Ghrelin regulation of glucose metabolism

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A R T I C L E   I N F O

A B S T R A C T

The a 28-amino acid peptide ghrelin was discovered in 1999 as a growth hormone (GH) releasing peptide. Soon after its discovery, ghrelin was found to increase body weight and adiposity by acting on the hypothalamic melanocortinergic system. Subsequently, ghrelin was found to exert a series of metabolic effects, overall testifying ghrelin a pleiotropic nature of broad pharmacological interest. Ghrelin acts through the growth hormone secretagogue-receptor (GHS-R), a seven transmembrane G protein-coupled receptor with high expression in the anterior pituitary, pancreatic islets, thyroid gland, heart and various regions of the brain. Among ghrelins numerous metabolic effects are the most prominent the stimulation of appetite via activation of orexigenic hypothalamic neurocircuits and the food-intake independent stimulation of lipogenesis, which both together lead to an increase in body weight and adiposity. Ghrelin effects beyond the regulation of appetite and GH secretion include the regulation of gut motility, sleep-wake rhythm, taste sensation, reward seeking behaviour, and the regulation of glucose metabolism. The latter received recently increasing recognition because pharmacological inhibition of ghrelin signaling might be of therapeutic value to improve insuin resistance and type 2 diabetes. In this review we highlight the multifaceted nature of ghrelin and summarize its glucoregulatory action and discuss the pharmacological value of ghrelin pathway inhibition for the treatment of glucose intolerance and type 2 diabetes.

1. Introduction

Obesity and diabetes are major health threats of our society, leading annually to more than 1.5 million casualties [1]. The obesity pandemic affects nowadays almost every culture and ethnic civilization, placing an enormous burden on modern health care systems. From the numerous co-morbidities associated with excess body fat are the most prominent type 2 diabetes, cardiovasclar diseases and certain types of, predominantly gastrointestinal, cancer [2,3]. Underscoring the relevance of adequate glucose buffering, type 2 diabetes represents as of today the most frequent cause of overweight-related death [4]. In line with obesity being the major risk factor for the development of type 2 diabetes, weight loss achieved by either dieting [5] or through pharmacology [6] or bariatric surgery [7,8] improves glucose handling and numerous clinical studies have demonstrated that placebo-subtracted weight loss in the magnitude of even 5% is sufficient to show meaningful improvements in systemic glucose metabolism and of other obesity linked co-morbidities [9–12]. Further underlining the direct relation between body weight and glucose control, weight loss induced by bariatric surgery most often results in complete resolution of type 2

diabetes, an observation that prompted the American Diabetes Association (ADA) to even recommend such surgical intervention under certain circumstances for the treatment of type 2 diabetes [13–15]. Since the correlation between body weight and glucose control is solidly confirmed by numerous preclinical and clinical studies [16,17], drugs to control body weight appear intuitively promising to also improve glucose metabolism. In line with this notion, prominent examples of such strategy is e.g. the administration of GLP-1 mimetics, which not only improve glycemic control via their insulinotropic action but that also indirectly improve glucose metabolism via their ability to decrease body weight through central regulation of food intake [18–21]. While a plethora of weight lowering drugs have been shown to offer beneficial effects on glycemia, including GLP-1 mimetics [22], thyroid hormone [23,24], amphetamines [25], serotonergics [26] or lipase inhibitors [27], hormones with the ability to increase body weight are commonly known to rather impair glucose metabolism. A prominent example of the latter is the gut-derived peptide hormone ghrelin, which increases body weight and body fat mass via activation of orexigenic hypothalamic neurocircuits and via food-intake independent stimulation of lipogenesis [28–31]. In this manuscript we will summarize the

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multifaceted nature of ghrelin with a special focus on its role to regulate glucose metabolism. A key central aspect is thereby the question of whether blocking of ghrelin signaling might be of therapeutic value to improve glucose metabolism?

2. Ghrelin production, activation and degradation

Ghrelin is derived from preproghrelin, a 117 amino-acid precursor that is produced by X/A-like cells within gastric oxyntic glands of the stomach [32]. Preproghrelin is cleaved into a small signal peptide, ghrelin and obestatin. Obestatin has previously been thought to play a role in food intake *via* acting on the G protein-coupled receptor 39 (GPR39) but this was not supported by all studies [33,34]. Cleaved from preproghrelin, the 28 amino acid peptide ghrelin is highly conserved among species with only two amino acids differing between the rat and human peptide [35].

Ghrelin promotes its biological action *via* binding to the growth hormone secretagogue receptor 1a (GHSR1a), a seven transmembrane G protein-coupled receptor with highest expression in the pituitary, pancreatic islets, adrenals, thyroid gland, the myocardium, the hypothalamic arcuate nucleus (ARC), hippocampus, the substantia nigra pars compacta (SNpc), the ventral tegmental area (VTA), and raphe nuclei [36,37]. In the feeding center of the hypothalamus, *GHSR1a* is localized in neurons that express neuropeptide Y (*Npy*) and Agouti related peptide (*Agrp*), well known neuropeptides stimulating food intake [38]. GHSR1 is present in two forms, the long form (GHSR1a), which is mediating most, if not all, of acyl-ghrelins metabolic effects and a truncated form, GHSR1b [36].

To activate its only known receptor, ghrelin needs to be post-translationally modified (acylated) to carry a fatty acid, preferably C:8 or C:10, on its third N-terminal amino acid position, which is a serine [35]. This rare post-translational modification is achieved by the ghrelin O-acyl-transferase (GOAT), a member of the membrane bound O acyltransferase (MBOAT) family [39,40]. GOAT is essential to acylate ghrelin *in vivo*, as demonstrated by the absence of acyl-ghrelin in plasma of mice deficient for GOAT [39,41–44].

The reported half-life of acyl-ghrelin varies between 30 min in rats to 240 min in humans [45]. Reflecting the species-related differences in ghrelin degradation, butyrylcholinesterase is the main enzyme inactivating ghrelin in humans whereas in rodents carboxylesterases allow for an 8-times faster des-octanoylation of ghrelin [45].

3. Physiological effects of unacylated ghrelin

While substantial evidence indicates that most metabolic effects of ghrelin require acylation of the peptide, there is accumulating evidence suggesting that also des-acyl ghrelin has physiologically relevant effects on systems metabolism, potentially *via* a receptor that yet still needs to be identified. In line with this notion, desacyl ghrelin affects differentiation of C2C12 skeletal muscle cells [46], prevents muscle atrophy [47], has protective effects on the heart [48,49] and affects glucose metabolism *via* pathways that are independent of GHSR1 [50–52]. When injected directly into the third ventricle of the hypothalamus, des-acyl ghrelin seems to acutely stimulate food intake through mechanisms that are independent of GHSR1a and Npy signaling [51]. When injected into the periphery, des-acyl ghrelin is either reported to not affect food intake [51] or to even decrease food intake [53]. Nevertheless, mice overexpressing des-acyl ghrelin under control of the FABP4 promoter seem to be protected from diet-induced obesity and show reduced body fat mass when fed with a standard chow diet [52]. These data align with a growing body of evidence testifying des-acyl ghrelin a certain potential to prevent diet-induced obesity and to improve HFD-induced derangements in glucose and lipid metabolism [54,55]. Interestingly, the glycemic effects of ghrelin to increase blood glucose through inhibition of insulin secretion seems to be antagonized by co-administration of des-acyl ghrelin [56]. Despite not supported by

all studies [57], also several human studies report positive effects of des-acyl ghrelin on insulin sensitivity [58,59]. In line with this notion, there is recent evidence suggesting that des-acyl ghrelin promotes survival of pancreatic β-cells and protects from streptozotocin-induced β-cell damage [60–63].

4. Ghrelins effects beyond the stimulation of food intake

The most prominent effect of ghrelin is its ability to stimulate food intake *via* activation of hypothalamic neurocircuits [28]. In line with this notion, in the hypothalamic arcuate nucleus (ARC), ghrelin increases the activity of neurons expressing neuropeptide y (*Npy*) and the agouti-related protein (*Agrp*) while at the same time inhibiting neurons that express proopiomelanocortin (*Pomc*) [29,38]. Ghrelin signaling *via* these neurons is essential for ghrelins orexigenic effect since ghrelin fails to increase food intake in mice lacking *Npy* and *Agrp* [64]. Intracerebroventricular (icv) injection of ghrelin further increases food intake in rats, but fails to do so when NPY and AgRP neurons were blocked [65], further underlining the importance of the hypothalamic melanocortinergic system. In line with its effect on the melanocortinergic system, a ying yang balance between ghrelin and leptin has been suggested and ghrelin accordingly seems to counteract food intake inhibition by leptin [66]. Beside its ability to stimulate food intake, ghrelin activates gastric emptying and motility, as well as gastric acid secretion (Fig. 1) [67,68]. Ghrelin further modulates food reward and taste sensation, increases locomotor activity, motivation towards food reward, and enhances olfactory sensitivity [69–74]. As a pulsatile hormone, ghrelin is also involved in sleep regulation as suggested by different studies [75–77].

Acutely, ghrelin seems to induce anxiolytic and anti-depressant like effects in mice, most likely *via* stimulating the activity of the HPA axis [78,79]. Under stress, also the preference for HFD seems to be affected by ghrelin signaling [80]. Collectively, these data suggest a role for ghrelin in sleep regulation, stress and depression. Ghrelin also enhances differentiation and fusion of skeletal muscles cells *in vitro* and impairs skeletal muscle atrophy in mice [46,47]. Ghrelin further increases myocardial contractility, has a protective effect on the heart, and plays a role in atherogenesis [81]. Acute or chronic administration of ghrelin improves left ventricular (LV) dysfunction, and limits LV abnormal development in patients with chronic heart failure. Ghrelin also increases exercise capacity in both rats and humans [82,83]. In healthy humans, forearm blood flow is further increased by ghrelin, suggesting also a role in vasodilatation [84].

Effects on energy expenditure are frequently reported upon administration of ghrelin. Single peripheral or central (icv) injection of ghrelin suppresses BAT sympathetic nerve activity, thereby decreasing BAT temperature *via* CNS-dependent mechanisms [85,86]. Chronic ghrelin treatment further decreases *Ucp1* mRNA expression in the BAT [87]. Corroborating a role of ghrelin in regulating BAT function, mice lacking ghrelin or administration of *GHSR* antisense mRNA increases BAT activity [88,89].

5. Preclinical studies on ghrelins role in glucose metabolism

Numerous studies have evaluated ghrelins effects on glucose metabolism (as reviewed in [30]). Ghrelin inhibition of insulin secretion has been shown in a variety of species including mice [90], rats [91], pigs [92] monkeys [93,94] and humans [95]. In line with ghrelins ability to decrease insulin release *in vivo*, levels of blood glucose are typically decreased in mice lacking either ghrelin or GHSR relative to wildtype controls [96]. When exposed to a HFD, mice deficient for ghrelin or its receptor show a better glucose tolerance and insulin sensitivity when compared to wildtype controls [97,98]. Underling ghrelins role in glucose metabolism, ghrelin deletion in ob/ob mice decreases hyperglycemia and enhances glucose-induced insulin secretion, thereby improving insulin sensitivity in peripheral tissues relative
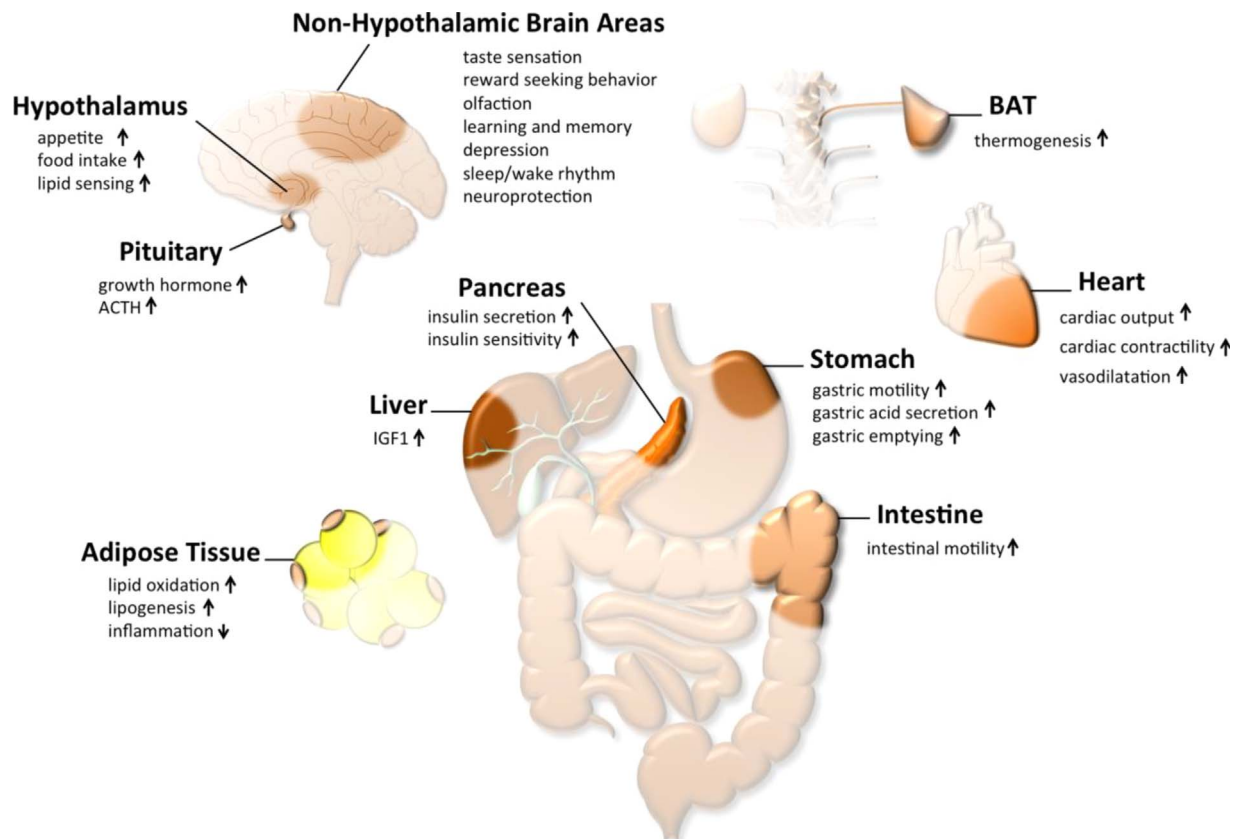
Fig. 1. Schematic on physiological effects of acyl-ghrelin.

to ob/ob controls [99].

The endocrine pancreas comprises four main cell types, the glucagon-producing α-cells, the insulin-prducing β-cells, the somatostatin producing δ-cells and the pancreatic polypeptide producing PP-cells [100,101]. Notably, a fifth endocrine cell type, the ghrelin-producing ε-cells have also been described [102–104] but their presence in mature adult islets remains subject of investigation [101]. Rats carrying a loss-of-function mutation in the cyclin-dependent kinase inhibitor p27 show an elevated number of ghrelin producing ε-cells, which coincides with increased food intake, higher fat mass and decreased glucose stimulation of insulin secretion [105]. In the pancreas, ghrelin is also produced in pancreatic α-cells [90,106–108] and blockade of pancreatic ghrelin enhances insulin secretion and prevents high-fat diet (HFD) induced glucose intolerance in mice [108,109]. Apart from ghrelin itself, also its receptor is expressed in the pancreatic α-cells and several lines of evidence suggests a role of ghrelin in affecting glucose metabolism not only by directly inhibiting glucose stimulation of insulin secretion but also via stimulation of α-cell glucagon secretion [110]. Supporting the glucoregulatory role of acyl-ghrelin, pharmacological inhibition of GOAT improves glycemic control and stimulates the release of insulin [41]. Despite not confirmed by all studies [111], the GOAT-ghrelin systems further seems to be essential for the prevention of hypoglycemia during extreme episodes of calorie restriction [43].

Ghrelins negative insulinotropic action is mediated by Gα$_i$-dependent GHSR1a signaling in the beta-cells [112] and involves interaction with the somatostatin receptor subtype-5 (SST5) [113]. Counter-intuitively, beyond its role as a negative regulator of insulin secretion, ghrelin seems to also have protective effects on the β-cells under conditions of type 1 diabetes [114,115] despite evidence showing that levels of ghrelin decline with the onset of type 1 diabetes [116,117].

Ghrelins well confirmed glycemic effects suggest that pharmacological inhibition of ghrelin action might offer beneficial effects in the treatment of type 2 diabetes. In line with this notion, GHSR1a

antagonism induces weight loss and improves glucose tolerance in rats, potentially via stimulation of glucose-dependent insulin secretion [118]. Similar results are reported from mice showing a MODY-type diabetes due to lack of the hepatocyte nuclear factor-1α (HNF1α). In these mice, pharmacological inhibition of ghrelin signaling by administration of the GHSR antagonist GHRP-6 improves glycemic control via restoration of insulin sensitivity [119]. Notably, GHSR1a shows a certain degree of intrinsic constitutive activity, potentially resulting in a certain degree of ligand-independent GHSR effects on glycemia [120,121]. Ghrelin receptor inverse agonists might thus be of pharmacological value to improve systems metabolism and administration of such GHSR inverse agonist has recently been shown to decrease body weight and adiposity and to improve glucose metabolism in zucker diabetic fatty (ZDF) rats [122]. Central icv administration of the GHSR1a inverse agonist [d-Arg1, d-Phe5, d-Trp7,9, Leu11]-substance P, was further shown to decrease food intake and body weight gain, supposedly via modulation of Npy expression [123].

Vaccination has been traditionally used to prevent infectious diseases, but the concept has over the last years been refined to also allow the pharmacological regulation of body weight. In line with this notion, rats vaccinated with ghrelin immunoconjugates display decreased body weight and adiposity due to lower food efficiency [124]. Another anti-obesity vaccine targeting the ghrelin system has recently been developed in mice. Ghrelin was here combined with a carrier protein (Pspa), which is normally used in pneumococcal vaccine. The vaccine was developed in a nanogel to allow intranasal administration and upon administration in mice, it decreases HFD-induced body weight gain both in wildtype mice and in ob/ob mice, thereby improving glucose tolerance and insulin sensitivity [125].

6. Clinical studies on ghrelins role in glucose metabolism

In line with a series of preclinical studies all testifying ghrelin a

hyperglycemic nature due to inhibition of insulin secretion [90,106–108], 65 min of continuous ghrelin infusion in healthy human volunteers suppresses glucose-stimulated insulin secretion and impairs glucose tolerance [95]. These data are supported by a series of other studies overall demonstrating that plasma levels of glucose increase while insulin levels decrease following ghrelin administration [91,126–129]. Notably, a link between ghrelin and insulin is also suggested by the fact that both hormones exhibit a reciprocal correlation over the day with insulin levels being high when ghrelin levels are low and vice versa [130,131]. Also epidemiological studies support the inverse relationship between ghrelin and indexes of impaired glucose tolerance and insulin resistance [132]. Single intravenous administration of ghrelin increases plasma glucose levels followed by drop in fasting insulin levels in lean [126] and obese subjects with or without polycystic ovarian syndrome [133], further supporting an inhibitory role of the ghrelin pathway in insulin secretion.

The Prader Willi syndrome (PWS) is a genetic disorder associated with the development of obesity. Patients with PWS are typically hyperphagic and show increased plasma levels of ghrelin [134,135], notably also relative to weight-matched non-PWS and lean subjects and both after fasting and post-prandially [134]. These data might indicate that hyperghrelinemia might underly the hyperphagia and obesity of PWS patients, suggesting that blocking of ghrelin action might be beneficial to decrease body weight and to improve glycemic control in these patients. In line with this notion AZP-531 (Alizé Pharmaceuticals), a stabilized peptide analog of unacylated ghrelin is in phase I clinical trials for the treatment of obesity in PWS patients and 14-day treatment of healthy and type 2 diabetic overweight/obese individuals with AZP-531 has recently been shown to decrease body weight and to improve glycemic control as indicated by decreased levels of Hba1c [136].

Pfizer recently developed a GHSR1a receptor antagonist, PF-05190457, that is currently in clinical evaluation for the treatment of T2D. This drug shows beneficial effect on glucose-dependent insulin secretion *in vitro*, and increases insulin secretion in isolated human islet [137,138]. Interestingly, PF-05190457 was stopped after the phase I clinical trials but nor for safety reasons [139] and it remains in clinical evaluation for the treatment of insomnia [140]. In summary, there is accumulating preclinical and clinical evidence overall supporting a beneficial effect of ghrelin pathway inhibition for the treatment of type 2 diabetes. Beyond ghrelin's direct glucoregulatory role, it has to be noted that also body weight loss due to ghrelin pathway inhibition might offer a certain potential to secondarily further improve glucose handling.

7. Conclusion

The endogenous ghrelin system has over the last decade emerged as being implicated in a myriad of metabolic effects that go well beyond it's initial classification as a hormone affecting food intake and GH secretion (Fig. 1). Along with ghrelins role in systemic metabolism, a variety of studies evaluated the therapeutic impact of ghrelin pathway modulation. While ghrelin agonism might offer potential to treat diabetic gastroparesis and anorexia associated with pathological underweight and cachexia [32], ghrelin receptor antagonism might be of therapeutic value to decrease body weight under certain conditions of obesity (as in patients with PWS) and also to improve glucose metabolism and type 2 diabetes. Interestingly, while ghrelins orexigenic effect is known for more than 1.5 decades, the peptide is always good for a surprise and it is not unlikely that other physiological effects of ghrelin are yet to be discovered.

Declaration of interest

The authors declare that there is no conflict of interest.

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