Ghrelin function in human obesity and type 2 diabetes: A concise review.

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Abstract

The 28 amino acid hormone, ghrelin has been found to have various effects on metabolism. This review will focus on the pathways integrated into ghrelin's effect within the hypothalamus, pancreas and adipocytes. The identification of molecules and pathways that regulate ghrelin mediated lipid retention could establish new mechanisms underlying cellular energy homeostasis. The impact of acyl-ghrelin on glucose metabolism and lipid homeostasis may allow for novel preventative or early intervention therapeutic strategies to treat obesity related type 2 diabetes and associated metabolic dysfunction.

1 Introduction

Type 2 diabetes mellitus (T2DM) and obesity are both chronic conditions associated with significant morbidity and mortality predominantly from associated chronic diseases. The World Health Organisation (WHO) states that obesity is a global epidemic, with over 1.9 billion obese adults. Obesity results from the dysregulation of energy expenditure resulting in an accumulation of white adipose tissue due to adipogenesis or lipid retention [1]. Therefore, the function of adipocytes, which includes the secretion of various hormones linked to the modulation of metabolism, is central to the development of obesity. A key hormone of interest, that is known to regulate cell proliferation and lipid retention, is the gastrointestinal peptide ghrelin [2]. The focus of this article is to review the mechanism(s) underlying ghrelin mediated lipid retention in adipocytes and its relevance to the aetiology of T2DM. The identification of new molecules modulating ghrelin mediated lipid retention in adipose tissue may be a therapeutic strategy for improving glucose homeostasis associated with obesity and T2DM.

2 Ghrelin

In 1999, Masayasu Kojima first described a 28 amino acid peptide hormone located in the stomach, with a distinctive N-octanoylated serine 3 residue [2]. This peptide was named 'ghrelin', from the Latin word 'Ghre' which means 'grow' due to its role as a growth hormone releasing peptide [2, 3]. The novel peptide was isolated from the gut of both human and rat as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [4]. GHS-R is transcribed in humans from the growth hormone receptor 1 (*GHR1)* gene, which encodes the full length functional receptor (GHS-R1α) and a splice variant truncated non-functional isoform (GHS-R1β) [5, 6]. GHS-R1α mRNA is expressed at low levels over a wide tissue distribution but it predominantly expressed in the anterior pituitary gland [5]. The highest levels of ghrelin are secreted from the X/A- like cells of the oxyntic glands located in the gastric fundus, with lower levels widely distributed throughout the body [7, 8]. Ghrelin is secreted

direct into the local gastric circulation and transported to the brain directly, requiring it to either cross the blood-brain barrier via a saturated transport system or via the blood stream to enter areas of the brain that are not protected by the blood brain barrier [9]. Ghrelin also modulates the hypothalamic arcuate nucleus (ARC), in an indirect manner, via activation of the vagus nerve and brain stem nuclei [9, 10]. Ghrelin circulates in two major forms; acylated (approximately 5% of total ghrelin) and desacyl (95% of total ghrelin) [11]. Previous work has demonstrated that the protein coding gene, membrane bound O-acyltransferase 4 (MBOAT4) is vital in the activation of ghrelin [12]. The human gene, MBOAT4 is located on chromosome 8 (8p12) and contains 6 exons. MBOAT4 transcribes a protein that was later termed ghrelin O-acyltransferase (GOAT), as this is the only enzyme known to post translationally acylate ghrelin. Both forms of ghrelin are observed to cross the barrier in a blood to brain direction. However, desacyl-ghrelin had reduced ability in crossing the barrier in a brain-blood direction [13]. Ghrelin's ability to cross the blood-brain barrier is the result of a combination of three systems; non-saturable, saturable blood-brain transport and saturable brain-blood transport, all of which are dependent on the unique post translational acylation and primary structure [13-15]. In the last 16 years research has demonstrated that ghrelin has various peripheral effects and caused a great interest into the manipulation of the ghrelin system as a pharmacological tool. Ghrelin has a homeostatic role that encompasses multiple areas of the body, with actions that include; downregulation of brown adipose tissue thermogenesis[16-18], modulation of non-hypothalamic brain regions producing an increased taste sensation[19] and stimulation of gastric emptying and motility[6, 20]. The actions of ghrelin may contribute to the development of T2DM and obesity[6] however this review will focus, in detail, on three main sites of ghrelin action; the hypothalamus, adipose tissue and the pancreas (Figure 1).

2.1 Ghrelin & the hypothalamus

2.1.1 Obesity

Ghrelin gene expression and plasma ghrelin levels change with food intake, such that in healthy humans acylated levels are elevated before feeding and decreased by feeding [21-23]. However, during prolonged fasting, plasma levels of acyl-ghrelin are not elevated and it is plasma desacyl-ghrelin levels that double [24, 25] . Indeed, ghrelin is the only known gastrointestinal hormone whose concentration is increased in the blood following calorie restriction. Ghrelin is often termed 'the hunger hormone' and is an appetite stimulatory peptide [26]. The mechanism by which ghrelin stimulates feeding is well documented, the route of stimulation has been linked to ghrelin's activation of Neuropeptide Y (NPY) neurons and agouti- related peptides (AGRP). NPY and AGRP containing neurons are located in the ARC where they are activated by elevation of intracellular calcium via phospholipase C/protein kinase C and adenylate cyclase/protein kinase A pathways [27]. In rodents, this calcium influx has also been linked to ghrelin's activation of phosphoinositide 3-kinase (PI3K) pathway due to the enhanced phosphorylation of Akt-Ser 473 [28]. As well as the activation of orexigenic neurons, neurons containing anorexigenic peptides (pro-opiomelanocortin) are suppressed in the presence of ghrelin [22, 29, 30]. Ghrelin's role in stimulating feeding is reported to be the direct effect of circulating ghrelin on the hypothalamus, as orexigenesis is present without intact vagal afferent signalling [31]. However, there are reports suggesting that vagotomy does result in the loss of ghrelin's orexigenic function [32]. This indicates that peripheral ghrelin induces an eating stimulatory effect via an intertwined relationship involving the afferent vagus nerve and direct endocrine signalling. The cross comparison of published findings, with respect to ghrelin's paracrine vs endocrine signalling method, does require caution in interpretation as alternative results may represent varying methodologies. Several published studies have reported an association between ghrelin and obesity with a main focus on plasma ghrelin levels being negatively correlated with body mass index (BMI) [29]. As well as in obese patients, total ghrelin levels are reduced in obese patients with T2DM and levels does not fluctuate throughout the day. Therefore, the total ghrelin

concentration does not return to baseline level as it does in lean patients after feeding [11, 33, 34]. The obese state has attenuated ghrelin levels resulting in abnormal hunger scores, upon the administration of acyl-ghrelin in humans, hunger profiles within obese subjects are returned to that of lean subjects [35]. The supressed level of ghrelin within diet induced obesity (DIO) can be linked to the decline in the expression of ghrelin and MBOAT4 in the stomach and GHS-R expression in the hypothalamus of mice [36]. DIO also has a detrimental effect on the neuroendocrine ghrelin system and causes ghrelin resistance in rodents through the down regulation and dysfunction of NPY/ AGRP neurons [36]. The apparent species-dependent discrepancy in DIO-induced ghrelin resistance warrants further research and may prevent the ghrelin system being a useful therapeutic target in DIO [37].

2.1.2 A role for ghrelin in human obesity?

Obesity is a multifactorial disease and its origin can span from various avenues including genetics. Genetic factors are strongly associated with obesity and cover two areas, monogenic and polygenic factors. Prader- Willi syndrome (PWS) is a key example of genetic obesity and is the result of a loss of paternal genes in the q11-13 region of chromosome 15. Patients with PWS experience excessive appetite (hyperphagia) which is linked to hyperghrelinemia [38]. PWS patients have increased fasting and post prandial ghrelin levels when compared to non PWS obese or lean patients [38]. This indicates a potential role for ghrelin as a cause of hyperphagia and obesity in PWS due to either an increase in ghrelin gene expression, a decline in transcription inhibitory factors or a reduction in ghrelin clearance [39]. The inhibition of the ghrelin system may allow for the treatment of individuals diagnosed with PWS. This may be achieved by i) ghrelin immunization, ii) MBOAT4 or GOAT inhibition, or iii) antagonizing GHS-R.

Zorrilla *et al*, demonstrated that ghrelin immunization through a vaccine approach produced a significant reduction in weight through metabolic efficiency rather than altering hyerphagia [40]. Further immunizations were carried out through the use of Spiegelmers, a single stranded mirror image oglionucleotide which binds to ghrelin to decrease its half-life [37, 41]. However, the use of

Spiegelmers only generated weight reduction on a short term basis [41]. Preventing the acylation of ghrelin through either the reduction in MBOAT4 transcription or GOAT function will allow for a decrease in circulating acyl-ghrelin. The use of an antagonist of the GHS-R, [D-Lys[3]]GHRP-6, has been shown in male mice to allow for an increase in glycaemic control and decrease in body weight [42]. The reduction in glucose levels was mirrored by a decrease in insulin levels, indicating that GHS-R antagonism may be a therapeutic pathway for treating both obesity and insulin resistance [42, 43].

Controversially, an antagonist is only efficacious when blocking the action of an agonist, yet, as previously mentioned, the low levels of circulating ghrelin present in obese subjects may invalidate this approach. However, further work has led to the development of GHS-R1α inverse agonists that work on the principle that GHS-R1α signals ~50% activity, independent of the presence of ghrelin [44]. The following inverse agonists; [D-Arg-1, D-Phe5, D-Trp7,9, Leu 11]- substance P, which prevent constitutive GHS-R1α activity, were administered intracerebroventricularly in rats, resulting in a reduction in food intake and weight gain [45]. Han Lee, and colleagues also noted that the neuronal depletion of GHS-R abolished ghrelin-induced food intake and was detrimental to the development of DIO [46]. These finding indicate that GHS-R is a key modulator in energy metabolism and plays a role in DIO. We propose that further research is needed to elucidate the possible adipogenic and lipogenic role of constitutive GHS-R1α signalling in the context of human obesity.

2.2 Ghrelin & Adipocytes

2.2.1 Adipogenesis

Ghrelin is involved in the regulation of metabolic hormones, with GHS-R's present within adipose tissue [4, 47]. In addition to stimulating growth hormone (GH) secretion and appetite, ghrelin has been shown to play a role in adiposity. Adipogenesis is a regulated process involving the differentiation of pre-adipocytes into mature adipocytes. This is controlled by specific transcription factors; peroxisome proliferator-activated receptor γ (PPARγ) and sterol-regulatory element binding protein-

1 (SREBP1). SREBP1 is encoded by the *SREBF1* gene which is transcribed into two splice variants, SREBP1-a and SREBP1-c [48]. Upon the addition of acyl-ghrelin and desacyl-ghrelin, PPARγ and SREBF1 mRNA expression levels increase in human visceral adipocytes during differentiation [11]. Therefore, in the presence of ghrelin, *in vitro* and *in vivo* studies have demonstrated that mRNA levels of PPARγ are increased resulting in the differentiation and proliferation of preadipocytes [11]. PPARγ activity can be influenced by key components that play a role in the mammalian insulin pathway, for example; mammalian target of rapamycin complex 1 (mTORC1) and the Akt/ Protein kinase B (Akt/PKB) complex [27]. In the presence of acyl-ghrelin, mTORC1 and Akt/PKB can enhance PPARγ activation promoting adipogenesis [27]. The presence of mTORC1 and Akt/PKB highlights ghrelin's ability to have multifactorial effects, with the synergy of acyl-ghrelin's adipogenic effect and insulin signalling. Both desacyl and acyl-ghrelin produce an increase in adipogenesis and a decrease in insulin sensitivity, however desacyl-ghrelin's role is not consistent across studies (Table 1) [27]. The elevation of SREBF1 was accompanied by a significant increase in lipid accumulation in visceral adipocytes [11]. This administration of ghrelin directly stimulated intracytoplasmic lipid accumulation via the increased production of various fat storage promoting enzymes including carboxylase, acetyl CoA, fatty acid synthase and lipoprotein lipase [11].

2.2.2 Lipid retention

Following chronic intravenous administration in mice, ghrelin has also been shown to increase mRNA expression of genes which promote the retention of cholesterol [49]. The reverse cholesterol transport pathway, the removal of excess cholesterol from peripheral tissues back to the liver for excretion and catabolism, is critical due to the pathways role in defence against atherosclerosis [50-52]. This cholesterol efflux is dependent upon the ATP binding cassette (ABC)A1 and ABCG1 [50, 53]. ABCG1 and ABCA1 are members of a superfamily of transporters that functions to transport cholesterol to the cell surface for removal by high density lipoproteins (HDL) [50, 52]. PPARγ induces the expression of ABC via nuclear cholesterol sensors, liver X receptors (LXR) i.e. LXRα and LXRβ [52]. LXR acts as a

transcription regulator for the genes associated with cholesterol efflux, which is activated when total cellular cholesterol levels are high [54]. However, when total cellular cholesterol levels are low, SREBP induces cholesterol biosynthesis [54]. So in the presence of ghrelin, SREBP1-c expression is increased whilst ABCG1 and LXRβ expression is decreased [49]. Even though these results indicate ghrelin has a role in increasing lipid retention within adipocytes, the relationship between cholesterol efflux, biosynthesis genes and ghrelin is disputed among studies. The hypothalamic activation of GHS-R1α in mice and rats results in the activation of Sirtuin 1 (SIRT1) to deacetylate the tumour suppressor gene p53, increasing phosphorylation of AMP-activated protein kinase (AMPK) which in turn inactivates fatty acid biosynthesis and activates fatty acid oxidation [55-57]. *In vivo*, administration of ghrelin to p53 null mice demonstrates a decrease in lipid metabolism modulating gene expression i.e. SREBP1c, indicating that p53 is essential for the action of ghrelin on adipose tissue [55]. Ghrelin administration has also been reported to activate the PPARγ-LXR-ABC pathway in a dose dependant manner, where ghrelin results in an increase in LXR and ABC expression in human THP-1 macrophages [58]. An increase within cellular fat mass could result in an increase in lipogenesis and substrate uptake and a decrease in lipolysis and export [1]. These processes could alter the intrinsic regulation of free fatty acids and cholesterol biosynthesis pathways that could lead to hypertriglyceridemia and other complications [59]. Due to the diversity of published data and various doses of acyl-ghrelin administered, further research into ghrelin mediated lipid retention especially within humans is needed.

2.3 Ghrelin & the pancreas

2.3.1 Glucose homeostasis

The process of glucose homeostasis is dependent on the liver and gut as sources for circulating glucose and adipose tissue as a peripheral organ for glucose utilisation. Ghrelin and GHS-R1α RNAs are expressed within the pancreas and β-cells, suggesting a possible relation for ghrelin in affecting glucose homeostasis via insulin function. In humans acyl-ghrelin has direct metabolic actions at a

peripheral level, influencing endopancreatic function and altering glucose's diabetogenic action [60, 61].

As well as acyl-ghrelin, desacyl-ghrelin has been shown to alter glucose metabolism, the intravenous administration of desacyl-ghrelin in humans has been demonstrated to promote a favourable influence on glucose metabolism, insulin sensitivity and the inhibition of lipolysis [60, 61]. The expression of GHS-R and ghrelin within the pancreas suggests local regulation of insulin secretion [62, 63], with acyl-ghrelin supressing insulin secretion both *in vivo* and *in vitro*. Dezaki and colleagues [64] reported that endogenous acyl-ghrelin supresses insulin secretion via a restriction on glucose induced cytosolic calcium concentration and insulin release within islet β cells of mice and rats. Furthermore, ghrelin inhibits glucose-induced membrane excitability, supressing cellular signalling [64]. However, the mechanism in which acyl-ghrelin regulates pancreatic islet function remains unclear with few studies in humans. Ghrelin's role in the attenuation of glucose stimulated insulin secretion was elucidated recently, indicating that ghrelin action on β cells may be through a non-direct manner. DiGruccio and colleagues demonstrated that the presence of GHS-R1 on δ cells mediated indirect ghrelin action on β cells [65]. Thus, GHS-R1a$^+$ δ cells, promoted the secretion of intermediate products such as somatostatin through a calcium ion cascade that blocked insulin secretion [65].

Studies in humans has shown that the acute intravenous administration of acyl-ghrelin via bolus injection and infusion are associated with an increase in plasma glucose levels in both healthy and obese patients, and this ghrelin mediated increase was seen to enhance second phase insulin response [8, 26, 66, 67]. In fasted mice this direct, non GH-mediated hyperglycaemic effect was blunted by the oral administration and intraperitoneal injection of a GHS-R antagonist, stabilising blood glucose levels [68]. Broglia *et al* [26] noted that acyl-ghrelin's initial stimulation of somatostatin levels caused an inhibition of insulin release. Further work by Arosio and colleagues [69] noted a similar relationship between acyl-ghrelin, somatostatin and glucose metabolism. However, the simultaneous rise in somatostatin and glucose indicates that ghrelin's effect on glucose metabolism is still unclear. The effect of ghrelin is suggested to be direct yet the query remains as to whether this effect is via the promotion of glycogen breakdown and/or the inhibition of glucose uptake. Upon longer term

administration of acyl-ghrelin in healthy men, at a constant infusion rate lasting 180 minutes at 5 pmol/Kg, glucose levels were raised but glucose-induced insulin secretion was blocked, with the blockage only being restored when ghrelin supply ceased [70]. However, acyl-ghrelin infusion in healthy humans caused peripheral insulin resistance post administration raising the possibility that insulin resistance may be attributed to raised GH and free fatty acid (FFA) secretion [63].

The literature indicates that acyl-ghrelin plays a detrimental role in glucose homeostasis, however studies indicate that desacyl-ghrelin plays a beneficiary role. In rodents and humans, desacyl-ghrelin was proven to enhance insulin levels in response to glucose load and shown to counteract acyl-ghrelins diabetogenic effect [71-73]. This a role for desacyl-ghrelin as a potent secretagogue reiterates the importance to consider desacyl and acyl-ghrelin as separate entities and a possible therapeutic pathway may lie within the desacyl-ghrelin and acyl-ghrelin ratio.

2.3.2 A role for ghrelin in type 2 diabetes?

An important contributor to the pathophysiology of T2DM is the failure of glucose uptake into the peripheral tissues such as adipose, skeletal muscle and liver. Decreased ghrelin levels within patients with T2DM are associated with an increase in abdominal adiposity and insulin resistance [74]. As previously mentioned ghrelin has a demonstrated role in fat metabolism and glucose homeostasis, and cross talk between lipid and glucose metabolism may result in a physiological role for ghrelin in insulin resistance. Cellular lipid accumulation that is observed upon ghrelin administration will have a knock on effect on glucose homeostasis. There are two hypothesese in place regarding lipid mediated insulin resistance. The first is an excess of visceral adiposity triggers the release of FFA into the circulation. An increase in hepatic FFA oxidation triggers insulin resistance and an increase in glucose output from the liver [75, 76]. Acyl-ghrelin infusion in humans has been associated with a rise in circulating FFA levels [35], this could result in a decrease in insulin sensitivity [76]. These findings suggest a causative role in the reported insulin resistance that occurs in healthy volunteers when given an acyl-ghrelin infusion [70, 77]. The second hypothesis is related to enlarged fat laden adipocytes

associated with release of FFA, physical stress and reactive oxygen species (ROS) production [78, 79]. Prolonged elevation of ghrelin increases visceral adiposity in mice and attenuates the transcription of LXRβ and ABCG1 which increases adipocyte volume due to a reduction in lipid export [49]. LXR isoforms have been reported to have anti-inflammatory properties, LXR ligand activation causes the inhibition of NFκB dependant induction of inflammatory genes [80]. This could allow for altered immune function, due to an increase in ROS and the release of damaging inflammatory agents such as tumor necrosis factor α (TNFα). When $Lxrab^{-/-}$ mice are placed under bacterial lipid peroxidase stress there is an increase in the expression of interlukin 1β, TNFα and nitric oxide synthase [81].

This indirect immuno-modulatory response may lead to insulin resistance and T2DM due to TNFα ability to induce the inhibitory phosphorylation of insulin receptor substrate (IRS)-1, leading to systemic insulin resistance [79]. The association between obesity and T2DM is well documented, allowing for ghrelin signalling to play a potential pharmacological role in its prevention and/or treatment. YIL-781 is a piperidine substituted quinazolinone derivative with a selective affinity for GHS-R1α, it is a competitive antagonist for GHS-R1α resulting in the blockage of the ghrelin binding domain [82]. Upon YIL-781 oral administration at 3 mg/kg to insulin resistant DIO rats it was shown to reduce fat mass, enhance glucose stimulated insulin secretion and promote weight loss [82]. Ghrelin's ability to cause hyperglycemia through GHS-R1α could underpin a therapeutic pathway that can alter insulin secretion and prevent glucose intolerance in T2DM [68]. The attenuation of rat islet β cells in the presence of ghrelin indicates that a ghrelin blockade may result in an increase in previously supressed glucose-induced insulin release. This will enable pancreatic islets to function at a higher yield, producing more insulin and restoring the body to normoglycemia [83]. However, the published data regarding the mechanism between ghrelin and insulin secretion is varied within humans. Tong et al [84], infused 12 healthy volunteers with ghrelin at 0.3, 0.9 or 1.5 nmol/kg/h, and show that ghrelin can reduce glucose stimulated insulin secretion. The range of ghrelin in this study fall within the physiological range in the circulation which may not be a true representative of the lower

concentration of ghrelin found in β cells [85]. Further insight in humans or human cells need to be explored in order to investigate species-dependent effects.

Exploration of novel research into ghrelin's relationship with obesity, lipid retention and glucose homeostasis may elucidate novel strategies to treat and prevent T2DM. As well as therapeutic avenues in T2DM, the treatment must take into consideration the physiological roles that ghrelin has in other regions of the body. The blocking of GHS-R1α may seem beneficial for obesity but may cause detrimental effects in other organs. Recently published data demonstrates that ghrelin has an AMPK-dependent protective role in substantia nigra dopamine neurons in a mouse model of Parkinson's disease [86]. Furthermore, acyl-ghrelin promotes new neurone formation and enhances cognition in the brains of adult rodents [87, 88]. With ghrelin reported to have effects on multiple organs in the body it is clear that new ghrelin-based therapies for T2DM need to consider potential adverse consequences, particularly for brain function.

3 Future work

In order to establish a possible therapeutic role for ghrelin within human obesity and T2DM previous research findings must be amalgamated and developed further to delineate ghrelin-specific pathways. The examination of the use GHS-R1α inverse agonist to prevent DIO will require the optimisation of dosage to ensure that blocking the receptor will not have detrimental effects on the other important actions of ghrelin. An *in vitro* study in human adipocytes would determine the effect that a physiological range of desacyl and acyl-ghrelin has on lipid retention and would allow for the molecular characterisation of this effect. This will lead to a better understanding of the cellular mechanisms involved, potentially leading to novel treatments for complications associated with atherosclerosis and hypertriglyceridemia. For instance, translating previous findings by Davies and colleagues [49], is important as it could provide evidence for the speculation that ghrelin immunization may prevent lipid retention within adipocytes. As previously mentioned the resultant

fat laden adipocytes could be detrimental and result in the development of T2DM, so in essence we envision that the prevention of lipid accumulation within adipocytes will prevent the indirect immune modulating response that may be leading to insulin resistance and T2DM. In addition, this area of research requires further insight into whether the effect of ghrelin on adipocytes is dependent on acylation of the hormone.

4 Summary

This review considers the important roles that ghrelin plays in regulating lipolysis, lipogenesis and adipogenesis within adipocytes. However, the studies compared in this review were performed in different species and predominantly in rodents. Further work is essential to understand the role of ghrelin in cellular energy balance within human adipocytes. Current literature report variations in route of administration, dosage and model species, allowing for the potential misinterpretation of ghrelin's physiological role. An expansion of this work using healthy and diseased humans and human cellular models treated with physiological levels of acyl and desacyl-ghrelin will increase our current knowledge of the pathways associated with glucose and lipid metabolism. Human studies will also allow for the elucidation of the therapeutic potential of ghrelin in various metabolic disturbances but in particular, DIO and its complications.

5 Acknowledgements

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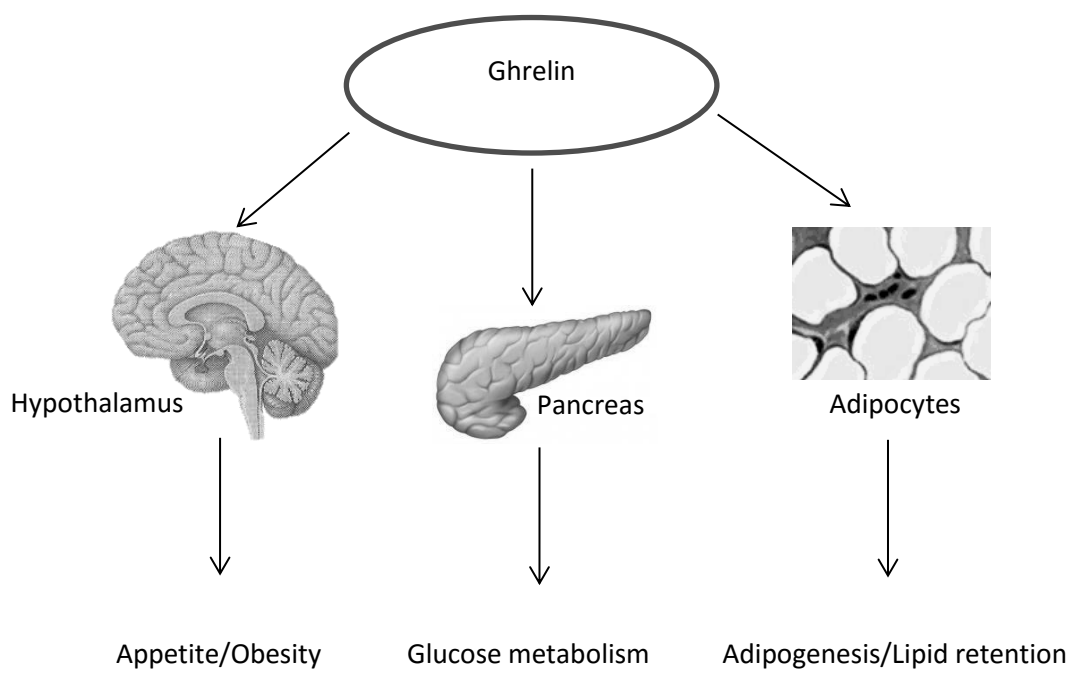
Figure 1. Ghrelin peripheral effects on specific tissues.

Model	Dose	Treatment	Effect	Reference
Healthy humans	1 µg/kg Acyl ghrelin	Intravenous	Decreased Insulin Sensitivity Reduced serum insulin levels	[26]
Healthy humans	Bolus; Des acyl ghrelin [1.1 µg/kg] IV; Des acyl ghrelin [4.0 µg/kg/h]	Bolus injection followed by continuous intravenous infusion	Des acyl ghrelin had no effect on insulin secretion	[89]
Male C57BL/6 mice	5 nmol/day Des acyl ghrelin & Acyl ghrelin	Chronic intracerebroventricular infusion	Hyperinsulinemia	[90]
Human macrophages (THP-1)	1, 10 & 100 nM ghrelin	Treated PMA-differentiated THP-1 macrophages with increasing doses of ghrelin	Promotes activation of the Akt/PKB pathway	[58]
3T3-L1	Acyl ghrelin 1-100 pmol, Des Acyl ghrelin 1-100 pmol	Preadipocytes incubated with des acyl ghrelin and acyl ghrelin for 24 hours	DAG and AG stimulated adipogenesis, fatty acid uptake and inhibited lipolysis	[91]
C57BL/6NHsd mice	100 nM Ghrelin	Wild type mouse pancreatic islets incubated with ghrelin	Reduced glucose stimulated insulin secretion	[65]

Table 1. Effects of ghrelin treatment on insulin secretion and adipogenesis.