

# Diabetes, Ghrelin And Related Peptides: From Pathophysiology To Vasculopathy

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**Abstract:** Ghrelin and obestatin are two different peptides originated from the same gene isolated in the stomach. Numerous actions have been described where these two peptides are implicated in metabolism, appetite regulation, glucose levels regulation, and a wide range of systemic effects. In this article, we summarize (1) the cellular receptors implicated in ghrelin and obestatin effects; (2) the role of ghrelin and obestatin in metabolism and appetite regulation; (3) their role in the glucose homeostasis regulation and its implication in diabetes patho-physiology; (5) the effects of ghrelin and obestatin in regulation of normal angiogenesis and (6) their possible role in the regulation of diabetes-induced pathologic angiogenesis.

**Keywords:** Ghrelin, obestatin, diabetes pathophysiology, appetite regulation, peptides, diabetes vascular complications.

## GHRELIN

Ghrelin is an acylated, 28-amino-acid peptide that promotes the release of GH in the hypothalamus. It is the natural ligand of the GHSR-1a receptor [1]. For it to act on the GHSR-1a ghrelin has an n-octanoic acid modification on serine 3 residue [2].

The ghrelin gene is located in chromosome 3 (3p25-26) [1], contains four preproghrelin-coding exons, and encodes a precursor of 117aa (preproghrelin) with 82% of homology between species [3]. As a result of alternative splicing of this gene, a 27 aa acylated peptide was identified with the same activity potency as ghrelin (des-Gln14-ghrelin) [4]. Another form of ghrelin, des-acyl ghrelin, exists at significant levels in both stomach and blood. This variant lacks the octanoyl chain in serine 3 and represents more than 90% of the circulating peptide [2, 4, 5]. In plasma, acyl ghrelin levels are 10–20 fmol/ml while total ghrelin levels are 100–150 fmol/ml (including both acyl and non-acyl forms) [6, 7]. Several minor forms of ghrelin were described with modifications on the peptide chain or on the acidic chain and are only present in low amounts [4, 8]. Some of these are independently produced and regulated and their levels are not directly related to those of ghrelin [3]. Finally, in a posttranslational process, this gene can originate a 23 aa peptide named obestatin that has some different and even opposite actions than ghrelin [9].

During adult life ghrelin is synthesized mainly in the gastric oxyntic mucosa in the X/A cells. Ghrelin is also produced in the X/A cells of the intestine and in some others tissues such as the pancreas, kidney, placenta, lymphatics,

gonads, adrenal, thyroid gland, heart, lung, pituitary, hypothalamus, eye [10], human B- and T-lymphocytes, neutrophils [1, 10-12], morula, blastocysts and embryos [13]. Ghelardoni observed that ghrelin gene expression and its protein were, in some tissues, dissociated [14].

In fetal life, ghrelin is mainly produced in the pancreas and lung [15]. The pancreas expresses ghrelin mRNA at midgestation being its mRNA levels six to seven times higher than in the fetal stomach [16]. In this period ghrelin's production in the stomach is very low and only increases after birth [17]. In the fetal lung, this peptide is highly expressed after the pseudoglandular stage of the development [18, 19].

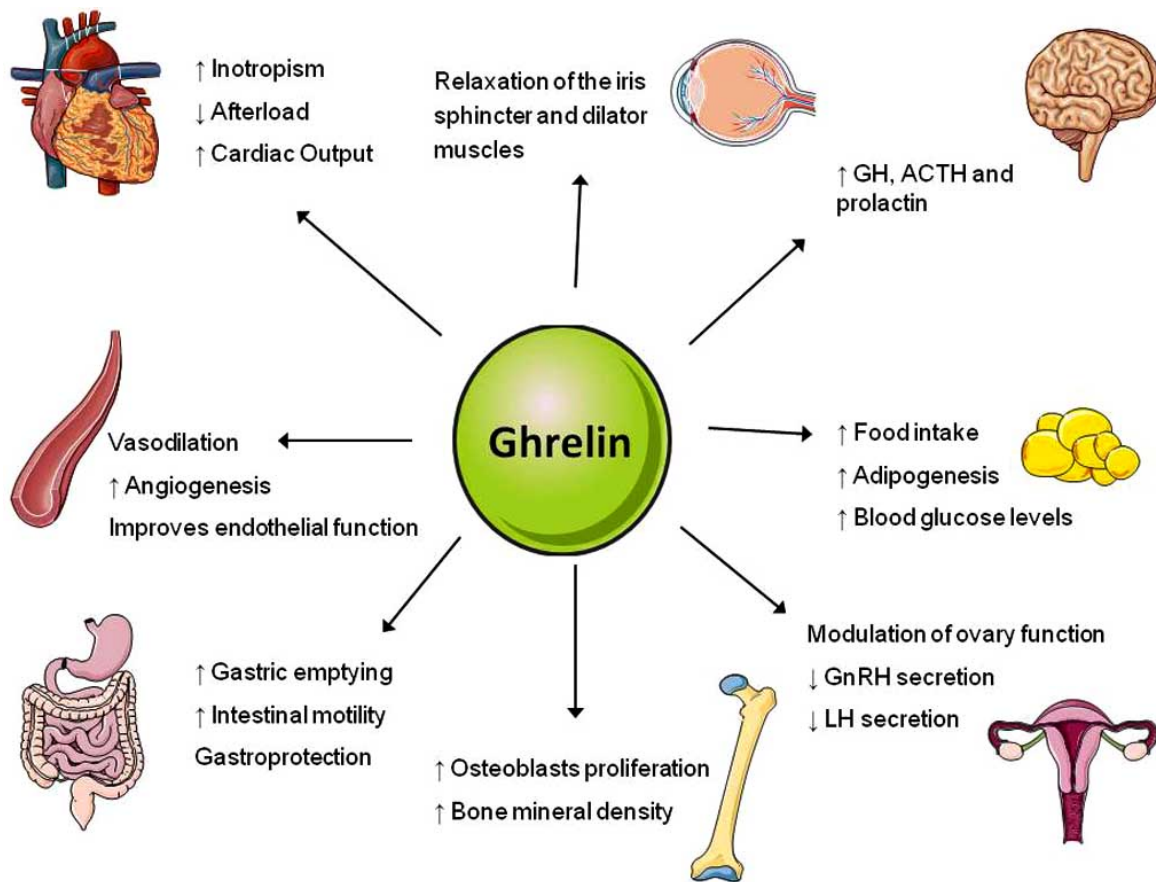
In plasma, ghrelin circulates associated to triglyceride-rich lipoproteins (TRL), high-density lipoproteins (HDLs), very high-density lipoproteins (VHDL) and, to some extent with low-density lipoproteins (LDL) [5, 20], while des-acyl ghrelin circulates as a free peptide. Lipoproteins contain a potent esterase, paraoxonase, which may be involved in deacylation of acyl-modified ghrelin [21]. Therefore, des-acyl ghrelin may represent either a precursor of acyl-modified ghrelin or the product of its deacylation, having been reported some biological effects, including the modulation of cell proliferation and, to a small extent, adipogenesis of the non-acylated form [22-24]. The main systemic effects of ghrelin are summarized in Fig. (1).

## GHRELIN RECEPTORS

Ghrelin exerts its action through the activation of the GHSR-1a receptor. This is a seven-transmembrane domain G-coupled-protein receptor that belongs to the G-protein-coupled receptors superfamily which includes the motilin, neuromedin U and neurotensin receptors [25]. This receptor has 366 aminoacids (41 KDa) and is encoded by a gene located in the chromosome 3q26.2 [25, 26]. Through an alternative splicing program, the gene originates two different forms of the receptor, respectively GHSR-1a and GHSR-1b.

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**Fig. (1).** Overview of the effects attributed to ghrelin in different organs and tissues.

GHSR-1a is the functional form whilst GHSR-1b does not have, until now, known biological activity [25, 27]. Recently it was proposed that the GHSR-1b could suppress the activity of GHSR-1a by interaction through heterodimerization [27]. GHSR-1b is expressed in numerous endocrine and non-endocrine tissues including heart, thyroid, pancreas, spleen and adrenal gland [28], being more expressed than GHSR-1a in some tumors [11, 29-31].

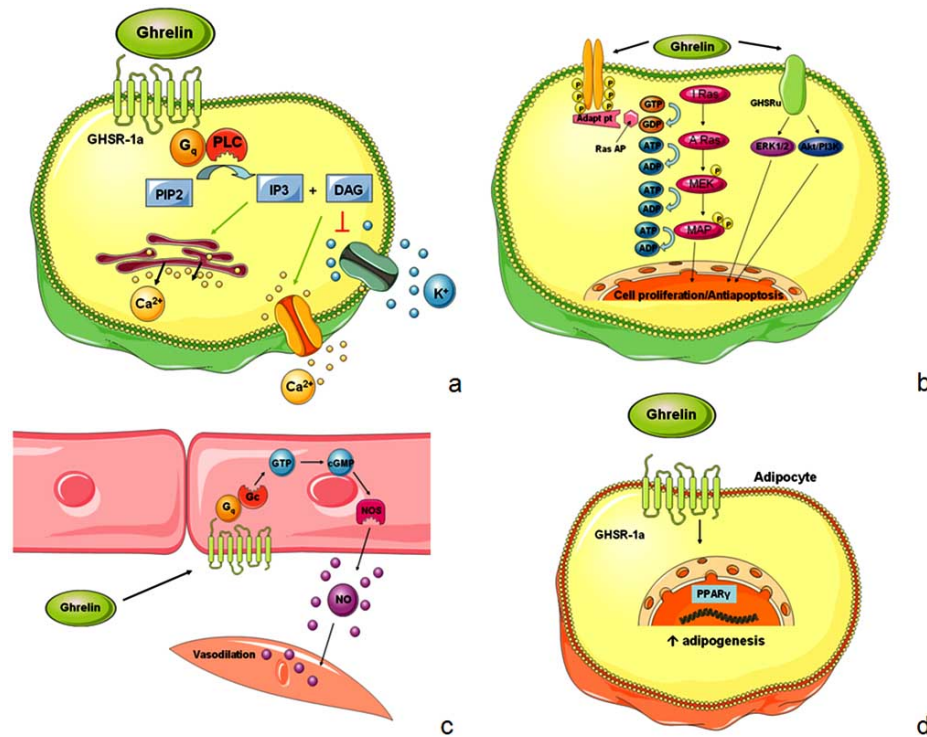
In its structure GHSR-1a has two different binding sites. The first one, the common binding site for ghrelin, is the transmembranar domain 3 [32], while the second was proposed to be an adenosine binding site [33]. This purine triggers a rise in intracellular calcium [34, 35]. However, recent reports proposed that this could be an action of adenosine in the  $A_{2B}R$  purinergic receptor [33, 34]. After its activation GHSR-1a promotes the activation of phospholipase C (PLC), phosphatidyl-inositol-4,5-biphosphate hydrolysis with the formation of diacylglyceride (DAG) and inositol triphosphate ( $IP_3$ ). While  $IP_3$  promotes a transient elevation of intracellular calcium from the sarcoplasmic reticulum, DAG induces depolarization by the inhibition of potassium channels and opening of the voltage-dependent L-type calcium channels of the cellular membrane [36].

Ghrelin also stimulates cell proliferation by activating several different pathways, including the mitogen-activated protein kinase (MAPK), extracellular signal-regulated protein kinases (ERK1/2), the transcriptional factor Elk1 [37], through the Akt/PI3K signaling [38] and through a tyrosine-kinase dependent pathway [22]. Another different pathway

for ghrelin action is the nitric oxide (NO)/cGMP signaling pathway, that promotes vasodilation [39, 40]. Finally, in differentiated adipocytes ghrelin increases the expression of peroxisome-proliferator-activated receptor gamma 2 (PPAR $\gamma$ 2), a nuclear receptor that regulates transcriptional pathways in adipogenesis, through a GHSR-1a dependent pathway [41]. The subcellular mediation of ghrelin's effects is illustrated in Fig. (2).

The main ghrelin receptor, GHSR-1a, was first identified in the pituitary and in the arcuate nucleus of the hypothalamus [25, 26]. Its expression was also described in other areas of the CNS and in some peripheral tissues as: thyroid gland, pancreas, spleen, myocardium, adrenal glands [11, 14, 42, 43], rat testis, ovary [44], human T lymphocytes [12], morula, blastocysts and embryos [13]. There were identified binding sites in the myocardium (highest binding capacity), adrenal gland, gonads, arteries, lung, liver, skeletal muscle, kidney, pituitary, thyroid gland, adipose tissue, veins, uterus, skin and lymph nodes [45]. In some of these tissues, the specific binding values were described as even higher than in the pituitary gland [45].

GHSR-1a is not the only receptor described for ghrelin actions. Both acylated and non-acylated forms of ghrelin can mediate some of ghrelin effects while only the acylated form can bind to GHSR-1a. The activation of cyclooxygenase by non-acylated, acylated and truncated forms of ghrelin indicates the existence of another active receptor different from GHSR-1a [10, 46-48].



**Fig. (2).** Intracellular pathways involved in ghrelin's effects. (a) Ghrelin binds to the GHSR-1a which is accoped to a Gq protein, leading to the activation of phospholipase C (PLC). This enzyme converts phosphatidylinositol bisphosphate (PIP<sub>2</sub>) into inositol triphosphate (IP<sub>3</sub>) and diacylglyceride (DAG). IP<sub>3</sub> induces Ca<sup>2+</sup> release from the sarcoplasmic reticulum while DAG inhibits K<sup>+</sup> channels, thereby inducing the opening of voltage-dependent L-type Ca<sup>2+</sup> channels in the cellular membrane. The increase of intracellular Ca<sup>2+</sup> results in membrane depolarization. (b) Ghrelin activates a tyrosine kinase receptor, which leads to the activation of the Ras protein. The double phosphorylation of the Ras protein results in the mitogen-activated protein kinase (MAPK), which needs another phosphorylation to enter the nucleus and regulate cell proliferation. Ghrelin also binds to an unknown receptor which activates ERK1 and ERK2 and Akt/PI3K, culminating in an antiapoptotic effect. (c) In the endothelial cells ghrelin stimulates a G-protein-coupled system which activates the guanylate cyclase (GC). This enzyme transforms guanidil triphosphate (GTP) into cyclic guanidil monophosphate (cGMP). cGMP leads to the activation of nitric oxide synthase (NOS) which increases nitric oxide's (NO) levels. NO enters the smooth muscle cell and promotes relaxation. (d) In adipocytes ghrelin binds to the GHSR-1a and stimulates the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ). PPAR $\gamma$  is a transcription factor regulating genetic transcription and thereby promoting adipogenesis.)

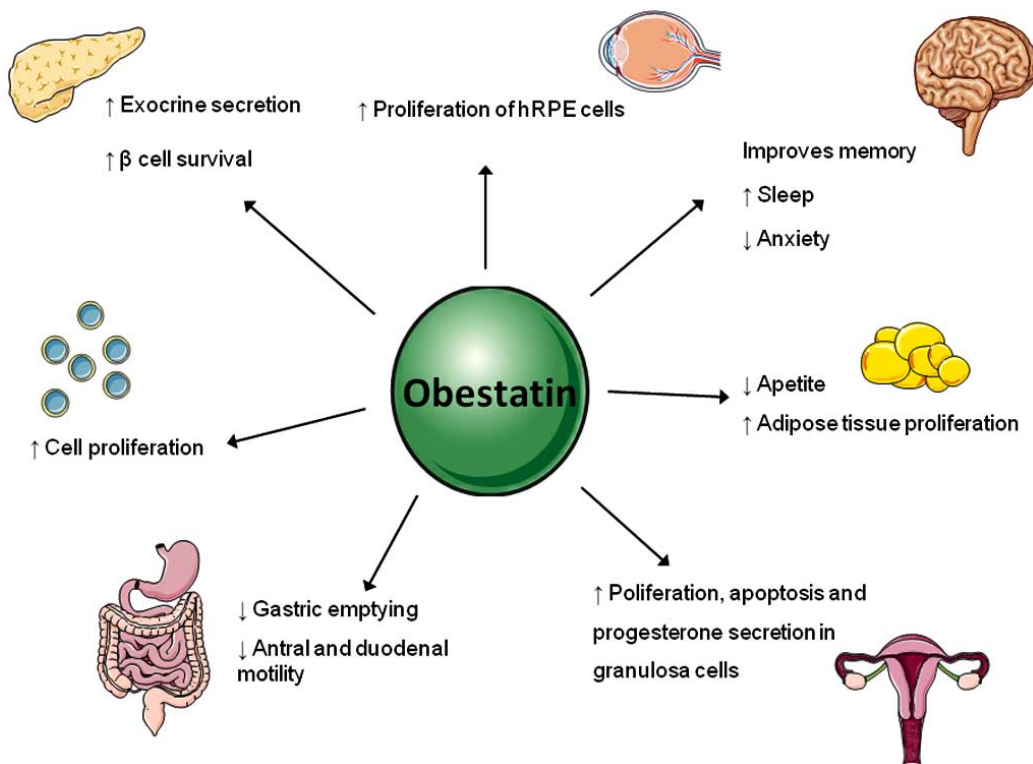
CD36, a multifunctional B-type scavenger receptor expressed in the adipose tissue, platelets, monocytes, macrophages, dendritic cells, microvascular endothelium and myocardium [49], is implicated in ghrelin's role in the protection of the myocardium from ischemia [50].

## OBESTATIN

From the same gene of ghrelin, through a posttranslational or an alternative splicing process, a peptide named obestatin is obtained [3, 9]. This is a 23 amino acid amidated peptide identical to residues 76-98 of the C-terminal peptide of preproghrelin, also isolated in the rat stomach [9]. Obestatin is present in the stomach [9, 51], duodenum, jejunum, colon, pancreas, spleen, mammary gland, breast milk, plasma [9, 52-56], pituitary and hypothalamus [9]. In energy metabolism and gastrointestinal functions obestatin was initially considered to have the opposite functions of ghrelin. Hence, while ghrelin stimulates feeding, obestatin suppresses body weight gain in rats [9]. While ghrelin blood levels vary in fasting, feeding and drinking conditions, obestatin blood levels are constant during these processes [9, 57]. While ghrelin has a prokinetic effect and stimulates the contraction of the jejunal smooth muscle [58, 59], obestatin decreases its contractile activity and suppresses gastric emptying. How-

ever, some of the gastrointestinal and metabolic effects of obestatin were not reproduced in subsequent reports [60]. It has been suggested that the originally described effects could be due to zinc action in the GPR39 receptor [61]. Another reason for the metabolic discrepancies can be related to the peculiar U-shape of the dose response curve [57]. The opposing effects of obestatin and ghrelin are controversial and some authors suggest that obestatin could act as a paracrine/autocrine factor, being degraded rapidly in the serum [62].

Some studies have demonstrated that obestatin stimulates cell proliferation in prostate cancer cells, ovarian cancer cells [3], gastric cancer cells [63] and ovarian granulosa cells [64]. Obestatin has also been reported to regulate sleep [65], to affect memory and anxiety [66], and to inhibit thirst [67]. In the pancreas, obestatin promotes pancreatic juice enzymes secretion [68], promotes the survival of  $\beta$  cells [69] and inhibits glucose induced insulin secretion [70]. Obestatin was co-localized with acetylcholine in the guinea pig myenteric plexus of the gastrointestinal (GI) system [53], suggesting that in the GI tract obestatin is a regulator of the cholinergic system [53]. In ocular tissues, obestatin promotes the proliferation of hRPE cells (human retinal pigment epithelium) in



**Fig. (3).** Overview of the effects attributed to obestatin in different organs and tissues.

a pathway related to ERK  $\frac{1}{2}$  activation [71]. Fig. (3) summarizes the principal effects of obestatin.

### OBSTATIN RECEPTOR

The main receptor involved in obestatin's actions is not known, but it was suggested that GPR39, a rodopsin type G coupled receptor, member of the ghrelin receptor superfamily, might fit the role [9, 72]. GPR39 expression was described in the stomach, small intestine, amygdala, hippocampus, pituitary and auditory cortex, while low levels were identified in several other brain regions [9, 72, 73]. However, the expression in the hypothalamus was not confirmed by "in situ hybridization" and real time quantitative polymerase chain reaction [74]. Recent reports suggest that obestatin may not be the major ligand of GPR39 [75]. It was also demonstrated that obestatin is not able to bind to GPR39 [75]. Purified obestatin does not stimulate cAMP and SRE (serum responsive element) response by cells expressing GPR39 [76, 77]. Finally, knockout animals for GRP39 have accelerated gastric emptying and more effective expulsion of distal located pallets [78] when compared to wild types.

Recently another receptor, the GLP-1 receptor, was proposed to mediate the effects of obestatin [69] in pancreatic  $\beta$  cells. The capacity of obestatin to bind to these cells was not confirmed in latter studies [79].

### ROLE OF GHRELIN AND OBESTATIN IN METABOLISM AND APPETITE REGULATION

Ghrelin plays a role in energy homeostasis and in food intake. Ghrelin's effects on appetite can be divided into short- and long-term. Ghrelin might be an important factor for meal initiation as it stimulates appetite and increases food intake [80-82]. Ghrelin's orexigenic actions are very rapid

and short-lived, as is required for a signal influencing individual meal-related behaviour. [80] Plasma ghrelin levels increase during fasting and decrease postprandially [81, 83]. Indeed, plasma ghrelin levels increase immediately before each meal and fall into basal levels within 1 hour after eating. [7] Ghrelin levels and hunger scores are positively correlated and the postprandial suppression of ghrelin levels is proportional to the ingested caloric load [84]. Lipids suppress ghrelin levels less effectively than carbohydrates or proteins do [85]. Although the kinetics of ghrelin response to proteins differs from that to carbohydrates, the overall magnitude of suppression rendered by isocaloric intake of these two nutrient types is relatively similar [86]. The decrease of ghrelin levels after food intake does not require luminal nutrient exposure in the stomach or duodenum, the major sites of ghrelin's production [85, 87]. Signals which mediate this response are not entirely understood but originate downstream in the intestine and from post-absorptive events [85, 87]. Ghrelin exerts its orexigenic effect by reaching the hypothalamus via three different ways: via the bloodstream into the arcuate nucleus, *via* peripheral ghrelin receptors on the vagus nerve [88] and *via* local hypothalamic ghrelin-secreting cells [1]. The arcuate nucleus is involved in food intake and adiposity [89]. Here, GHSR-1a is expressed [1] and ghrelin stimulates neurones containing the orexigenic factors neuropeptide Y and agouti related peptide [80, 81, 90, 91] and inhibits neurones producing the anorexigenic peptides pro-opiomelanocortin, cocaine, and amphetamine related transcript [90]. Neurones in the arcuate nucleus stimulate orexin containing neurones in the lateral hypothalamic area to increase appetite [92]. The primary effect of ghrelin injections on meal patterns is the decrease of the inter-meal intervals, thus increasing meal number without affecting meal size [93].

In addition to its orexigenic effects ghrelin plays a role in the regulation of long-term energy homeostasis. Continuous or repeated ghrelin administration increases body weight [9, 80, 82, 94]. Beyond stimulating overall food intake, ghrelin increases the preference for fat diets and induces adipogenesis [41, 80, 82]. It can also decrease adipocyte apoptosis [95], lipolysis [41, 96], energy expenditure [9] and sympathetic nervous system activity [97], body temperature [98], proinflammatory cytokines production [12] and locomotor activity [99]. Therefore, ghrelin plays a role in all aspects of energy homeostasis, tending to promote weight gain. It has been demonstrated that blockade of endogenous ghrelin signaling in the brain reduces spontaneous food intake and body weight [80, 89]. This reveals the importance of basal ghrelin tone to maintain normal appetite. Ghrelin levels respond to changes in body weight [82]. Ghrelin secretion is upregulated under conditions of negative energy balance and downregulated in the setting of a positive energy balance [100]. Plasma ghrelin levels are increased in patients with anorexia nervosa [7, 100] and after recovery these levels decrease [101]. Similarly, GHSR-1a expression in the hypothalamus rises in acute or chronic food restriction [102]. This is consistent with the appetite stimulating effect induced by ghrelin. Conversely, in obesity ghrelin plasma levels are decreased, suggesting a physiological adaptation to a positive energy balance [103, 104]. Importantly, ghrelin levels inversely correlate with body mass index and fasting serum insulin levels [103, 105]. However, when obese subjects are at fasting conditions their ghrelin levels increase more than in lean people. After each meal, ghrelin is less suppressed and as a result their weight gain is supported [106]. When obese subjects lose weight, plasma ghrelin levels increase and the range of increase is positively correlated to the extent of the weight loss [104]. This result is independent on diet changes [107]. Hence, ghrelin levels alterations compensate bidirectional changes in body weight, consistent with the hypothesis that ghrelin contributes to the known adaptative metabolic responses to such alterations.

Stimulatory [108] and inhibitory [109, 110] effects on food intake have been attributed to des-acyl ghrelin. Its effects on appetite seem to be exclusively centrally mediated [108, 109]. Des-acyl ghrelin increases the expression of cocaine, urocortin and amphetamine related transcript in the paraventricular and arcuate nuclei [109]. Overexpression of des-acyl ghrelin, in rats, leads to a decrease in body weight

and fat pad mass weight. It has also been reported a decrease in plasma des-acyl ghrelin levels, but not in acylated ghrelin levels, in non-obese mice that eat more food [111]. Restricted feeding decreases plasma des-acyl ghrelin levels in non-obese and increases in obese animals [111]. Hence, des-acyl ghrelin seems to have opposite effects to that of acylated ghrelin to generate a negative energy balance [109]. However, more studies are necessary to explain the exact role of des-acyl ghrelin on food intake.

The role of obestatin on food intake and body weight is not well established. Some studies do not attribute any effect to it [112, 113] whilst others indicate an inhibitory effect on food intake and a decrease in body weight [9, 114]. Intracerebroventricular injection of obestatin decreases water intake. As a result, the effects of obestatin on food intake may be secondary to an action of the peptide to inhibit water drinking, a phenomenon known as dehydration anorexic effect [67]. Obestatin levels do not vary with a fixed energy meal, but are lower in obese subjects supporting a possible role for obestatin in long-term body weight regulation [115]. The effects of ghrelin, des-acyl ghrelin and obestatin in metabolism regulation are summarized in Table 1.

The short-term regulation of plasma levels of both total ghrelin and the active form of ghrelin is delayed in obese animals. Hypoglycaemia induced by insulin restored the delayed regulation, suggesting that blood glucose levels are involved in the delayed regulation in obese animals [116].

## GLUCOSE METABOLISM

Ghrelin is involved in glucose metabolism. Ghrelin injection in humans increases plasma glucose levels [117] Ghrelin levels are positively correlated to blood glucose levels [118, 119] and negatively to plasma insulin levels [118, 120, 121]. It has been identified ghrelin producing cells in the endocrine pancreas but the type of cell differs from one study to another. Ghrelin producing cells have been co-localized with  $\alpha$  cells [118, 122, 123],  $\beta$  cells [124], and PP cells [122]. However, a specific ghrelin producing cell has been identified, the  $\epsilon$  cell, which does not express any of the other pancreatic hormones [125, 126]. All these results were obtained using different methodologies and therefore, ghrelin may be expressed in different islet cell types depending on conditions such as age or species [118]. Even though the stomach is the major site of ghrelin production [1, 7] it is the pancreatic ghrelin, through an autocrine/paracrine way, that regulates pancreatic insulin release [120]. Ghrelin inhibits insulin secretion from islet  $\beta$  cells [117], an effect which is

**Table 1. Summary of the Metabolic Effects of Ghrelin and its Related Peptides**

	Ghrelin	Des-acyl Ghrelin	Obestatin
Food intake	↑	↑↓	↑↓
Body weight	↑	↓	↓=
Glucose levels	↑	↓	?
Insulin secretion	↓	↓	↑↓=
Insulin sensitivity	↓	↑	?

↑ increase; ↓ decrease; = no effect; ? unknown effect

secretion from islet  $\beta$  cells [117], an effect which is dependent on GHSR-1a [118]. Therefore, the hyperglycaemic effect is due to that pancreatic action, but also to other effects attributed to ghrelin. Ghrelin stimulates glucose synthesis and output from hepatocytes [127, 128] and hampers insulin's capacity to inhibit endogenous glucose production [129, 130]. Therefore, inhibition of insulin release and other mechanisms are involved in the increase of blood glucose levels induced by ghrelin.

In the pancreas, insulin is secreted in two phases and ghrelin suppresses both peaks of insulin release. Dezaki *et al.* have described the signaling mechanisms involved in ghrelin's effect and proposed a pathway to explain how ghrelin inhibits insulin secretion. Ghrelin acts in the GHS-R expressed in  $\beta$  cells. Indeed, GHSR-1a mRNA and protein are expressed in the pancreas of rats and humans [11, 123, 124]. In the pancreas GHSR-1a is linked to  $G\alpha_{i2}$  protein sensitive to PTX [121]. This connection of GHSR-1a to a  $G\alpha_{i2}$  protein is different from the one described on the pituitary that promotes the release of GH, where GHSR-1a is linked to a  $G\alpha_{11}$  protein [25]. The  $G\alpha_{i2}$  protein leads to the activation of voltage-dependent Kv channels causing a rapid repolarization and shortening the bursts of the action potentials. This attenuates the increase in intracellular  $Ca^{2+}$  induced by glucose and thereby insulin secretion [118, 121] (Fig. 4).

Ghrelin may also interfere with insulin secretion in a chronic pattern. The deletion of ghrelin is associated with a reduction in uncoupling protein-2 (UCP2) expression [131]. UCP2 is a mitochondrial protein that modulates the efficiency of ATP production by dissociating the substrates of oxidation from ATP synthesis. This effect of UCP2 has a negative impact on insulin secretion [132]. Hence, chronic deprivation of ghrelin augments glucose dependent insulin

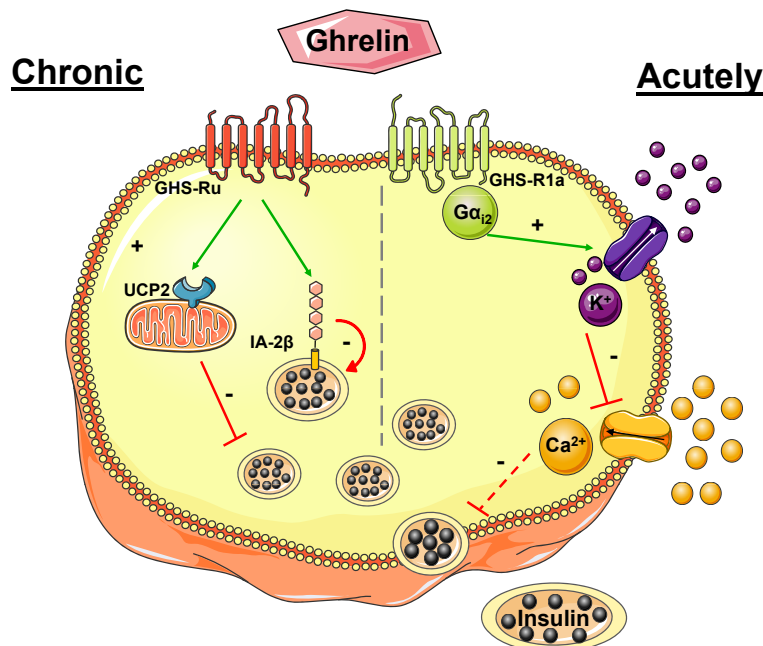
secretion from the pancreatic  $\beta$  cells by diminishing UCP2 expression [131]. Ghrelin also induces the expression of IA-2 $\beta$ , a  $\beta$  cell autoantigen for type 1 diabetes, which is an integral membrane glycoprotein expressed in neuroendocrine tissues and is localized to secretory granules [133]. IA-2 $\beta$  inhibits glucose stimulated insulin secretion. The inhibitory effect of ghrelin in insulin secretion, after glucose stimulation, is at least in part due to an increase in IA-2 $\beta$  [133]. Thus, ghrelin influences negatively pancreatic insulin release. These effects are illustrated in Fig. (4).

Insulin and glucose levels seem to be physiological and dynamic modulators of plasma ghrelin concentrations through an inhibitory effect [100, 134, 135].

Both stimulatory [69] and inhibitory [136] effects have been attributed to obestatin regarding insulin secretion. Another study showed no effect of obestatin in insulin's release from the pancreas [114]. A decrease in plasma obestatin levels is associated with diabetes and impaired glucose regulation [137]. Hence, the effects of obestatin in glucose metabolism are still not completely understood. The effects of ghrelin, des-acyl ghrelin and obestatin in glucose metabolism regulation are summarized in Table 1.

Ghrelin, des-acyl ghrelin and obestatin stimulate cell proliferation and inhibit apoptosis in pancreatic  $\beta$  cells [69, 138].

Des-acyl ghrelin is also capable of regulating glucose metabolism. When given with acylated ghrelin, des-acyl ghrelin prevents the increase of glucose levels [130]. Des-acyl ghrelin improves insulin sensitivity [130]. However, more studies are necessary to determine the role of des-acyl ghrelin in glucose homeostasis.



**Fig. (4).** Subcellular pathways involved in the effects of ghrelin in the pancreatic  $\beta$  cell. Ghrelin may affect pancreatic  $\beta$  cell function and insulin secretion in an acute and chronic pattern. Acutely Ghrelin binds to the GHSR-1a which is accopled to a  $G\alpha_{i2}$  protein leading to the opening of  $K^+$  channels sensitive to voltage. This repolarizes the plasmatic membrane and closes  $Ca^{2+}$  channels. As a consequence insulin secretion is inhibited. Chronic: Ghrelin increases the expression of uncoupling protein-2 (UCP2), a mitochondrial protein that dissociates substrates of oxidation from ATP synthesis and IA-2 $\beta$ , a  $\beta$  cell auto-antigen for type 1 dibetes. These two molecules inhibit insulin secretion.

## DIABETES PATHOPHYSIOLOGY

As a hormone involved in insulin secretion, conditions in which this secretion is impaired, like diabetes mellitus, changes plasma ghrelin levels. Ghrelin plasma levels are reduced in type 1 diabetes mellitus [139]. Another study refers a lack of suppression on postprandial ghrelin levels in patients with type 1 diabetes mellitus; after insulin treatment, postprandial plasma ghrelin levels decreased [134]. Plasma ghrelin levels are decreased in obese type 2 diabetic patients. This decrease may play a causative role in the development of type 2 diabetes mellitus [140]. Some polymorphisms of the ghrelin gene may be associated with type 2 diabetes mellitus. Although the Arg51Gln polymorphism is not frequently expressed in the general population it is significantly represented in type 2 diabetic patients. Thus, this polymorphism may be a risk factor for type 2 diabetes mellitus [141, 142].

Ways to modulate ghrelin's effect on insulin secretion and glucose blood levels are being studied to better understand ghrelin's effects in the pathogenesis of diabetes mellitus.

## ANGIOGENESIS AND DIABETIC RETINOPATHY

Angiogenesis is the process involving the growth of new vessels from pre-existing ones which occurs in both physiological and pathological settings and is characterized by a complex cascade of events. The successful execution of this cascade requires the carefully balanced interplay of growth-promoting and growth-inhibiting angiogenic factors. Identified activators of angiogenesis include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) families, transforming growth factor (TGF)- $\alpha$  and TGF- $\beta$ , angiopoietin-1, and angiopoietin-2. Inhibitors of angiogenesis include thrombospondin, angiostatin, endostatin, and pigment epithelium-derived factor (PEDF). Other endogenous peptides have been reported to play a role in angiogenesis, including ghrelin; however its role is controversial [143].

One of the major complications of diabetes is diabetic retinopathy. The development and progression of diabetic retinopathy is strongly related to pathologic angiogenesis. In diabetic retinopathy, chronic hyperglycaemia leads ultimately to retinal ischemia, the driving force for the expression of angiogenic stimuli and consequently angiogenesis. VEGF plays a central role in the pathogenesis of diabetic retinopathy, both increasing vascular permeability and vascular proliferation.

Chronic hyperglycaemia induces pericyte apoptosis [144], hemodynamic alterations, endothelial dysfunction, inflammation and changes in the expression of growth factors. The major pathogenic pathways include the formation of advanced glycation end products (AGEs), increased activity of aldose reductase and production of reactive oxygen species (ROS) [145].

AGEs are a reflex of prolonged hyperglycaemia and have been correlated with the onset and severity of diabetic retinopathy in animal models [146]. AGEs result from the non-enzymatic glycation of proteins. Cell damage occurs as a result of dysfunction of a wide variety of intracellular and extracellular proteins [145]. AGEs are related with the production of ROS, are known to cause basement membrane

thickening [147], and may induce vascular leakage and an increase in VEGF production [148].

In hyperglycaemic states, aldose reductase reduces glucose into sorbitol. Because the oxidation of sorbitol into fructose is a slow reaction, accumulation of sorbitol occurs inside the cell. Increased sorbitol levels may lead to osmotic cellular damage and endothelial dysfunction [145, 149]. Pericytes are affected by the increased levels of sorbitol and lose their primary contractile function [150]. Pericyte loss eventually leads to endothelial loss and capillary closure and consequently, hypoperfusion.

ROS production is increased in hyperglycemic and hypoxic states [151]. ROS induce structural changes in the microvascular retinal environment. They contribute to AGE's formation, basement membrane thickening and pericytes and endothelial cell loss and capillary closure [152], leading to poor perfusion and hypoxia. ROS also induce the expression of endothelin-1. Endothelin-1 is a potent vasoconstrictor agent that also increases extracellular matrix production [153] contributing to thickening of the basement membrane and to the vasoconstriction seen in diabetic retinopathy.

The increased oxidative stress promotes leukostasis and inflammation. All these mechanisms contribute to the breakdown of the blood retinal barrier [151, 154] and to hypoxia.

Hypoxia upregulates the cellular production of hypoxia inducible factor 1- $\alpha$  (HIF1- $\alpha$ ), a molecule that is important for cellular viability in hypoxic states [155]. HIF1- $\alpha$  stimulates the production of VEGF; oxidative stress also increases VEGF expression [156]. VEGF is a key growth factor in inducing diabetic retinopathy. It induces vascular permeability and angiogenesis. When injected into healthy non-human primate eyes, it can reproduce many of the pathological complications of diabetes including pericyte loss, hemorrhage, macular edema, and retinal vascular proliferation [157]. VEGF promotes neovascularization from existing retinal capillaries. Angiogenic proliferation occurs in areas of transition of good to poor perfusion as well as on the optic nerve head. It also increases vascular leakage by effect on endothelial tight junctions promoting intraretinal edema [151]. VEGF acts through two tyrosine kinase receptors, VEGFR-1 and VEGFR-2. VEGFR-2 is the primary mediator of the angiogenic and proliferative effects of VEGF. In diabetic retinopathy, VEGFR-2 levels are increased [158]. PEDGF is a potent anti-angiogenic agent, but its levels are reduced in diabetic retinopathy [149].

Finally hyperglycaemia also induces the synthesis of DAG in vascular cells, leading to activation of protein kinase C (PKC). PKC has many vascular effects such as inducing permeability and cellular proliferation. It is activated by VEGF and induces VEGF expression [159]. Erythropoietin has also been linked to the pathogenesis of diabetic retinopathy [160].

## GHRELIN, ANGIOGENESIS AND ITS POTENTIAL ROLE IN DIABETIC RETINOPATHY

The role of ghrelin in angiogenesis is some now controversial, with some evidences showing a pro-angiogenic while others describe an anti-angiogenic effect of ghrelin and its associated peptides. Conconi *et al.* [161] showed that hu-

man umbilical vein endothelial cells (HUVECs) express ghrelin and GHSR-1a mRNAs and that ghrelin inhibits FGF-2-induced proliferation of HUVECs cultured *in vitro* and *in vivo*, in the chick embryo chorioallantoic membrane. These findings suggest that ghrelin acts as an anti-angiogenic factor. Similar findings have been showed in brain microvascular endothelial cells in the rat [162]. Earlier authors suggested that ghrelin gene product may act as a survival factor directly on the cardiovascular system inhibiting doxorubicin-induced apoptosis of porcine aortic endothelial cells, *via* ERK1/2 and PI3K/Akt signaling cascades [46]. Belloni and colleagues did not confirm this data and found that ghrelin did not affect the basal apoptotic rate of HUVECs cultured in normal growth medium [163].

Other studies suggest that ghrelin acts as an angiogenic factor. Li *et al.* found expression of ghrelin and its receptor mRNA in human dermal microvascular endothelial cells (HMVECs) and showed that ghrelin increases these cells proliferation, migration and angiogenesis through activation of ERK2 signaling [164]. Later, the same group suggested that ghrelin is one of the factors responsible for aging-related angiogenesis impairment [165].

It seems that ghrelin's secretion is upregulated under negative energy balance conditions, whereas it is down-regulated in a state of positive energy balance. Plasma ghrelin concentrations in diabetic patients with long-term poor glycemic control are lower than those in patients with good glycemic control. In patients with triopathy (i. e. retinopathy, nephropathy and neuropathy) plasma ghrelin concentrations were significantly lower than those in patients without diabetic complications. However, there are no significant differences in the plasma ghrelin concentrations in diabetic patients with and without macroangiopathy and retinopathy [166]. A study that investigated the relationship between oxidative stress, inflammation and atherosclerosis in the general population did not find a correlation with ghrelin levels [167]. Therefore, the impact of ghrelin in physiological and pathological angiogenesis is not fully understood.

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