Ghrelin regulation of glucose metabolism

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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, cardiovascular 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].

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 testing 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 ghrelin 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 insulin 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.
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 segmental 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 (GHSR1la), which is mediating most, if not all, of acyl-ghrelin metabolic effects and a truncated form, GHSR1b [36].

To activate its only known receptor, ghrelin needs to be posttranslationally 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-acyltransferase (GOAT), a member of the membrane bound O acyltransferase (MBOAT) family [39,40]. GOAT is essential to acylate ghrelin and obestatin. Obestatin has previously been thought to play a role in vasodilatation [84].

Numerous studies have evaluated ghrelin’s 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 ghrelin’s 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 ghrelin 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 to 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. Ghrelin’s 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 ghrelin’s 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, 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 atherosogenesis [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 stimulating the activity of the HPA axis [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 ghrelin’s role in glucose metabolism

Numerous studies have evaluated ghrelin’s 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 ghrelin’s 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 ghrelin’s 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 to...
The endocrine pancreas comprises four main cell types, the glucagon-producing α-cells, the insulin-producing 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].

Ghrelin’s 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 (Psps), 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 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-S31 (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-S31 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 its initial classification as a hormone affecting food intake and GH secretion (Fig. 1). Along with ghrelin’s 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 [52], 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 ghrelin 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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References

[1] C.D. Mathers, D. Loncar, Projections of global mortality and burden of disease from 2002 to 2030, PLoS Med. 3 (2006) e442.
[2] E.-E. Celle, C. Rodrigues, K. Woll, K. Thisted, M.J. Thun, Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults, N. Engl. J. Med. 348 (2003) 1625–1638.
[3] C.B. Ebbeling, D.B. Pawlak, D.S. Ludwig, Childhood obesity: public-health crisis common sense cure, Lancet 360 (2002) 473–482.
[4] G.B.R.O. Collaborators, A. Ashin, M.H. Forouzanfar, M.B. Reitsma, P. Sur, K. Estept, et al., Health effects of overweight and obesity in 195 countries over 25 years, N. Engl. J. Med. 377 (2017) 13–27.
[5] H. Raynor, P.G. Davies, C.G. Jeffery, M.D.H. Nadelon, S. Mezrick, V. Ubley, et al., Medical nutrition therapy and weight loss questions for the evidence analysis library prevention of type 2 diabetes: systematic reviews, J. Acad. Nutr. Diet. 117 (2017) 1578–1611.
[6] G. Servatova, C.M. Agosto, Current pharmacotherapy for obesity, Nat. Rev. Endocrinol. 14 (2018) 12–24.
[7] A. Ardestanti, D. Rhoads, A. Tavakkoli, Insulin cessation and diabetes remission after bariatric surgery in adults with insulin-treated type 2 diabetes, Diabetes Care 38 (2015) 659–664.
[8] Z. Landau, G. Karplus, A. Hanukoglu, S. Abiri, A. Levy, F. Serour, Laparoscopic sleeve gastrectomy (LSG) in adolescents with morbid obesity, Harefuah 150 (2011) 765–768 816, 815.
[9] S.B. Heymufeld, S.A. Wadden, Mechanisms, pathophysiology, and management of obesity, N. Engl. J. Med. 376 (2017) 254–266.
[10] F. Magkos, G. Fraterrigo, J. Yoshino, C. Luecking, K. Kirbach, S.C. Kelly, et al., Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity, Cell Metab. 23 (2016) 591–601.
[11] R.R. Wing, W. Lang, T.A. Wadden, M. Safford, W.C. Knowler, A.G. Bertoii, et al., Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes, Diabetes Care 34 (2011) 1481–1486.
[12] J. Lindstrom, A. Loudantara, M. Mannelin, M. Rastas, V. Salminen, J. Eriksson, et al., The Finnish diabetes prevention study (DPS): lifestyle intervention and 3-year results on diet and physical activity, Diabetes Care 26 (2003) 3230–3236.
[13] S. Chakravarti, All in one: researchers create combination drugs for diabetes and obesity, Nat. Med. 22 (2016) 694–696.
[14] F. Robino, D.M. Nathan, R.H. Eckel, P.R. Schauer, K.G. Alberti, P.Z. Zimmet, et al., Metabolic surgery in the treatment algorithm for type 2 diabetes: a joint statement by international diabetes organizations, Diabetes Care 39 (2016) 861–877.
[15] J.P. Brito, V.M. Montori, A.M. Davis, Metabolic surgery in the treatment algorithm for type 2 diabetes: a joint statement by international diabetes organizations, Jama 317 (2016) 653–656.
[16] Q.X. Paulson, J. Hong, V.B. Holcomb, N.P. Nunez, Effects of body weight and alcohol consumption on insulin sensitivity, Nutr. J. 9 (2010) 14.
[17] K.M. Godde, Y. Pritham Raja, Pharmacotherapy of obesity: clinical trials to clinical practice, Curr. Diab. Rep. 17 (2017) 34.
[18] C. Clemenstsen, B. Finan, K. Fischer, R.Z. Tom, B. Legutko, L. Seherrer, et al., Dual melanocortin-4 receptor and GLP-1 receptor agonism amplifies metabolic benefits in diet induced obese mice, EMBIO Mol. Med. 7 (2015) 288–298.
[19] B. Finan, T. Ma, N. Ootttayi, T.D. Muller, K.M. Habegger, K.M. Heppner, et al., Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans, Sci. Transl. Med. 5 (2013) 204ra151.
[20] S.J. Sjölin, R. Gutierrez-Aguilar, M. Scott, D.A. D'Allessio, D.A. Sandoval, R.J. Seeley, Neuronal GLP1R mediates iraglutide's anorectic but not glucose-lowering effect, J. Clin. Invest. 124 (2014) 2456–2463.
[21] B. Finan, C. Clemenstsen, T.D. Muller, Emerging opportunities for the treatment of metabolic diseases: glucagon-like peptide-1 based multi-agonists, Mol. Cell. Endocrinol. 418 (Pt 1) (2015) 42–54.
[22] B. Finan, B. Yang, N. Ootttayi, D.I. Smiley, T. Ma, C. Clemenstsen, et al., Rdionally designed monomeric peptide triagonist corrects obesity and diabetes in rodents, Nat. Med. 21 (2015) 27–36.
[23] J.D. Baxter, P. Webb, Thyroid hormone mimetics: potential applications in atherosclerosis, obesity and type 2 diabetes, Nat. Rev. Drug Discov. 8 (2009) 308–320.
[24] E. Flies, L.P. Klieverik, A. Kalsheek, Novel neural pathways for metabolic effects of thyroid hormone, Trends Endocrinol. Metab. 21 (2010) 230–236.
[25] C.K. Haddock, W.S. Poston, P.L. Dill, J.P. Foresi, M. Ericsson, Pharmacotherapy for obesity: a quantitative analysis of four decades of published randomized
G. Collden, M.H. Tschop, T.D. Muller, Therapeutic potential of targeting the hypothalamic circuit regulating energy homeostasis, Neuron 37 (2003) 649–652.

M.A. Cowley, R.G. Smith, S. Diano, M. Tschop, N. Pronchuk, K.L. Grove, et al., The GI functions of GPR39: novel biology, Curr. Opin. Pharmacol. 12 (2012) 647–652.

I. Depoortere, GOAT links dietary lipids with the endocrine control of energy balance, Nat. Med. 15 (2009) 741–745.

J. Yang, T.J. Zhao, J.L. Goldstein, M.S. Brown, Inhibition of ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of caloric-restricted mice, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 7467–7472.

H. Chai, L.K. Zhang, Y.Z. Pang, C.S. Pan, Y.F. Qi, L. Chen, et al., Cardioprotective effects of ghrelin, Curr. Pharm. Des. 17 (2011) 5865–5871.

J.N. Nagaya, M. Uematsu, M. Kojima, Y. Ikeda, F. Yoshihara, W. Shimizu, et al., Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety in rodents and humans, J. Neurosci. 31 (2011) 5841–5846.

V. Tolle, M.H. Bassant, P. Ziajari, F. Poidsen-Juizat, C. Tomasetto, J. Epelbaum, et al., Ultradian rhythm of ghrelin secretion in relation with GH, feeding behavior, and sleep-wake patterns in rats, Endocrinology 143 (2002) 1353–1361.

E. Szentirmai, I. Hajdu, F. Obor, J.M. Krueger, Ghrelin-induced sleep responses in ad libitum fed and food-restricted rats, Brain Res. 1088 (2006) 131–140.

J.C. Weihek, A. Wichniak, M. Ising, H. Brunner, E. Friess, K. Held, et al., Ghrelin promotes slow-wave sleep in humans, Am. J. Physiol. Endocrinol. Metab. 298 (2010) E529–E535.

K. Yoshida, H. Yamaguchi, Y. Sun, R.G. Smith, A. Yamanaka, T. Sakurai, et al., Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restrains anxiety after acute stress, Biol. Psychiatry 72 (2012) 457–465.

F. Bregli, C. Gottero, F. Promad, C. Gauina, G. Muccioli, M. Papotti, et al., Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acute ghrelin in humans, J. Clin. Endocrinol. Metab. 89 (2004) 3062–3065.

J. Toog, H.W. Davis, S. Sumner, S.C. Bennett, A. Haque, M. Bidninghamer, et al., Acute administration of unacylated ghrelin has no effect on basal or stimulated insulin secretion in healthy humans, Diabetes 63 (2014) 2309–2319.

R. Barazzoni, M. Zanetti, C. Ferreira, P. Vinci, A. Pirillo, M. Mucci, et al., Relationships between desacylated and acylated ghrelin and insulin sensitivity in obese subjects with the metabolic syndrome, J. Clin. Endocrinol. Metab. 92 (2007) 3935–3940.

H. Cederberg, V.M. Koivisto, J. Jokelainen, H.M. Surcel, S. Keinanen-Kiukasniemi, U. Rajala, Unacylated ghrelin is associated with changes in insulin sensitivity and lipid profile during an exercise intervention, Clin. Endocrinol. (Oxf.) 76 (2012) 39–45.

R. Granata, F. Settanni, M. Julien, R. Nago, G. Togniato, A. Trometer, et al., Des-acyl ghrelin fragments and analogues promote survival of pancreatic beta-cells and human pancreatic beta-cell lines, Exp. Diabetol. Res. 2013 (2013) 2585–2596.

R. Granata, F. Settanni, L. Biancone, L. Trovato, R. Nango, F. Bertuzzi, et al., Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 5,5'-cyadinosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3-kinase/Akt signaling, Endocrinology 148 (2007) 512–529.

R. Granata, F. Settanni, L. Trovato, S. Destefanis, D. Gallo, M. Martinetti, et al., Unacylated as well as acylated ghrelin promotes cell survival and inhibit apoptosis in HIT-T15 pancreatic beta-cells, J. Endocrinol. Invest. 29 (2006) C91–C22.

R. Granata, M. Volante, F. Settanni, C. Gauna, G. Behe, M. Annunziata, et al., Unacylated ghrelin and obestatin increase cell mass and prevent diabetes in STZ-treated rats, J. Mol. Endocrinol. 41 (2013) 17–25.

H.Y. Chen, M.E. Trumbauer, A.S. Chen, D.T. Weingarth, J.R. Adams, E.G. Frazier, et al., Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein, Endocrinology 145 (2004) 2667–2612.

D.M. Nakata, N. Murooka, M. Tsuchiya, T. Kato, K. Kangawa, et al., A role for ghrelin in the central regulation of feeding, Nature 409 (2001) 194–198.

S.P. Kalra, N. Ueno, P.S. Kalra, Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action, J. Nutr. 135 (2005) 1331–1335.

Y. Masuda, T. Tanaka, N. Inomata, N. Ohnuma, S. Tanaka, Z. Itoh, et al., Ghrelin stimulates gastric acid secretion and motility in rats, Biochem. Biophys. Res. Commun. 276 (2000) 905–909.

A. Asakawa, A. Inui, K. Tsuchiya, H. Yuzuriha, T. Nagata, N. Ueno, et al., Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin, Gastroenterology 120 (2001) 1430–1435.

R. Granata, M. Tschop, T.D. Muller, Therapeutic potential of targeting the ghrelin pathway, Int. J. Mol. Sci. 18 (2017).

J.V. Zhang, P.G. Ren, O. Avian-Kretzer, C.W. Lu, R. Rauch, C. Klein, et al., Obestatin a peptide encoded by the ghrenin gene, opposes ghrelin’s effects on food intake, Science 310 (2005) 996–999.

I. Depoortere, GI functions of GPR39: novel biology, Curr. Opin. Pharmacol. 12 (2012) 647–652.

M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, K. Kangawa, Ghrelin is a growth-hormone-releasing acylated peptide from stomach, Nature 402 (1999) 656–660.

S. Gnanapavan, B. Kola, S.A. Bustin, D.G. Morris, P. McGee, P. Fairclough, et al., Acylated and unacylated ghrelin promotes cell survival and inhibit apoptosis in HIT-T15 pancreatic beta-cells, J. Endocrinol. Invest. 29 (2006) C91–C22.

W. Zhang, B. Chai, J.Y. Li, H. Wang, M.W. Mulholland, E. Zhang, et al., Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats, Gastroenterology 129 (2005) 8–25.

P.J. Delhanty, M. Huisman, I.Y. Baldos-Rojas, I. van den Berge, A. Grehoorst, T. Abirbat, et al., Des-acyl ghrelin analogs prevent high-fat-diet-induced dysregulation of glucose homeostasis, FASEB J. 27 (2013) 1690–1700.

P.J. Delhanty, S.J. Neggers, A.J. van der Lely, Des-acyl ghrelin: a metabolically active peptide, Endocr. Dev. 25 (2011) 112–121.

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Peptides 100 (2018) 236–242

[83] N. Nagaya, J. Moriya, Y. Yasumura, M. Uematsu, F. Ono, W. Shimizu, et al., Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure, Circulation 110 (2004) 3674–3679.

[84] H. Okumura, N. Nagaya, M. Emomoto, E. Nakagawa, H. Oya, K. Kangawa, Vasodilatory effect of ghrelin, an endogenous peptide from the stomach, J. Cardiovasc. Pharmacol. 39 (2002) 779–783.

[85] T. Yamanashi, Y. Sakata, K. Takuma, H. Yoshimatsu, Centrally administered ghrelin suppresses sympathetic nerve activity in brown adipose tissue of rats, Neurosci. Lett. 349 (2003) 75–78.

[86] A. Mano-Otagiri, H. Ohata, A. Iwasaki-Sekino, T. Nemoto, T. Shimabashi, Ghrelin suppresses noradrenaline release in the brown adipose tissue of rats, J. Endocrinol. 201 (2009) 341–349.

[87] T. Tsujino, T. Masaki, K. Takuma, H. Yoshimatsu, Ghrelin regulates adiposity in white adipose tissue and UCPI mRNA expression in brown adipose tissue of mice, Regul. Pept. 150 (2010) 193–203.

[88] A. Mano-Otagiri, A. Iwasaki-Sekino, T. Nemoto, H. Ohata, Y. Shuto, H. Nakabayashi, et al., Genetic suppression of ghrelin receptors activates brown adipocyte function and decreases fat storage in rats, Regul. Pept. 160 (2010) 161–168.

[89] L. Lin, P.K. Saha, X. Mu, I.O. Henshaw, L. Shao, B.H. Chang, et al., Ablation of ghrelin receptor reduces adiposity and improves insulin sensitivity during aging by regulating fat metabolism in white and brown adipose tissues, Aging Cell 10 (2011) 996–1010.

[90] M.K. Reimer, G. Pacini, B. Ahren, Dose-dependent inhibition by ghrelin of insulin secretion in the mouse, Endocrinology 144 (2003) 916–921.

[91] E.M. Eigo, J. Rodriguez-Gallardo, R.A. Silverstone, J. Marco, Inhibitory effect of ghrelin on insulin and pancreatic somatostatin secretion, Eur. J. Endocrinol. 146 (2002) 241–244.

[92] C.R. Reynolds, A.N. Elias, C.S. Whisnant, Effects of feeding pattern on ghrelin and insulin secretion in pigs, Domest. Anim. Endocrinol. 39 (2010) 99–106.

[93] S.V. Angeloni, N. Glynn, G. Ambrosini, M.J. Garant, J.D. Higley, S. Suomi, et al., Characterization of the rhesus monkey ghrelin gene and factors influencing ghrelin gene expression and fasting plasma levels, Endocrinology 145 (2004) 2197–2205.

[94] V.S. Varghese, M.E. Tejero, J.M. Profitt, S.A. Cole, L.A. Cox, M.C. Mahaney, et al., Characterization of ghrelin in pedigreed baboons: evidence for heritability and pleiotropy, Obesity (Silver Spring) 16 (2008) 804–810.

[95] J. Tong, R.L. Pfrigo, H.W. Davis, M. Bidlingmaier, S.E. Kahn, D.E. Cummings, et al., Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans, Diabetes 59 (2010) 2145–2151.

[96] Y. Sun, N.P. Butte, J.M. Garcia, R.G. Smith, Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance, Endocrinology 149 (2008) 843–850.

[97] Y. Sun, P. Wang, H. Zheng, R.G. Smith, Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor 1a, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 3618–3623.

[98] J.M. Zigman, Y. Nakano, R. Coppari, N. Balthasar, J.N. Marcus, C.E. Lee, et al., Characterization of ghrelin in pedigreed baboons: evidence for heritability and pleiotropy, Obesity (Silver Spring) 16 (2008) 804–810.

[99] B. Holst, A. Cygankiewicz, T.H. Jensen, M. Ankersen, T.W. Schwartz, High constitutive activity is an intrinsic feature of ghrelin receptor protein: a study with a functional monomeric GHS-R1a receptor reconstituted in lipid discs, J. Biol. Chem. 287 (2012) 9360–9361.

[100] G.R. Lussier, K. Belleville, P. Sarret, F. Boudreau, Ghrelin inhibition restores glucose homeostasis in hepatic nuclear factor-1alpha (MODY3) deficient mice, Diabetologia 53 (2010) 3534–3540.

[101] M. Damian, J. Marie, J.P. Leyris, J.A. Fehrentz, P. Verde, J. Martinez, et al., In high constitutive activity is an intrinsic feature of ghrelin receptor protein: a study with a functional monomeric GHS-R1a receptor reconstituted in lipid discs, J. Biol. Chem. 287 (2012) 9360–9361.

[102] P.S. Petersen, D.P. Woldbye, A.N. Madsen, K.L. Egerod, C. Jin, M. Lang, et al., In vivo effects of acute ghrelin administration in pigs, Domest. Anim. Endocrinol. 39 (2010) 90–98.

[103] Z. Negrelli, L. Bernasconi, M. Hutter, L. Whiting, C. Pietra, C. Giuliano, et al., Ghrelin receptor inverse agonists as a novel therapeutic approach against obesity-related metabolic disease, Diabetes Metab. 33 (2007) 1740–1750.

[104] F. Brial, S. Iwasaki, A.J. Moss, J. Chang, J. Otsui, K. Inoue, et al., Vaccination against weight gain, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 15236–15241.

[105] T. Aregani, Y. Yuki, S. Sawada, M. Meijima, K. Ishige, K. Akiyoshi, et al., Nanog-based nasal ghrelin vaccine prevents obesity, Mucosal Immunol. 10 (2017) 1351–1360.

[106] F. Broglio, E. Arvat, A. Benso, C. Gotti, G. Muccioli, M. Papotti, et al., Ghrelin a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans, J. Clin. Endocrinol. Metab. 86 (2001) 5083–5086.

[107] F. Negligu, C. Gotti, A. Benso, F. Poudram, S. Desteianiu, C. Gauna, et al., Effects of ghrelin on the insulin and glycemic responses to glucose arginine, or free fatty acids load in humans, J. Clin. Endocrinol. Metab. 88 (2003) 4268–4272.

[108] M. Guido, D. Romualdi, L. De Marinis, T. Porcelli, M. Giuliani, B. Costantini, et al., Administration of exogenous ghrelin mimics the effect of acute ghrelin administration in human obesity: syndrome: effects on plasma levels of growth hormone, glucose, and insulin, Fertil. Steril. 88 (2007) 125–130.

[109] F. Tassone, F. Broglio, S. Desteianiu, S. Rovere, A. Benso, C. Gotti, et al., Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity, J. Clin. Endocrinol. Metab. 88 (2003) 5478–5483.

[110] D.E. Cummings, J.Q. Purnell, R.S. Frayo, K. Schimidova, B.E. Wisé, D.S. Weigle, A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans, Diabetes 50 (2001) 1714–1719.

[111] D.E. Flanagan, M.L. Evans, T.P. Monsod, F. Rife, R.A. Heptulla, W.V. Tamborlane, et al., The in vivo effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure, Circulation 110 (2004) 3674–3679.

[112] K. Dezaki, H. Hoshoda, M. Sakei, S. Hashiguchi, M. Watanabe, K. Kangawa, et al., Endogenous ghrelin in pancreatic islets represses insulin release by attenuating Ca2+ signaling in beta-cells: implication in the glycemic control in rodents, Diabetes 53 (2004) 3142–3151.

[113] K. Dezaki, H. Sone, M. Koizumi, M. Nakata, M. Kakei, H. Nagai, et al., Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance, Diabetes 55 (2006) 3486–3493.

[114] J.C. Chuang, I. Sakata, D. Kohno, M. Perrelli, S. Osborne-Lawrence, J.J. Repa, et al., Ghrelin directly stimulates glucagon secretion from pancreatic alpha-cells, Mol. Endocrinol. 25 (2011) 1600–1611.
J. Kong, J. Chuddy, I.A. Stock, P.M. Loria, S.V. Straub, C. Vage, et al., Pharmacological characterization of the first in class clinical candidate PF-05190457: a selective ghrelin receptor competitive antagonist with inverse agonism that increases vagal afferent firing and glucose-dependent insulin secretion ex vivo, Br. J. Pharmacol. 173 (2016) 1452–1464.

A Study Of PF-05190457 In Healthy Volunteers And Type-2 Diabetic Patients

ClinicalTrials.gov NIH https://clinicaltrials.gov/ct2/show/NCT01372163?term = PF-05190457&rank = 3 (2012).

W.S. Denney, G.E. Sonnenberg, S. Carvajal-Gonzalez, T. Tuthill, V.M. Jackson, Pharmacokinetics and pharmacodynamics of PF-05190457: the first oral ghrelin receptor inverse agonist to be profiled in healthy subjects, Br. J. Clin. Pharmacol. 83 (2017) 326–338.