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EDITORIAL

Ghrelin's second life: From appetite stimulator to glucose regulator

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Abstract

Ghrelin, a 28 amino acid peptide hormone produced by the stomach, was the first orexigenic hormone to be discovered from the periphery. The octanoyl modification at Ser^3, mediated by ghrelin O-acyltransferase (GOAT), is essential for ghrelin's biological activity. Ghrelin stimulates food intake through binding to its receptor (GRLN-R) on neurons in the arcuate nucleus of the hypothalamus. Ghrelin is widely expressed throughout the body; accordingly, it is implicated in several other physiological functions, which include growth hormone release, gastric emptying, and body weight regulation. Ghrelin and GRLN-R expression are also found in the pancreas, suggesting a local physiological role. Accordingly, several recent studies now point towards an important role for ghrelin and its receptor in the regulation of blood glucose homeostasis, which is the main focus of this review. Several mechanisms of this regulation by ghrelin have been proposed, and one possibility is through the regulation of insulin secretion. Despite some controversy, most studies suggest that ghrelin exerts an inhibitory effect on insulin secretion, resulting in increased circulating glucose levels. Ghrelin may thus be a diabetogenic factor. Obesity-related type 2 diabetes has become an increasingly important health problem, almost reaching epidemic proportions in the world; therefore, antagonists of the ghrelin-GOAT signaling pathway, which will tackle both energy- and glucose homeostasis, may be considered as promising new therapies for this disease.

Key words: Ghrelin; Blood glucose; Pancreas; Diabetes; Insulin

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INTRODUCTION

Ghrelin, a 28 amino acid peptide hormone predominantly produced by the stomach[1], is the endogenous ligand of the growth hormone secretagogue receptor 1a isoform (GHS-R1a), presently renamed ghrelin receptor (GRLN-R)[2]. Soon after the identification of ghrelin[3], it became clear that ghrelin's effect extends beyond the stimulation of growth hormone (GH) secretion. Ghrelin's involvement in appetite stimulation was discovered as a side-effect in a study investigating the effect of ghrelin injection on GH-release in healthy humans[4]. Ghrelin was soon identified to be the only known orexigenic hormone from the periphery, which stimulates food intake in a dose-dependent manner in rodents[5-8] and humans[9,10]. Many papers have been dedicated to ghrelin's orexigenic

effect, which has long been considered its main physiological function. More recent research now supports the idea that ghrelin may play an equally or even more important role in the regulation of blood glucose homeostasis. This review will give a summary of research data pointing out the importance of ghrelin in the regulation of blood glucose homeostasis. We will start with a general introduction about ghrelin and its expression throughout the body, including the pancreas. The relation with insulin will be discussed, followed by an overview of possible therapeutic implications resulting from these findings. The main goal of this review is not to provide an integral overview of previous research, but to indicate the importance of ghrelin in the regulation of blood glucose and implications for the treatment of disorders like diabetes.

GHRELIN AND THE GHRELIN RECEPTOR

Ghrelin is mainly produced by the X/A like cells, a distinct endocrine cell type found in the mucosal layer of the stomach and, to a lesser extent, in the small and large intestines[1]. Smaller amounts are also detected in the pancreas, hypothalamus, heart, kidneys, lungs, testes, liver, and thyroid[11,12].

Active ghrelin contains a unique posttranslational modification, an n-octanoyl group on the third serine residue, performed by ghrelin O-acyltransferase (GOAT), the 4th and highly conserved member of the membrane-bound O-acyltransferases superfamily (MBOAT4)[13,14]. GOAT is localized to the gastric ghrelin cells, suggesting that ghrelin becomes octanoylated at its production site[15]. GOAT transcripts predominantly occur in the stomach and pancreas in humans, while in mice they are found in the stomach, small intestine, colon, and, to a lesser extent, in the testis[13,14]. According to the latest reports, acyl ghrelin makes up only 25% of the total amount of circulating ghrelin[16]. The amount of circulating acylated ghrelin does not always parallel total ghrelin concentrations, suggesting that ghrelin acylation and secretion are regulated separately[17,18]. In addition to n-octanoyl ghrelin, other acyl groups can be transferred to ghrelin, giving rise to other forms, such as decanoylated ghrelin, the second most abundant form of acylated ghrelin in circulation. The acyl groups added to ghrelin reflect the fatty acid content of the diet[18], as enrichment of the diet with heptanoic acid causes ghrelin to be preferentially acylated with heptanoate, rather than with octanoic acid[19]. The non-modified, unacylated form of ghrelin, which is the most abundant form of circulating ghrelin, does not bind to the ghrelin receptor and was first believed to be biologically inactive. Several studies have since established a physiological role for unacylated ghrelin[20-22], probably through binding to a distinct, but unidentified, receptor[22,23].

Alternative splicing of the *GRLN-R* gene results in the formation of two receptor forms, the full length GRLN-R1a, which is believed to be the biologically active form, and the truncated GRLN-R1b[24,25]. GRLN-R1a is activated by octanoyl ghrelin and synthetic growth hormone secretagogues while the GRLN-R1b is not activated by ghrelin and its physiological function remains unknown, despite its wide expression. GRLN-R1a is also widely expressed throughout the body. The highest expression levels of GRLN-R1a have been found in the hypothalamus[26], where GRLN-R1a is expressed on growth hormone releasing hormone-expressing neurons[27,28] and NPY/AgRP neurons in the arcuate nucleus[29], consistent with its role in growth hormone release and appetite stimulation. Other expression sites of GRLN-R1a mRNA include the pituitary, thyroid, pancreas, spleen, myocardium, the adrenal gland, and the intestinal myenteric plexus[11,30]. GRLN-R immunoreactivity was also observed within neuronal cell bodies and fibers in the human stomach and colon[31].

The variety of ghrelin and GRLN-R expression sites already suggests a broad range of different physiological functions. In addition to its stimulatory effects on food intake in humans[9,10] and rodents[6,32,33], ghrelin also stimulates body weight gain, not only by promoting food intake, but also through an increased adipogenesis[8,34]. Ghrelin also has prokinetic properties in the gastrointestinal tract. Peripheral ghrelin administration accelerates gastric emptying in conscious rats[35,36] and mice[37,38]. Ghrelin infusion stimulates gastric emptying in healthy volunteers[39] and in patients with idiopathic[40], diabetic[41] and neurogenic[42] gastroparesis, and induces hunger contractions in the fasting state[43]. In addition, ghrelin has many other physiological functions, including cardiovascular effects[44] and stimulatory effects on learning and memory[45,46]. Recently, more and more attention has been given to the regulatory effects of ghrelin on glucose homeostasis[47,48].

To unravel the physiological functions of ghrelin signaling, different transgenic and knockout mice models have been developed. Mice with a genetic deletion of ghrelin[49-51], the ghrelin receptor[52,53], or GOAT[19] display only subtle phenotypic changes on a standard laboratory diet and a lower body weight compared to wild-type mice on a high fat diet, probably due to less accumulation of body fat[19,53,54]. Ablation of ghrelin in ob/ob mice markedly improved glucose tolerance by increasing serum insulin levels, but did not result in a reduced body weight[55]. Another study reported that ghrelin or ghrelin receptor ablation did not prevent diet-induced obesity in adult mice, but increased insulin sensitivity[56]. When these mice were subjected to 50% caloric restriction, ghrelin and GRLN-R knockout mice had lower blood glucose levels than their wild-type littermates, as also observed in a recent study using GOAT knockout mice[57], suggesting that ghrelin is involved in the counterregulatory glucose response during negative energy balance. While several compensatory mechanisms on food intake and body weight may have evolved in these knockout mice, these studies support the hypothesis that ghrelin and the GRLN-R may be non-essential regulators of appetite. It seems that ghrelin and its receptor have an important role in the regulation of blood glucose ho-

meostasis, which may represent a more important physiological function than the earlier reported effects on food intake regulation.

EXPRESSION OF GHRELIN AND THE GHRELIN RECEPTOR IN THE PANCREAS

The endocrine cells of the pancreas are assembled in dispersed islets containing traditionally four different types of cells, the insulin releasing β-cells, which make up the major cell population within an islet, the glucagon producing α-cells, the somatostatin producing δ-cells, and the pancreatic polypeptide containing PP cells. Moreover, the pancreas is an important source of ghrelin. Release of ghrelin by the pancreas was assessed by comparing the ghrelin concentration in the pancreatic vein with that in the pancreatic artery in rats. Acylated and unacylated ghrelin levels were found to be significantly higher in the pancreatic vein, indicating that the pancreas not only produces ghrelin[58], but also expresses GOAT, the enzyme responsible for its acylation[14].

The exact location and cellular origin of pancreatic ghrelin has been a matter of debate, and conflicting results have appeared. The first study to report that the pancreas is an important production site for ghrelin[59] also stated that ghrelin production is restricted to the insulin producing β-cells of the human pancreas. In contrast, Date *et al*[47] reported that ghrelin is exclusively expressed in the glucagon producing α-cells in rats and humans, supported by the overlapping immunohistological stainings for ghrelin and glucagon observed in another study[58]. The existence of a fifth separate islet cell population in the human pancreas, producing ghrelin and devoid of any other islet hormones, was postulated for the first time by Wierup *et al*[60] and is no longer debated. These pancreatic cells were found to be quite numerous (up to 10% of the endocrine cells) from midgestation to early postnatally, sometimes forming an almost continuous layer at the islet periphery[60]. Only a few ghrelin cells remain visible on the mantle of the islets in adult pancreata of humans[60,61] and rats[62]. Therefore, the major source of ghrelin in fetal circulation is probably the pancreas, not the stomach[63]. Normal mouse pancreas also contains a small population of ghrelin-producing cells, which were named "epsilon" cells. In the neonatal mouse pancreas, about 30% of the ghrelin cells were found to coexpress glucagon, whereas two-thirds (67%) of the ghrelin cells represented a unique islet cell population[64].

Deletion of Nkx2.2 or Pax4 in mice, two transcription factors involved in the differentiation of insulin-producing cells, leads to an enormous increase in ghrelin-producing ε cells[64]. Based on these results, it has been postulated that insulin-producing β-cells and ghrelin-producing ε cells share a common precursor[64].

Guan *et al*[26] first demonstrated a weak GRLN-R mRNA signal in the rat pancreas during *in situ* hybridization. Indeed, not only transcripts for ghrelin, but also for its receptor are expressed in pancreatic tissue of both humans[11,59,65] and rats[3,47]. Immuno-histochemical studies in rat pancreatic tissue revealed that the GRLN-R is localized to most of the α-cells and to some, but not all, β-cells[66]. The latter was confirmed in human pancreatic islets[67], supporting the idea of an autocrine/paracrine response of both β- and α-cells to ghrelin.

EFFECTS OF GHRELIN ON INSULIN SECRETION AND VICE VERSA

Glucose homeostasis is controlled by two key processes: insulin secretion by the pancreatic β-cells and insulin sensitivity of the peripheral tissues. The presence of the GRLN-R on pancreatic β-cells already suggested a role for ghrelin in the function of the β-cell, leading to the hypothesis that ghrelin also has a regulatory role in insulin secretion.

The observed inverse relationship between the circulating levels of ghrelin and insulin in healthy humans[68] suggested inhibitory feedback between ghrelin and insulin. Indeed, insulin is able to suppress circulating ghrelin concentrations, independent from changes in glucose concentrations[69]. The decrease of plasma ghrelin is induced by hyperinsulinemia and not by the resulting plasma glucose decrease, because plasma ghrelin was similarly suppressed when glucose was kept constant in a euglycemic study[70]. A direct effect of physiological insulin concentrations on ghrelin secretion was also shown in the isolated perfused rat stomach[71]. The inhibitory effect of insulin on ghrelin secretion was confirmed in several other studies[72,73], while some reports did not confirm this observation, probably because of the different experimental conditions[74,75].

Ghrelin, in turn, has been proven to affect insulin secretion, which was first demonstrated by Broglio *et al*[76], who showed that acute ghrelin administration in healthy volunteers resulted in prompt increases in blood glucose levels, followed by a decrease in insulin levels, independent from GH. Numerous other studies have investigated ghrelin's (acylated, unless otherwise indicated) effects on glucose and insulin metabolism, sometimes showing ambiguous results. An overview is given in the following paragraphs.

In vitro studies

Exogenous ghrelin: Studies on the effect of exogenous ghrelin on insulin release *in vitro* are summarized in Table 1.

Studies with β-cell lines, isolated β-cells, and pancreatic perfusion experiments considerably contributed to the understanding of the basic mechanisms governing the role of ghrelin in glucose and insulin metabolism. Date *et al*[47] reported an increased cytosolic Ca^{2+} concentration and stimulated insulin release in isolated rat islets treated with ghrelin (1 pmoL/L) in the presence of a stimulatory (8.3 mmoL/L), but not basal (2.8 mmoL/L) glucose concentration[47]. Ghrelin at 1 pmoL/L modestly potentiated glucose-induced Ca^{2+} responses in a small portion of β-cells, but ghrelin had a clear inhibitory ef-

Table 1 Summary of *in vitro* and *in vivo* studies investigating the effect of exogenous ghrelin on insulin release

Study	Species	Dose	Effect on insulin
In vitro studies			
Isolated rat islets			
Date *et al*[47], 2002	Rat	1 pmol/L ghrelin +2.8 mmol/L glucose	No effect
		1 pmol/L ghrelin +8.3 mmol/L glucose	Increase
Dezaki *et al*[58], 2004	Rat	1 pmol/L-0.1 nmol/L ghrelin +8.3 mmol/L glucose	No effect
		10 nmol/L ghrelin +8.3 mmol/L glucose	Decrease
		10 nmol/L ghrelin +2.8 mmol/L glucose	No effect
Colombo *et al*[79], 2003	Rat	1 pmol/L-1 μmol/L ghrelin +16.7 mmol/L glucose	Dose-dependent decrease
Qader *et al*[80], 2008	Rat	10 nmol/L-1 μmol/L ghrelin +8.3 mmol/L glucose	Decrease
Reimer *et al*[81], 2003	Mouse	10 nmol/L ghrelin +3.5-5.5 mmol/L glucose	No effect
		0.01-1 nmol/Lghrelin +8.3-22.2 mmol/L glucose	Decrease
Qader *et al*[80], 2008	Mouse	1 pmol/L ghrelin +12 mmol/L glucose	Decrease
		10 nmol/L-1 μmol/L ghrelin +12 mmol/L glucose	Increase
Pancreas perfusion			
Egido *et al*[85], 2002	Rat	10 nmol/L ghrelin +5.5 mmol/L glucose	No effect
		10 nmol/L ghrelin +5.5-9 mmol/L glucose	Decrease
Dezaki *et al*[77], 2006	Rat	10 nmol/L ghrelin +8.3 mmol/L glucose	Decrease
Desacyl ghrelin			
Dezaki *et al*[77], 2006	Rat	10 nmol/L desacyl ghrelin +8.3 mmol/L glucose	No effect
Pancreatic tissue fragments			
Adeghate *et al*[86], 2002	Rat	1 nmol/L ghrelin	Increase
β cell lines			
Wierup *et al*[62], 2004	INS-1	0.1-100 nmol/L ghrelin +3 mmol/L glucose	No effect
		0.1-100 nmol/L ghrelin +15 mmol/L glucose	Decrease
Gauna *et al*[87], 2006	INS-1	10 nmol/L ghrelin +20 mmol/L glucose	Increase
Doi *et al*[83], 2006 Wang *et al*[84], 2010	MIN 6	1-10 nmol/L ghrelin +3.3 mmol/L glucose	No effect
		1-10 nmol/L ghrelin +22.2 mmol/L glucose	Decrease
Granata *et al*[67], 2007	HIT-T15	100 nmol/L ghrelin +1.25 mmol/L glucose	No effect
		100 nmol/L ghrelin 7.5-15 mmol/L glucose	Increase
Desacyl ghrelin			
Gauna *et al*[87], 2006	INS-1E	10 nmol/L desacyl ghrelin +20 mmol/L glucose	Increase
Granata *et al*[67], 2007	HIT-T15	100 nmol/L desacyl ghrelin +1.25-15 mmol/L glucose	Increase
In vivo studies			
Dezaki *et al*[58], 2004	Mouse overnight fasted	1-10 nmoL/kg (*ip*) ghrelin +1 g/kg (*ip*) glucose	Decrease
Reimer *et al*[81], 2003	Mouse 3 h fasting	5 nmoL/kg (*iv*) ghrelin	No effect

		+1 g/kg (*iv*) glucose 50-150 nmoL/kg (*iv*) ghrelin +1 g/kg (*iv*) glucose	Decrease
Cui *et al*[89], 2008	Rat overnight fasted	0.3 pmoL/kg/mL (40 min, 1 mL/h, *iv* portal vein or *ip* femoral vein) ghrelin +13.3 mg/kg/min (10-40 min, *iv* portal vein or *ip* femoral vein)	Decrease (portal vein) No effect (femoral vein)
Broglio *et al*[76], 2001	Healthy humans overnight fasted	0.3 nmoL/kg (*iv*) ghrelin	Decrease
Akamizu *et al*[91], 2004	Healthy humans overnight fasted	0.3-1.5 nmoL/kg (*iv*) ghrelin	Decrease after 90 min only with 1.5 nmol/kg
Lucidi *et al*[92], 2005	Healthy humans overnight fasted	7.5-15 pmoL/kg/min (*iv*, 2 h infusion)	No effect
Gauna *et al*[93], 2004	Adult-onset GH-deficient patients overnight fasted	0.3 nmoL/kg ghrelin, *iv*	No effect
Tong *et al*[48], 2010	Healthy humans, 10-12 h fast	0.3-1.5 nmoL/kg/h (*iv*, 65 min) 0.3-1.5 nmoL/kg/h (*iv*, 65 min) +11.4 g/m^2 body surface area glucose (*iv*, after 55 min)	No effect Dose-dependent decrease
Desacyl ghrelin			
Gauna *et al*[90], 2007	Rat overnight fasted	3-30 nmoL/kg desacyl ghrelin (*iv*) +1 g/kg (*iv*) glucose	Increase
Broglio *et al*[94], 2004	Healthy humans overnight fasted	0.3 nmoL/kg desacyl ghrelin (*iv*)	No effect
Gauna *et al*[93], 2004	Adult-onset GH-deficient patients overnight fasted	0.3 nmoL/kg desacyl ghrelin (*iv*)	No effect

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

fect at relatively high concentrations (10 nmoL/L)[58,77], which is consistent with the majority of literature reports. Although this concentration is higher than that of circulating ghrelin, ranging from 100 pmoL/L to 3 nmoL/L[78], it is generally conceived that the level of hormone working in a paracrine or autocrine manner is higher than that working in an endocrine manner. Acylated ghrelin could also dose-dependently suppress glucose-induced insulin secretion in isolated adult rat and mouse islets[79-81], in isolated islets from rat neonates[82], and in β-cell lines[62,83,84]. In a pancreas perfusion study, an *in vitro* system that retains the intact circulation in pancreatic islets excluding the influence of other organs, the infusion of ghrelin into the isolated pancreas also inhibited the insulin response to increasing glucose concentrations, arginine, and carbachol[77,85].

Only a few studies reported a stimulatory effect of ghrelin on insulin secretion. Ghrelin could stimulate insulin release in pancreatic tissue fragments from normal and diabetic rats[86]. Accordingly, both acylated (AG) and unacylated ghrelin (UAC) could exert an insulinotropic effect in the INS-1E rat[67,87] and HIT-T15 hamster[67] insulinoma derived β-cell lines in the presence of a static glucose concentration.

In addition its effect on insulin secretion, both acylated and unacylated ghrelin promote cell proliferation and counteract apoptosis of pancreatic β-cells in INS-1E β cell lines and in human islets independent from the GRLN-R[67].

In vitro studies

Endogenous ghrelin: To examine the effects of endogenous ghrelin produced in the islets, isolated rat islets and pancreata were treated or perfused, respectively, with GRLN-R antagonists or an antiserum against active ghrelin, both resulting in increased glucose-induced insulin release in the absence of exogenous ghrelin[77]. In the same study, glucose-induced insulin release from isolated islets of ghrelin knockout mice was significantly greater than that of wild-type mice, despite the similar density and size of the islets. These results indicate an insulinostatic effect of endogenous ghrelin in the islets. In contrast, the GRLN-R antagonist YIL-781 only blocked the inhibitory effect of ghrelin on glucose-induced insulin secretion in dispersed pancreatic islets, but had no effect in the absence of ghrelin[88].

In vivo studies

Exogenous ghrelin: Studies on the effect of exogenous ghrelin on insulin release *in vivo* are summarized in Table 1.

Systemic administration of exogenous ghrelin decreases glucose-induced insulin secretion in mice[58,81] and rats[89]. In mice fasted for 3 h, intraperitoneal (ip) injection of ghrelin diminished the 1-min insulin response after administration of an intravenous glucose load (1g/kg)[81], which was confirmed in overnight fasted mice[58]. When ghrelin was simultaneously injected with glucose, the glucose levels at 30 min and 60 min were higher and the insulin levels at 5 min and 10 min were markedly attenuated in comparison to control values. Overnight fasted mice also displayed significantly elevated glucose levels at 30 min after *ip* administration of ghrelin, an effect that could be blocked completely by simultaneous administration of the GRLN-R antagonist, [D-Lys^3]GHRP-6, indicating that ghrelin increases blood glucose *via* specific interaction with the GRLN-R[58]. Accordingly, ghrelin infusion into the hepatic portal vein inhibited glucose-induced insulin secretion in rats[89]. In contrast, UAG infusion at pharmacological doses enhanced the insulin response to an intravenous glucose load potently and dose-dependently, an effect that was abolished by co-administration of AG[90].

There is little and inconsistent information concerning the effect of ghrelin on insulin release in humans. The first study with exogenous ghrelin administration in healthy humans confirmed that acute administration of ghrelin (1 μg/kg) after an overnight fast resulted in prompt increases in blood glucose concentrations, followed by a slight, but significant, decrease in insulin levels, which may further increase blood glucose[76]. A decrease in insulin levels was also observed in a study from Akamizu *et al*[91]. However, Lucidi *et al*[92] could not confirm these changes in insulin and glucose levels when physiological increases (two to three-fold increments) in plasma ghrelin levels were reached, indicating that ghrelin only affects glucose metabolism at pharmacological doses.

Until now, only a few studies investigated the effect of exogenous ghrelin administration on glucose-stimulated insulin secretion. A recent study reported a reduction in glucose-induced insulin in an intravenous glucose tolerance test after exogenous ghrelin administration[48] in healthy humans. However, because ghrelin infusion raised ghrelin levels by about 4.5, 15.4, and 22.6 fold, the clinical relevance of the latter study can be questioned.

Administration of ghrelin not only affects insulin release, but also insulin sensitivity. In adult-onset GH-deficient patients, ghrelin reduced insulin sensitivity up to 6 h after administration, whereas co-administration of AG ghrelin and UAG ghrelin neutralized the insulin desensitizing effects of AG administration and even improved insulin sensitivity[93]. Broglio *et al*[94] also showed that administration of UAG alone did not affect insulin and glucose levels, but antagonized the effects of AG on insulin secretion and glucose levels in humans. That the relationship between AG and UAG may have an impact on metabolism has been suggested from clinical studies, which show an indirect relationship between circulating AG/UAG ratio and insulin resistance[95], and a decreased AG/UAG ratio in fasting, relatively insulin sensitive, subjects[17].

In vivo studies

Endogenous ghrelin: Intraperitoneal injection of the GRLN-R antagonist, [D-Lys^3]GHRP-6, in mice markedly decreased fasting glucose concentrations, and resulted in an attenuated glucose elevation and enhanced insulin response after an *ip* glucose challenge[58]. Similarly, injection of [D-Lys^3]GHRP-6 in overnight fasted rats resulted in an enhanced insulin response during an intravenous glucose tolerance test (GTT)[90]. In gastrectomized rats, which have dramatically lower acylated ghrelin levels (around 16% of control animals), [D-Lys^3]GHRP-6 increased plasma insulin concentrations to a similar extent as in normal rats, indicating that locally produced ghrelin in the pancreas is the major regulator of insulin release[77].

Numerous studies were performed in ghrelin, ghrelin receptor and recently also in GOAT knockout mice, to investigate whether endogenous ghrelin can influence blood glucose homeostasis. Ghrelin knockout mice presented normal blood glucose and plasma insulin levels in the fed and fasted state on a standard laboratory diet[49,50]. However, during a GTT, ghrelin knockouts had reduced blood glucose and increased insulin levels compared to the wild-types. In addition, an insulin tolerance test showed a more pronounced decrease in glucose levels occurring 30 min after insulin injection in the ghrelin knockout animals on a standard diet, pointing towards increased insulin sensitivity compared to the wild-types. The latter was confirmed by an increased performance in euglycemic hyperinsulinemic clamp studies[55]. When placed on a high fat diet immediately after weaning, wild-type mice became glucose intolerant and insulin resistant, while ghrelin knockout animals were able to maintain normal glucose levels because of a markedly enhanced insulin secretion[54,77]. Their body weight and body fat percentage were lower.

Similar results were obtained in GRLN-R knockout mice, which showed resistance to diet-induced obesity and enhanced insulin sensitivity when exposed to a high fat diet immediately after weaning[53,96]. In addition, RQ values were found to be higher in GRLN-R knockout mice indicating a preference for carbohydrates as fuel, regardless of the diet[96].

Whereas one study found no difference in glucose tolerance during an *ip* GTT in GOAT knockout mice[19], Zhao *et al*[57] showed that GOAT knockout mice, during caloric restriction (fasted for 16 h), experience an improved glucose tolerance and an increased insulin secretion during an oral GTT. Accordingly, mice that were pretreated during an *ip* glucose challenge with the bisubstrate analog, GO-CoA-Tat, which antagonizes GOAT, showed a significant increase in insulin response that was accompanied by a reduction in blood glucose[97]. This effect was not observed in ghrelin knockout mice, indicating that the effect on glucose regulation is mediated by acyl ghrelin.

MECHANISMS INVOLVED IN THE INHIBITORY EFFECT OF GHRELIN ON INSULIN SECRETION

Insulinoma-associated protein 2β (IA-2β), a membrane glycoprotein localized to secretory granules, is a β cell autoantigen for type 1 diabetes, and about 50% of newly diagnosed patients have autoantibodies against IA-2β[98,99]. Administration of ghrelin increased IA-2β mRNA expression in mouse pancreas, brain, and insulinoma (MIN6 and βTC3) cell lines. Moreover, both ghrelin administration and stable overexpression of IA-2β could attenuate glucose-induced insulin secretion. These findings strongly suggest that ghrelin exerts its inhibitory effects on insulin secretion at least partly through enhancement of IA-2β expression[83]. Another recently identified pathway implicated in the effect of ghrelin on glucose-stimulated insulin secretion (GSIS) and independent from IA-2β, is the AMPK-uncoupling protein 2 (UCP2) pathway[84], which is also involved in the control of food intake in

the hypothalamus[100]. UCP2, which uncouples oxidative phosphorylation from ATP synthesis, diminishes the glucose-stimulated insulin secretion in β cells[101]. The link between ghrelin and UCP2 was hypothesized based on the observation that ghrelin knockout mice, which have an enhanced glucose-induced insulin secretion, also displayed reduced expression of UCP2 mRNA in the pancreas[55]. Conclusive evidence was provided by down-regulating UCP2 with the siRNA technique in MIN6 cells, which enhanced GSIS and blocked ghrelin's inhibitory effect on GSIS[84].

GHRELIN RECEPTOR AS A THERAPEUTIC TARGET FOR THE TREATMENT OF DIABETES

Diabetes mellitus (DM) is a chronic disease with increasing worldwide prevalence. In 2000, 171 million individuals were estimated to have diabetes and this is expected to increase to 366 million by 2030[102]. Currently, more than 220 million people worldwide have diabetes. There are two main types of DM: type 2 diabetes or non insulin-dependent diabetes mellitus (NIDDM) accounts for 90% of patients, whereas type 1 or insulin-dependent diabetes mellitus (IDDM) accounts for most other diabetic patients and generally appears before the age of 40 years. Both types of diabetes are characterized by fasting hyperglycemia and abnormal glucose excursion after administration of a glucose load. In the following paragraphs, the role of GRLN-R as a therapeutic target for type 1 and 2 diabetes will be discussed. For both types of diabetes, it is important to preserve and protect viable β-cells. Type 2 diabetes is also characterized by insulin resistance, which means that improving insulin sensitivity is important for the outcome of this disease.

DM type 1

Type 1 diabetes results from autoimmune destruction of the β-cells, leading to a dependency of exogenous insulin administration for maintaining blood glucose homeostasis in these patients. Inverse patterns of plasma ghrelin and insulin concentrations have been described in a 24 h observation period in normal subjects[68]. Likewise, fasting insulin and ghrelin concentrations were found to be negatively correlated in both lean and obese subjects[103]. However, plasma ghrelin data obtained from type 1 diabetic patients are often conflicting, probably dependent on whether these patients are treated or not with insulin in addition to the timing of sampling. In newly diagnosed children with type 1 diabetes, ghrelin levels were low prior to insulin treatment and did not respond to meal tests[104]. This was confirmed in another study, which reported that total and acylated ghrelin concentrations were decreased, compared to healthy children[105]. Another study observed higher plasma total ghrelin concentrations in type 1 diabetic patients and these levels declined by 29% after insulin treatment[106]. Murdolo *et al*[107] showed that the lack of insulin in untreated type 1 diabetic patients prevented post-prandial ghrelin suppression, which may contribute to the hyperphagia that is often observed in these patients. Accordingly, gastric and plasma ghrelin concentrations were reported to be increased in untreated streptozotocin (STZ)-induced diabetic rats, an animal model of type 1 diabetes[108-110]. Conclusive evidence for the contribution of ghrelin signaling pathway to the development of STZ-induced diabetic hyperphagia has been provided in two studies with ghrelin[111] and ghrelin-receptor knockout mice[112].

Therapeutic applications of ghrelin receptor antagonists in type 1 diabetes

Treatment of mice, five days after the induction of STZ-induced diabetes, with a GRLN-R antagonist reduced blood glucose levels and normalized plasma glucagon levels[111]. In addition, daily food intake was reduced. However, in STZ-induced diabetic GHS-R or ghrelin knockout mice hyperphagia was reduced, but no differences in blood glucose levels were observed compared with wild-type mice[111,112]. These data suggest that ghrelin receptor antagonists may only be efficient in the treatment of type 1 diabetes when a small percentage of insulin-producing β-cells remain intact, particularly at early onset.

Therapeutic applications of ghrelin receptor agonists in type 1 diabetes

Growing evidence exists for a potential therapeutic role for ghrelin receptor agonists during type 1 diabetes, at least at an early stage. Ghrelin administration for 7 d was able to promote β-cell regeneration in newborn rats treated with STZ, thereby preventing the development of hyperglycemia at an adult age[113]. Surprisingly, similar protective effects were observed after treatment with unacylated ghrelin, which resulted in an increased area and number of pancreatic islets in STZ-treated rats[114]. Likewise, daily exogenous ghrelin injections in 90% pancreatectomized rats enhanced endocrine and exocrine pancreatic regeneration. Acylated ghrelin treatment increased the number of β-cells, resulting in increased insulin production and an improved glucose tolerance[115]. However, twice daily ghrelin administration for 4 wk, in adult mice 8 wk after STZ-diabetes induction, did not change body weight, food intake, blood glucose levels, or plasma insulin levels, indicating that ghrelin did not improve or worsen diabetic conditions[116]. This strongly suggests that ghrelin may only affect diabetes type 1 in its earliest stage, when some viable β-cells remain. Ghrelin administration may then be able to prevent further progression of the disease, while later administration may have the opposite effect and may further inhibit insulin secretion. This indicates that the timing of administration is very important. Long-term treatment may further impair the function of the remaining β-cells; therefore, it is also important to take the duration of treatment into account.

In *in vitro* studies, both acylated and unacylated ghrelin have been demonstrated to promote proliferation and to

inhibit apoptosis (induced by serum-starvation or cytokines) of HIT-T15 and INS-1E β cell lines and human islets of Langerhans[67,117]. Acylated ghrelin prevents lipotoxicity-induced apoptosis in MIN6 β-cells[118]. The comparable effects of both acylated and unacylated ghrelin on β-cell survival may be mediated *via* an as yet unidentified receptor, because high-affinity binding sites for both peptides were identified on the cell membrane of HIT-T15 cells, which do not express the ghrelin receptor[67].

Ghrelin also stimulates the expression of islet associated protein 2β (IA-2β) mRNA, a major auto-antigen for type 1 diabetes, in mouse pancreas, brain and insulinoma (MIN6 and βTC3) cell lines[83].

DM type 2

The key pathogenic feature of type 2 DM is insulin resistance leading to a compensatory hypersecretion of insulin, ultimately leading to β-cell dysfunction. Type 2 diabetes, which is far more common than type 1 diabetes, is tightly associated with obesity, making it difficult to distinguish between the effects of diabetes alone, or in conjunction with obesity. Obese subjects have decreased circulating ghrelin levels[103] and several studies have found that low ghrelin levels are associated with elevated fasting insulin concentrations and the prevalence of type 2 diabetes and insulin resistance[119]. Indeed, total plasma ghrelin, as well as unacylated ghrelin, concentrations were found to be lower in insulin-resistant obese adults relative to equally obese insulin-sensitive controls[120], indicating a link between ghrelin and insulin sensitivity.

Therapeutic applications of ghrelin receptor antagonists in type 2 diabetes

Although inconclusive data about the effects of ghrelin on insulin secretion were reported, most studies observed an inhibitory effect of ghrelin on glucose-induced insulin secretion *in vitro* using isolated pancreatic islets, β-cell lines, or perfused pancreata, which was confirmed *in vivo* in rodents and humans (Table 1). Transgenic mice overexpressing ghrelin showed glucose intolerance caused by decreased insulin secretion[121]. Another study revealed that MK-677, a ghrelin mimetic, decreased insulin sensitivity and increased fasting blood glucose in aged healthy volunteers in a 1 year study[122].

Accordingly, improved insulin sensitivity was observed in ghrelin-receptor knockout mice[53,96,123] and the high fat diet-induced glucose intolerance is largely prevented by an enhanced insulin release in ghrelin knockout mice[77]. Ghrelin deletion in ob/ob mice also promoted insulin sensitivity and insulin release in response to a glucose challenge[55], by decreasing pancreatic UCP2 expression. By deleting the insulinostatic effect of ghrelin, the maximum capacity of glucose-induced insulin release may be increased enabling the β-cells to secrete more insulin to meet the increased insulin demand during obesity. Oral glucose tolerance testing in GOAT knockout mice, which lack the active form of ghrelin, showed a markedly better glucose tolerance and enhanced insulin secretion on a regular and a high fat diet, compared to their wild-type littermates[57].

Immunoneutralization of endogenous ghrelin with anti-ghrelin antiserum, or by GRLN-R antagonism, resulted in an enhanced glucose-induced insulin release in perfused pancreas[77] and in isolated islets[58]. *In vivo*, the ghrelin receptor antagonist, YIL-781 improved body weight and glucose tolerance in rats with insulin resistant diet-induced obesity[88]. The GOAT-inhibitor, GO-CoA-Tat improved glucose tolerance and reduced weight gain in wild-type mice, but it remains to be investigated whether this compound is also effective in animal models of type 2 diabetes[97]. The first non-peptidic, small molecule antagonists of GOAT, have recently been discovered[124].

Unacylated ghrelin prevented the ghrelin-induced decrease in insulin secretion and insulin sensitivity, but did not induce any changes in these parameters when it was given alone[94]. It is therefore unlikely that this compound will find any application in patients with type 2 diabetes, who have low plasma ghrelin levels.

All these results indicate that ghrelin is a diabetogenic factor and that counteraction of ghrelin augments glucose-dependent insulin secretion in pancreatic β-cells and improves insulin sensitivity in peripheral tissues, with beneficial effects on body weight. This reinforces the concept that ghrelin antagonists and/or GOAT inhibitors may provide a good therapeutic option for the treatment of type 2 diabetes and obesity.

Table 2 gives an overview of the ghrelin receptor antagonists that are currently under development. It is intriguing to observe that, despite 10 years of ghrelin research, the development of ghrelin antagonists is still in its infancy. One of the problems may be the discrepancy obtained with ghrelin antagonists *in vivo* and *in vitro*[125] or the restraint of pharmaceutical companies to develop drugs which target a multifactorial disease such as obesity related type 2 diabetes, where the redundancy in orexigenic signals may lead to unpredictable results.

CONCLUSION

Besides ghrelin's well-described effects on food intake and growth hormone secretion, more attention has been given recently to its contribution to the regulation of blood glucose levels in the body. Although this effect may be mediated by direct mechanisms, most evidence suggests indirect regulation through insulin. Despite controversial results, many studies point out that ghrelin is able to inhibit insulin secretion *in vitro* and *in vivo,* which provides excellent therapeutic perspectives for type 2 diabetes. Ghrelin receptor antagonists for example, could be used to improve insulin release and insulin sensitivity. However, only a few antagonists are currently under investigation in clinical studies and the development of new potent and selective antagonists of the ghrelin receptor are warranted. Moreover, antagonists of ghrelin-O-acyltransferase have recently been discovered and their development may provide new perspectives.

Table 2 Ghrelin antagonists under development for the treatment of obesity and type 2 diabetes

Ghrelin antagonists Drug	Company	Target	Status
TZP-301	Tranzyme Pharma	Obesity, metabolic syndrome	Lead optimization
Two families of ghrelin antagonists	Helsinn Pharmaceuticals	Obesity, metabolic syndrome	Lead optimization
AEZS-123	Aeterna Zenartis	Obesity, alcohol abuse	Preclinical
Ghrelin antagonist	Novartis (Elixir)	Type 2 diabetes, obesity	Preclinical

Ghrelin agonists may be a promising therapeutic avenue to preserve and improve the function of the remaining β-cells in both type 1 and type 2 diabetes, by promoting β cell regeneration at an early stage of the disease. In addition, ghrelin agonists are useful to accelerate diabetic gastroparesis, which often impairs quality of life in diabetic patients. Long-term studies will be needed to investigate whether the developed ghrelin agonists do not induce desensitization of the ghrelin receptor. To prevent this issue, the half-life of these agonists should not be too long. Moreover, ghrelin has a wide range of other physiological functions, such as cardiovascular effects, anxiety, sleep, memory, and mood, which may lead to unwanted side effects. Additional research is needed to further address these issues.

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