small intestine and protects against peripheral sensory nerve damage in streptozotocin-induced diabetic rats [83].

The antioxidant effects of ghrelin may be mediated, at least in part, by UCP2. UCP2 is a mitochondrial membrane protein thought to uncouple oxidative phosphorylation from ATP synthesis through proton transfer, releasing thermal energy [84]. UCP2 can also act as a free radical scavenger, counteracting ROS to prevent tissue damage. Ghrelin has been suggested to support the sustained activity of hypothalamic neuropeptide Y/agouti-related protein neurons by protecting them from the ROS produced by β -oxidation by upregulating UCP2 activity [85]. Ghrelin also signals through an UCP2-mediated pathway to activate mitochondrial respiration to preserve nigrostriatal dopaminergic neuronal viability and function [86]. Ghrelin also appears to promote UCP2 expression in the pancreas to modulate insulin release. Ghrelin-mediated upregulation of UCP2 inhibits insulin release through an AMPK-dependent pathway [44], and ablation of ghrelin reduces pancreatic UCP2 expression and increases glucose-stimulated insulin release [87]. UCP2, therefore, appears to play a role in mediating the effects of ghrelin on neuronal and endocrine cell function.

Diabetic gastroparesis is a gut motility disorder typified by delayed gastric emptying, and may present with nausea, vomiting, bloating, heartburn and early satiety [88]. The risk of developing gastroparesis is 30-fold higher in Type 1 diabetics and eightfold higher in Type 2 diabetics than in healthy controls [89]. It is widely believed that autonomic neuropathic damage caused by oxidative stress through hyperglycemia is responsible for diabetic gastroparesis [90,91]. Gut motility is mediated partly through neural signaling from the vagus nerve [92] and ghrelin has been shown to signal to vagal nerve efferents and afferents [93]. One human study reported that plasma ghrelin in diabetics with gastroparesis was not suppressed by oral glucose loading to the same degree as in nongastroparetic diabetic individuals [94]. In addition, administering exogenous ghrelin to patients with diabetic gastroparesis can enhance their gastric emptying [95]. Dysfunction of the vagal afferent signaling usually mediated through the release of ghrelin may, thus, be

responsible for the development of gastroparesis in chronic diabetes.

Endothelial dysfunction is a major complicating factor in diabetes, more commonly seen in chronic disease [96]. A loss of endothelial viability may lead to cardiovascular disease, nephropathy, retinopathy and neuropathy. Ghrelin appears to exert a significant antioxidant effect on vascular endothelial and nervous tissue, protecting against the effects of ROS [97,98]. Vascular endothelial decline is responsible for the development of most diabetes-related disease and, thus, it may be possible that ghrelin agonists have potential in the treatment of complications such as proliferative diabetic retinopathy.

Proliferative diabetic retinopathy may cause blindness in diabetes. It is associated with elevated levels of ROS, which are thought to contribute to microvascular damage in primary retinopathy, causing the loss of viable retinal blood vessels and creating hypoxic conditions favorable for the neovascular changes observed in secondary retinopathy [99]. Interestingly, it has recently been shown that intravitreal delivery of ghrelin analogs Dap3-ghrelin and GHRP6 significantly reduced retinal degeneration in a rat model of oxygen-induced retinopathy [100]. However, the pathological angiogenesis usually observed in secondary retinopathy was increased with ghrelin administration in this model. The evidence that ghrelin may cause pathological angiogenesis may contraindicate use as a therapy for now, but further investigation into the effects of targeting the ghrelin system in diabetic disease is required.

Apoptosis & β -cell proliferation

β -cell apoptosis is commonly observed in diabetic pancreatic tissue and represents an important step in the decline of pancreatic function. It has recently been suggested that des-acyl ghrelin fragments promote the survival of human β -cells and islets under conditions of both serum starvation and cytokine-induced cell death [101]. Increased cytokines are thought to precipitate β -cell destruction in Type 1 diabetes. When des-acyl ghrelin was administered to streptozotocin rats, blood glucose levels decreased and animal survival rates were increased to approximately 80% after just 9 days of treatment. Thus, des-acyl ghrelin may be exerting a protective effect against hyperglycemic damage in pancreatic β -cells. This is

supported by previous *in vitro* investigations from the same group, which showed that both acylated and des-acyl ghrelin stimulated proliferation in HT-T15 β -cells despite serum starvation and treatment with cytokines [42]. The leptin-deficient *ob/ob* murine model of obesity has a diabetic phenotype. It has recently been shown that in *Ghsr^{-/-}:ob/ob* mice there is profound hyperglycemia, reduced insulin release and reduced glucose tolerance compared with ghrelin deficient *ob/ob* mice, and that these differences are not secondary to changes in body weight [102]. Ablation of the GHS-R in these mice also caused an upregulation of unfavorable β -cell regulators. The ablation of the GHS-R would be expected to reduce food intake and increase energy expenditure, thereby reducing further progression of insulin resistance in these diabetic mice. However, this model suggests that the impairment of insulin secretion observed in the absence of ghrelin signaling may at least be partly due to compromised β -cell function. This suggests that ghrelin is antidiabetic in nature and protective against the decline in β -cell function. It also highlights that GHS-R antagonists may be ineffective in treating, or may even worsen, the progression of diabetes. These findings are further complicated by another study that found that an *ob/ob* mouse also deficient in ghrelin (rather than GHS-R) had increased insulin sensitivity and attenuated hyperglycemia compared with *ob/ob* mice that express ghrelin [87]. In comparison, the findings in the *Ghsr^{-/-}:ob/ob* mouse suggest that there may be nonghrelin-mediated effects of the GHS-R on glucose handling [102]. These very different phenotypes suggests ghrelin and GHS-R signaling may have subtle and complex effects on glucose homeostasis, the resolution of insulin sensitivity and perhaps β -cell survival.

Conclusion & future perspective

This review has highlighted the complexity in the interactions between ghrelin and insulin in the control of glucose tolerance and insulin sensitivity. It has yet to be elucidated whether the observed changes in ghrelin are causative or secondary to the development of diabetes. The mechanism or mechanisms by which ghrelin regulates insulin dynamics remains unknown, but the conflicting evidence on the resulting changes in insulin release as a result of ghrelin administration, as documented in the literature, suggests a degree of complexity. Ghrelin may

directly influence insulin signaling by interfering with islet function, preventing insulin synthesis and release, perhaps contributing to the development of diabetes [103]. It may be that ghrelin indirectly affects insulin release by stimulating the release of GH, or by inhibiting the actions of downstream signals such as GLP-1 [29]. It is currently unclear whether the characteristically low levels of ghrelin in obese individuals precede and contribute to insulin resistance, or whether the decrease in ghrelin is a consequence of insulin resistance [20,50]. There have been conflicting reports regarding whether current pharmacological diabetes treatments modulate ghrelin levels, suggesting that antidiabetic drugs may partly exert their effects through ghrelin signaling, but the data remain inconclusive [104–106]. Most evidence suggests that blocking ghrelin signaling may be beneficial in the treatment of diabetes, by preventing ghrelin-induced inhibition of insulin release and possibly by preventing the putative negative effects that ghrelin exerts on insulin sensitivity. However, in terms of using ghrelin as a therapy to treat diabetes, further basic research on the relationship between insulin and ghrelin signaling is required. This relationship is clearly complex, and probably dependent on disease type and progression, severity of insulin resistance, the existence of comorbid disease and genetic background. Any antidiabetic effects of ghrelin may possibly be outweighed by its pathological angiogenic properties in, for example, secondary retinopathy [100]. GHS-R agonists and antagonists may both be effective if administered at an appropriate time point in disease progression, such as in early prediabetics and late adult diabetic neuropathies, respectively. The pleiotropic nature of ghrelin complicates pharmacological targeting of this signaling pathway, but it seems possible that the ghrelin system represents a viable therapeutic target for the prevention or treatment of diabetes.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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