Adipobiology
ISSN 1313-3705
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REVIEW

ROLE OF GHRELIN GENE-DERIVED PEPTIDES IN THE CONTROL OF ENERGY BALANCE AND CARDIOMETABOLIC HOMEOSTASIS

Amaia Rodríguez[1,3], Javier Gómez-Ambrosi[1,3], Victoria Catalán[1,3], Gema Frühbeck[1,2,3]

[1]Metabolic Research Laboratory and [2]Department of Endocrinology and Nutrition, Clínica Universidad de Navarra, Pamplona, Spain; [3]CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Spain

Abstract

The human *GHRL* gene encodes a 117-aminoacid peptide, preproghrelin that is proteolytically processed to yield two peptides: ghrelin and obestatin. Ghrelin is secreted to the bloodstream in two major forms, acylated ghrelin (with an *n*-octanoylation in the third serine residue) and desacyl ghrelin (the form lacking *n*-octanoylation). Ghrelin acylation, promoted by ghrelin *O*-acyltransferase (GOAT), is essential for binding to the growth hormone (GH) secretagogue receptor (GHS-R) 1a and for the main endocrine functions of acylated ghrelin, including the stimulation of GH release, induction of food intake and stimulation of adipogenesis. Although devoid of binding to GHS-R 1a, desacyl ghrelin also displays orexigenic and adipogenic actions, whereas the role of obestatin in the regulation of energy balance remains unclear. The discovery of the widespread distribution of ghrelin and its receptor in the cardiovascular system opened a new research field in the role of ghrelin in the control of blood pressure and myocardial function. Indeed, ghrelin inhibits the apoptosis of cardiomyocytes and endothelial cells, ameliorates left ventricular function and reduces fibrosis after myocardial injury in experimental models. In humans, ghrelin improves endothelial function by increasing nitric oxide (NO) bioavailability, normalizes the altered balance between endothelin-1 and NO in patients with metabolic syndrome and exerts performance-enhancing effects on myocardial function of patients with chronic heart failure. This review focuses on advances in cardiometabolic effects of ghrelin gene-derived products in rodents and humans, and the possible role of ghrelin as a therapeutic molecule for treating cardiometabolic diseases.

Adipobiology 2011; 3: 21-30

Keywords: ghrelin, obestatin, obesity, adipogenesis, cardiometabolic diseases, left ventricular remodelling

Received 20 December 2011, revised 28 December 2011, accepted 29 December 2011.
Correspondence: Amaia Rodríguez, PhD, Metabolic Research Laboratory, University of Navarra, Irunlarrea 1, 31008 Pamplona, Spain. Tel: +34 948 42 56 00 (ext. 6544); Fax: +34 948 42 56 52; E-mail: arodmur@unav.es

Introduction

The adipose tissue acts as an active endocrine organ, secreting a large number of hormones, growth factors, enzymes, cytokines, complement factors and matrix proteins, collectively termed adipokines (1,2). The physiological and pathophysiological relevance of adipokines in the homeostasis of the cardiovascular system resides in their effects on blood pressure, fibrinolysis, angiogenesis, coagulation, vascular remodelling, insulin sensitivity and immunity, among others (1,3,4). In this respect, adipokines participate either directly or indirectly in the regulation of several processes that contribute to the development of inflammation, atherogenesis, hypertension, and insulin resistance (2).

Obesity was classified as a major modifiable risk factor for cardiovascular diseases by the American Heart Association and, by the American College of Cardiology guidelines for secondary prevention of coronary artery disease (5,6). Upper body excess adiposity is associated with increased incidence of cardiometabolic diseases

(CMD) including atherosclerosis, hypertension, type 2 diabetes mellitus and the metabolic syndrome (7-9). Moreover, it has been recently described that percentage of body fat is *per se* positively associated with well-known circulating cardiovascular markers (10). The molecular mechanisms underlying the onset of obesity-associated CMD have not been completely disentangled. In this sense, ghrelin gene-derived products might constitute an important link between both pathologies.

Ghrelin constitutes a peptide hormone that stimulates appetite and induces a positive energy balance, leading to body weight gain (11,12). Additionally, ghrelin exerts several cardiovascular effects, including cardioprotective effects against ischemia, enhancement of vasodilation, cardiotropic effects, and regulation of blood pressure (13). The present review focuses on advances in cardiometabolic effects of ghrelin gene-derived products in rodents and humans.

1. Discovery of the components of the ghrelin system

1.1. Growth hormone (GH) secretagogue receptor (GHS-R)

GHS-R was cloned from the pituitary and arcuate, ventromedial and infundibular hypothalamus of swine and humans in 1996 (14). GHS-R is a seven transmembrane G-protein coupled receptor that acts on the pituitary gland and hypothalamus to stimulate GH release (14). The human *GHSR* gene maps on chromosome 3, at 3q26.31, and two transcripts are produced from the alternative splicing of this gene: GHS-R 1a and 1b. The transcript GHS-R 1a excises an intron, encodes a protein with 366 aminoacids with seven transmembrane domains and it is considered the functional ghrelin receptor (14,15). The second transcript GHS-R 1b retains the intron, encodes a C-terminally truncated isoform of the ghrelin receptor, consisting of 289 aminoacids and five transmembrane domains, and it does not stimulate GH release. Nevertheless, GHS-R 1b can attenuate the activity of GHS-R 1a by the formation of heterodimers (16).

1.2. G-protein-coupled receptor 39 (GPR39)

The G-protein-coupled receptor 39 (GPR39) was cloned together with another receptor, GPR38, from human foetal brain cDNA in 1997 (17). In 2005, Zhang and colleagues reported that GPR39 was the receptor for obestatin, a peptide encoded by the ghrelin gene (18). Nevertheless, other authors have failed to reproduce GPR39-binding ability to obestatin *in vitro* (18, 19). Current evidence is weighted heavily against a role for obestatin as a GPR39 agonist. The human *GPR39* gene is located on chromosome 2, at 21q21-q22, and generates two transcripts by alternative splicing: GPR39-1a and 1b (20). The transcript GPR39-1a encodes a full-length receptor of 435 aminoacids, whereas the GPR39-1b encodes a protein that is identical in sequence to the full-length receptor between aminoacids 1–285, but after Leu285, the GPR39-1b transcript encodes Ser-Glu-Ser, followed by a stop codon; GPR39-1b is assumed to be non-functional (21). GPR39-1a is expressed selectively throughout the gastrointestinal tract, including the liver and pancreas as well as in the kidney and adipose tissue, whereas the truncated GPR39-1b form has a widespread expression, including the central nervous system, with the highest expression in the stomach and small intestine (20).

1.3. Ghrelin gene products (acylated ghrelin, desacyl ghrelin and obestatin)

In 1999, the endogenous ligand for GHS-R, ghrelin, was isolated from rat stomach (11). Ghrelin is a 28-aminoacid peptide hormone, synthesized by X/A-like cells in the mucosa of the gastric fundus (11, 22). Stomach and intestine constitute the two major ghrelin-secreting tissues (11, 22, 23), but ghrelin is also synthesized to a lesser extent in other tissues, including pancreas, kidney, gonads, heart or adipose tissue (24). The human *GHRL* gene maps on chromosome 3, at 3p26.25, comprises 4 exons and 3 introns and encodes a 117-aminoacid polypeptide called preproghrelin (Fig. 1) (25,26). This polypeptide contains a 23-aminoacid sequence signal at the N-terminal region, which is first cleaved by a putative signal peptidase resulting in proghrelin with 94 aminoacids. The prohormone convertases 1 and 3 (PC1/3), PC2 and furin are the endoproteases responsible for the cleavage of proghrelin after Arg28, generating the N-terminal mature 28-aminocid ghrelin peptide as well as the C-terminal fragment, called obestatin (25,27). After this cleavage, the lysine residue of obestatin is cleaved by carboxypeptidase E. Two major forms of ghrelin are present in plasma and stomach: acylated ghrelin (~5% of total ghrelin with the *n*-octanoyl modification at Ser3) and desacyl-ghrelin (~95% without the acylation) (28). Other shorter forms of ghrelin have been recently described, like des-Gln14-ghrelin, but their role remains unknown (29).

1.4. Ghrelin O-acyltransferase (GOAT)

Yang (30) and Gutiérrez (31) independently reported the enzyme that catalyzes the octanoylation of ghrelin in the endoplasmic reticulum, the ghrelin *O*-acyltransferase (GOAT), in 2008. GOAT is a porcupine-like enzyme that belongs to the family of membrane-bound *O*-acyltransferases (MBOAT), previously known as MBOAT4 (32). The expression sites of GOAT include stomach, intestine, colon and testis in rodents, and mainly in

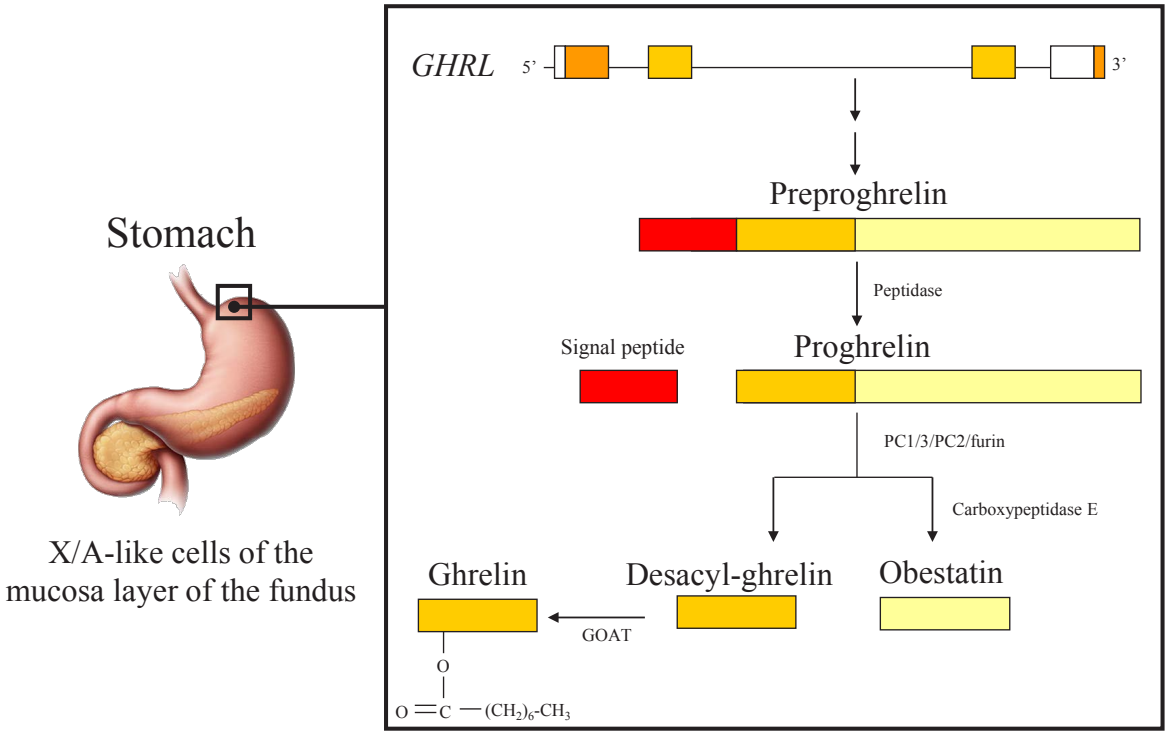

Figure 1. Synthesis and processing of ghrelin gene-derived peptides. Ghrelin is synthesized in the X/A-like cells of the mucosa of the gastric fundus. The human *GHRL* gene is located on chromosome 3 and comprises 4 exons and 3 introns. The *GHRL* gene encodes a polypeptide of 117 aminoacids, called preproghrelin. This precursor has a signal peptide that is cleaved by the action of peptidases resulting in the proghrelin molecule. This peptide is processed by PC 1 and 3 (PC1/3), PC2, furin and carboxypeptidase E, resulting in two peptides, obestatin and desacyl ghrelin. GOAT is the enzyme responsible of the esterification of an octanoic acid at Ser3 residue in ghrelin peptide.

stomach, intestine and pancreas in humans (33). The human *MBOAT4* gene is located on chromosome 8, at 8p12, and encodes a protein of 435 aminoacids in which the residues Asp307 and His338 are essential for its enzymatic activity (30, 31). It has been demonstrated *in vitro* that GOAT activity can be potently inhibited by an octanoylated ghrelin pentapeptide and other end-products, suggesting a negative feedback regulation on the production of acylated ghrelin (34). On the other hand, GOAT is regulated by nutrient availability, depends on specific dietary lipids as acylation substrates and links ingested lipids to energy expenditure and body fat mass (35).

2. Control of body weight and adiposity induced by ghrelin gene-derived peptides

2.1. Orexigenic effects of ghrelin

Ghrelin plays a major role in the short- and long-term regulation of appetite and body weight, in addition to its ability to stimulate GH release (12,36-38). Circulating ghrelin levels are characterized by a preprandial rise and a postprandial fall, supporting its role in meal initiation (39). Moreover, total serum ghrelin concentrations increase steadily during long-term fasting in rodents and humans (40), and are reduced after refeeding (40). In addition, administration of exogenous ghrelin stimulates appetite and increases food intake by the stimulation of hypothalamic neuropeptide Y/agouti-related peptide neurons expressing GHS-R 1a (41). The molecular mechanisms underlying the orexigenic effects of ghrelin involve the activation of hypothalamic AMP-activated protein kinase and inactivation of the *de novo* fatty acid synthetic pathway, resulting in decreased malonyl-CoA levels and leading to the activation of carnitine palmitoyltransferase 1 (42). Despite its orexigenic effect, obesity, insulin resistance, type 2 diabetes or the metabolic syndrome are associated with a paradoxical decrease in circulating total ghrelin levels (11,43,44). Nevertheless, these pathologies are associated with a dramatic reduction of plasma desacyl ghrelin concentrations, the most abundant circulating isoform of the hor-

mone, while plasma levels of acylated ghrelin remain unchanged or increased (45,46).

It was initially proposed that, contrary to the orexigenic effects of ghrelin, obestatin suppressed food intake and decreased body weight gain in rats, acting through the ghrelin receptor family member GPR39 (18). Obestatin levels are increased in fasted rats and, after re-feeding, plasma obestatin levels are significantly decreased but recovered quickly (40). Nevertheless, current knowledge of the secretory profile of obestatin is inadequate to make a correlation with specific feeding-related behaviours (47). In addition, obestatin degrades rapidly in serum and, unlike ghrelin, is not able to cross the blood-brain-barrier by a saturable transport system to allow the peptide to interact with its putative receptor GPR39 in the brain (48). Moreover, Nogueiras and colleagues failed to observe any effect of obestatin on food intake, body weight, body composition, energy expenditure, locomotor activity, respiratory quotient, or hypothalamic neuropeptides involved in energy balance regulation in rats (19). Therefore, the role of obestatin in the regulation of food intake remains unclear, and further investigations are required.

2.2. Adipogenic effects of ghrelin and obestatin

The adipose tissue also constitutes an important target for the adipogenic actions of ghrelin in rodents and humans (45). Ghrelin, GOAT and the receptors of ghrelin-related peptides, GHS-R 1a and GPR39, are expressed in human adipose tissue, suggesting an autocrine/paracrine effect of ghrelin in this tissue (45, 49-51). *GHRL* gene expression increases during adipogenesis and preproghrelin knockdown reduces insulin-mediated adipogenesis in 3T3-L1 adipocytes (52). Acylated ghrelin modulates preadipocyte proliferation and promotes their differentiation to mature adipocytes by increasing the expression of master adipogenic factors, peroxisome proliferator-activated receptor γ (PPARγ) and sterol regulatory element-binding protein (SREBP) (45, 53). In this sense, acylated and desacyl ghrelin directly stimulate the expression of several fat storage-related proteins, including acetyl-CoA carboxylase, fatty acid synthase, and lipoprotein lipase through both central mechanisms (54) and directly acting on human visceral adipocytes (45), thereby stimulating intracytoplasmic lipid accumulation. In addition, acylated ghrelin, desacyl ghrelin and obestatin attenuate isoproterenol-induced lipolysis through phosphatidyl-inositol-3 kinase (PI3K)-dependent mechanisms in murine 3T3-L1 cells as well as in isolated rat visceral adipocytes (55, 56). It has been recently discovered an antagonist of GOAT that reduces body weight in wild-type mice but not in ghrelin-deficient mice (57).

Recent *in vitro* studies suggest that obestatin also modulates adipocyte metabolism and adipogenesis. Obestatin expression is increased during adipocyte differentiation and obestatin treatment in 3T3-L1 adipocytes, in combination with isobutyl-methyl-xanthine and dexamethasone, regulates the expression of the transcription factors PPARγ and CCAAT-enhancer-binding proteins α, β, λ (C/EBPα, C/EBPβ, C/EBPγ), and hence promotes adipogenesis (58). Obestatin also decreases lipolysis in 3T3-L1 cells (56).

3. Role of ghrelin gene-derived peptides in the control of the cardiovascular system

Ghrelin acts on the pituitary and hypothalamus to stimulate GH release, while GH and its mediator, insulin-like growth factor (IGF)-1, are anabolic hormones that are essential for myocardial development and performance. Nevertheless, ghrelin has cardiovascular effects through GH-dependent and -independent mechanisms, as explained below. The analysis of ghrelin binding sites in the cardiovascular system by Katugampola and colleagues (59) showed a higher density of receptors in the myocardium of the right atrium than in the left ventricle (LV), whereas aorta and pulmonary artery expressed more receptors than saphenous vein or coronary artery. In this regard, the discovery of ghrelin receptors in the cardiovascular system opened a new research field on the role of ghrelin in the control of blood pressure and myocardial function (60,61).

3.1. Ghrelin acts as a cardioprotective hormone

The myocardium constitutes a source of ghrelin and obestatin (60), and GHS-R 1a is expressed in cardiomyocytes and other myocardial tissues (62), supporting the autocrine/paracrine effects of ghrelin in the heart. Acylated and desacyl ghrelin protect cardiomyocytes and endothelial cells against apoptosis through the activation of an intracellular survival pathway (63), whereas contradictory data have been reported regarding the effect of obestatin on cardiomyocyte viability (64, 65). Acylated ghrelin, des-Gln14-ghrelin and desacyl ghrelin show negative inotropic effects on papillary muscle by activating the release of cyclooxygenase metabolites in endocardial endothelium (66). Ghrelin improves the cachexia and the altered myocardial energy metabolism in rats with heart failure (67,68). In humans, plasma ghrelin and obestatin levels are increased in cachexia associated with chronic heart failure (CHF) (69, 70). The beneficial hemodynamic effects of ghrelin on patients with CHF seem to be attributable to both a positive inotropism of GH and a fall in cardiac overload. Furthermore, intravenous administration of

ghrelin in healthy individuals and patients with CHF improves the cardiac function by increasing the cardiac index and stroke volume index (71,72). Nevertheless, hearts of patients with CHF exhibit an impaired ghrelin production which might reflect maladaptive processes and a probably compensatory increase in GHS-R 1a expression (62).

Ghrelin exerts performance-enhancing effects on myocardial function. Chronic administration of ghrelin after myocardial infarction has been shown to attenuate LV enlargement and myocardial fibrosis by the suppression of cardiac sympathetic activity in rodents (67,73). The analysis by tissue Doppler imaging in healthy, young, normal-weight men with chronic intravenous administration of ghrelin showed an increase in the tissue tracking, which reflects an improvement in the global longitudinal systolic contraction of LV amplitude (74). Common variants in the human *GHSR* gene, which are associated with parameters of LV mass and geometry independently of blood pressure and BMI, have been identified in the general population (57). Moreover, a single-nucleotide polymorphism in the *GHRL* gene 5' flanking area (-501A>C) associated with LV mass index after adjustments for age, sex and systolic blood pressure has been recently described (75). In this sense, circulating acylated ghrelin concentrations are increased in patients with LV hypertrophy compared with those with appropriate LV mass, which might reflect the LV remodelling during the progression to LV hypertrophy (46).

3.2. Hypotensive effect of ghrelin

Growing evidence supports that ghrelin reduces arterial pressure and systemic vascular resistance. Plasma ghrelin and obestatin levels are increased in hypertensive rats (76). Intracerebroventricular administration of ghrelin into the nucleus of the solitary tract (brain region involved in the regulation of cardiovascular system) induces a decrease in blood pressure, heart rate and also suppressed sympathetic renal activity in experimental animals (77,78). Intravenous administration of obestatin does not change blood pressure values of spontaneously hypertensive rats (SHR) (79). In humans, intravenous administration of ghrelin in healthy individuals and patients with CHF decreases mean arterial pressure, without changes in heart rate (69,70). In patients with the metabolic syndrome, high plasma concentrations of acylated ghrelin are associated with significantly higher blood pressure values, even after adjusting for BMI (46). Given the physiological relevance of acylated ghrelin, its increased production may represent a compensatory hypotensive mechanism.

Ghrelin causes vasorelaxation through different molecular mechanisms. Firstly, ghrelin improves endothelial dysfunction in GH-deficient rats by increasing endothelial nitric oxide (NO) synthase (eNOS) expression in the aorta, suggesting that this vascular effect is mediated by a GH-independent mechanism (80). Ghrelin directly stimulates the production of NO from vascular endothelial cells using PI3K-dependent signalling pathways that mimic those of insulin (81). In humans, intraarterial infusion of ghrelin induces vasodilation through GH/IGF-1-independent mechanisms in healthy individuals (82) and reverses endothelial dysfunction in patients with metabolic syndrome by increasing NO bioactivity (83). Secondly, ghrelin inhibits vascular oxidative stress through the inhibition of vascular NAD(P)H oxidases activity in SHR (84). In this regard, systemic administration of desacyl ghrelin, but not acylated ghrelin, prevented diabetes-induced endothelial progenitor cell damage by modulating the NADPH oxidase regulatory protein Rac1 and improved the vasculogenic potential in individuals with type 2 diabetes (85). Ghrelin also inhibits proinflammatory cytokine production, mononuclear cell binding, and nuclear factor-κB (NFκB) activation in human endothelial cells *in vitro*, and hence, reduces the endothelial inflammation (86). Finally, ghrelin exerts an endothelium-independent depressor action in rat aorta by inhibiting angiotensin II-induced vasoconstriction (87). Furthermore, ghrelin constitutes an effective endothelium-independent vasodilator of the long-lasting constrictor endothelin-1 in human internal mammary arteries producing responses similar to those of adrenomedullin (88). Infusion of ghrelin in patients with metabolic syndrome restores the balance between vasoconstrictor endothelin-1 and vasodilator NO mediators (89). In summary, ghrelin increases NO bioavailability, prevents vascular oxidative stress and antagonizes the contraction of potent vasoconst rictor peptides, thereby preserving vascular homeostasis.

4. Conclusions

In summary, ghrelin exerts important cardiovascular actions in addition to its orexigenic and adipogenic effects, as shown in Figure 2. On the contrary, the contradictory observations of the role of obestatin in the viability of cardiomyocytes raise some questions regarding its involvement as a cardioprotective hormone (64,65). Further studies will be necessary to establish the specific contribution of obestatin in the myocardium. Likewise, further investigations in novel mutations, single-nucleotide polymorphisms or differential expression levels of molecules of the ghrelin system are needed to broaden our understanding of their role in the onset of obesity-associated CMD.

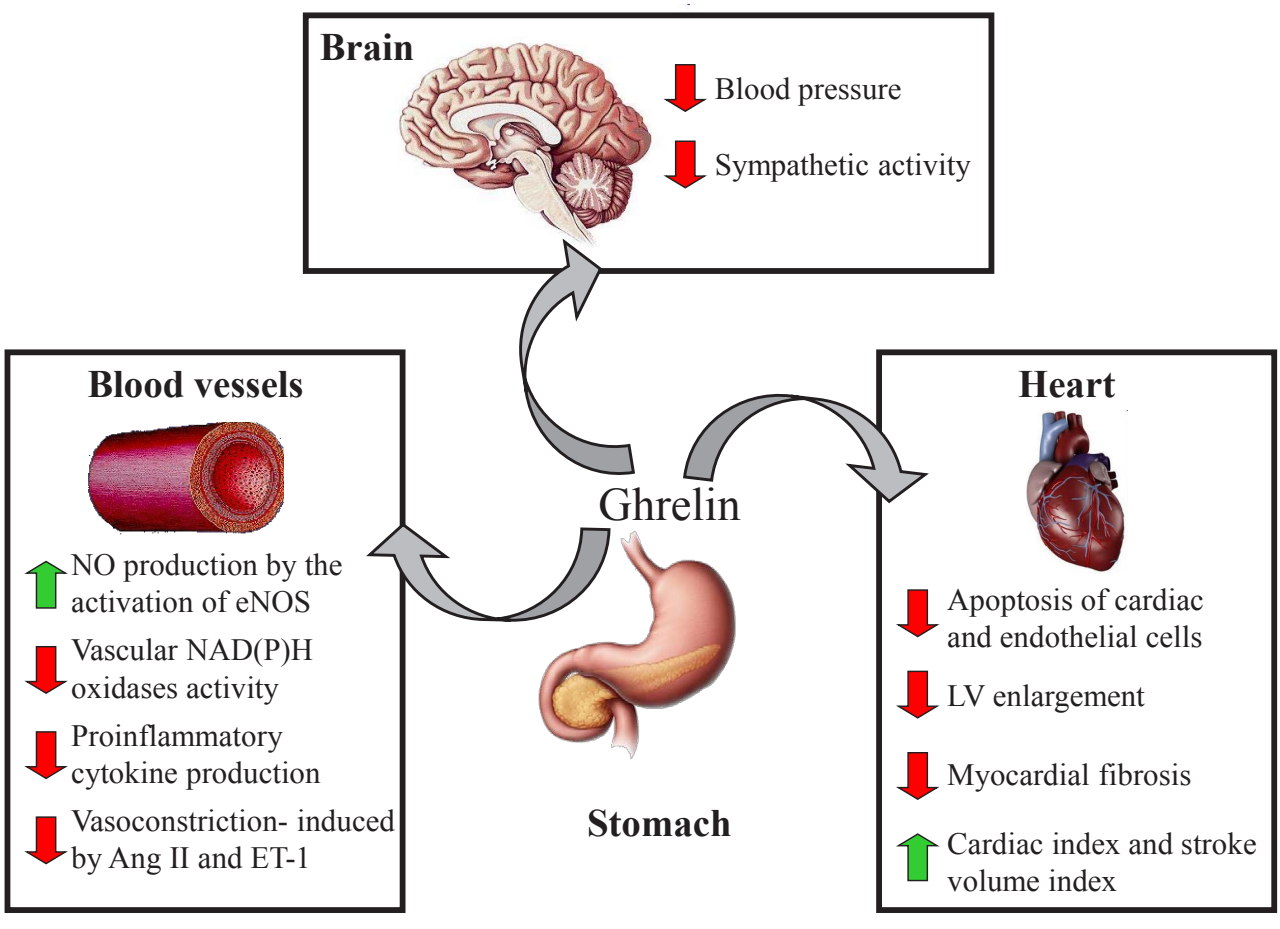

Figure 2. Central and peripheral effects of ghrelin in the control of cardiovascular homeostasis. By activating vagal afferents or via the bloodstream, ghrelin acts on the nucleus of the solitary tract inducing a decrease in blood pressure, heart rate and also a suppression of sympathetic renal activity. Cardiac effects of ghrelin include the inhibition of cardiomyocyte and endothelial cell apoptosis, the improvement of LV function and the reduction of fibrosis after myocardial injury, as well as the increase in cardiac index and stroke volume index after bolus injection of ghrelin. Vascular effects of ghrelin include the increase in NO bioavailability by increasing eNOS and by reducing the vascular NAD(P)H oxidases activity, the inhibition of cytokine release from blood vessels as well as the antagonism of Ang II and ET-1-induced vasoconstriction.

Acknowledgments

This work was funded by the Instituto de Salud Carlos III (FIS PS09/02330 and FIS PI10/01677). CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn) is an initiative of the Instituto de Salud Carlos III, Spain.

References

1. Frühbeck G. The adipose tissue as a source of vasoactive factors. *Curr Med Chem Cardiovasc Hematol Agents* 2004; 2:197-208.
2. Rodríguez A, Catalán V, Gómez-Ambrosi J, Frühbeck G. Visceral and subcutaneous adiposity: are both potential therapeutic targets for tackling the metabolic syndrome? *Curr Pharm Des* 2007; 13:2169-2175.
3. Chaldakov GN, Stankulov IS, Hristova M, Ghenev PI. Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Curr Pharm Des* 2003; 9:1023-1031.
4. Chaldakov GN, Beltowsky J, Ghenev PI, Fiore M, Panayatov P, Rancic G, *et al.* Adipoparacrinology: vascular periadventitial adipose tissue *(tunica adiposa)* as an example. *Cell Biol Int* 2012; 36:327-330.
5. Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. *Circulation* 1998; 97:2099-2100.

6. Smith SC, Jr., Blair SN, Bonow RO, Brass LM, Cerqueira MD, Dracup K, *et al*. AHA/ACC Guidelines for Preventing Heart Attack and Death in Patients With Atherosclerotic Cardiovascular Disease: 2001 update. A statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 2001; 38:1581-1583.
7. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, *et al*. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet* 2005; 366:1640-1649.
8. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity (Silver Spring)* 2006; 14:336-341.
9. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2010; 375:181-183.
10. Gómez-Ambrosi J, Silva C, Galofre JC, Escalada J, Santos S, Millán D, *et al.* Body mass index classification misses subjects with increased cardiometabolic risk factors related to elevated adiposity. *Int J Obes (Lond)* 2012; 36:286-294.
11. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone releasing acylated peptide from stomach. *Nature* 1999; 402:656-660.
12. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; 407:908-913.
13. Tesauro M, Schinzari F, Caramanti M, Lauro R, Cardillo C. Cardiovascular and metabolic effects of ghrelin. *Curr Diabetes Rev* 2010; 6: 228-235.
14. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, *et al*. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; 273:974-977.
15. Tanaka M, Miyazaki T, Yamamoto I, Nakai N, Ohta Y, Tsushima N, *et a*l. Molecular characterization of chicken growth hormone secretagogue receptor gene. *Gen Comp Endocrinol* 2003; 134:198-202.
16. Leung PK, Chow KB, Lau PN, Chu KM, Chan CB, Cheng CH, *et al*. The truncated ghrelin receptor polypeptide (GHS-R1b) acts as a dominant-negative mutant of the ghrelin receptor. *Cell Signal* 2007; 19:1011-1022.
17. McKee KK, Tan CP, Palyha OC, Liu J, Feighner SD, Hreniuk DL, *et al*. Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors. *Genomics* 1997; 46:426-434.
18. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, *et al*. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 2005; 310:996-999.
19. Nogueiras R, Pfluger P, Tovar S, Arnold M, Mitchell S, Morris A, *et al*. Effects of obestatin on energy balance and growth hormone secretion in rodents. *Endocrinology* 2007; 148:21-26.
20. Egerod KL, Holst B, Petersen PS, Hansen JB, Mulder J, Hokfelt T, *et al*. GPR39 splice variants versus antisense gene LYPD1: expression and regulation in gastrointestinal tract, endocrine pancreas, liver, and white adipose tissue. *Mol Endocrinol* 2007; 21:1685-1698.
21. Popovics P, Stewart AJ. GPR39: a Zn^{2+}-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. *Cell Mol Life Sci* 2011; 68:85-95.
22. Frühbeck G, Díez Caballero A, Gil MJ. Fundus functionality and ghrelin concentrations after bariatric surgery. *N Engl J Med* 2004; 350:308-309
23. Frühbeck G, Rotellar F, Hernández-Lizoain JL, Gil MJ, Gómez-Ambrosi J, Salvador J, *et al*. Fasting plasma ghrelin concentrations 6 months after gastric bypass are not determined by weight loss or changes in insulinemia. *Obes Surg* 2004; 14:1208-1215.
24. Chen CY, Asakawa A, Fujimiya M, Lee SD, Inui A. Ghrelin gene products and the regulation of food intake and gut motility. *Pharmacol Rev* 2009; 61:430-481..
25. Zhu X, Cao Y, Voogd K, Steiner DF. On the processing of proghrelin to ghrelin. *J Biol Chem* 2006; 281:38867-38870.
26. Garg A. The ongoing saga of obestatin: is it a hormone? *J Clin Endocrinol Metab* 2007; 92:3396-3398.
27. Takahashi T, Ida T, Sato T, Nakashima Y, Nakamura Y, Tsuji A, *et al*. Production of n-octanoyl-modified ghrelin in cultured cells requires prohormone processing protease and ghrelin O-acyltransferase, as well as n-octanoic acid. *J Biochem* 2009; 146:675-682.
28. Hosoda H, Kojima M, Matsuo H, Kangawa K. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun* 2000; 279:909-913.
29. Hosoda H, Kojima M, Matsuo H, Kangawa K. Purification and characterization of rat des-Gln14-Ghrelin, a second endogenous ligand for the growth hormone secretagogue receptor. *J Biol Chem* 2000; 275:21995-22000.
30. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 2008; 132:387-396
31. Gutiérrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, *et al*. Ghrelin octanoylation mediated by

an orphan lipid transferase. *Proc Natl Acad Sci U S A* 2008; 105:6320-6325
32. Hofmann K. A superfamily of membrane-bound O-acyltransferases with implications for wnt signaling. *Trends Biochem Sci* 2000; 25:111-112
33. Al Massadi O, Tschöp MH, Tong J. Ghrelin acylation and metabolic control. *Peptides* 2011; 32:2301-2308
34. Yang J, Zhao TJ, Goldstein JL, Brown MS. Inhibition of ghrelin O-acyltransferase (GOAT) by octanoylated pentapeptides. *Proc Natl Acad Sci U S A* 2008; 105:10750-10755
35. Kirchner H, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, et al. GOAT links dietary lipids with the endocrine control of energy balance. *Nat Med* 2009; 15:741-745
36. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, et al. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; 141:4325-4328.
37. Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, et al. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 2001; 50:2540-2547.
38. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, *et al.* A role for ghrelin in the central regulation of feeding. *Nature* 2001; 409:194-198.
39. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; 50:1714-1719.
40. Guo ZF, Ren AJ, Zheng X, Qin YW, Cheng F, Zhang J, *et al.* Different responses of circulating ghrelin, obestatin levels to fasting, re-feeding and different food compositions, and their local expressions in rats. *Peptides* 2008; 29:1247-1254.
41. Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, *et al.* Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* 2004; 145:2607-2612.
42. López M, Lage R, Saha AK, Pérez-Tilve D, Vézquez MJ, Varela L, *et al.* Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab* 2008; 7:389-399.
43. Tschöp M, Weyer C, Tataranni A, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; 50:707-709.
44. Poykko SM, Ukkola O, Kauma H, Kellokoski E, Horkko S, Kesaniemi YA. The negative association between plasma ghrelin and IGF-I is modified by obesity, insulin resistance and type 2 diabetes. *Diabetologia* 2005; 48:309-316.
45. Rodríguez A, Gómez-Ambrosi J, Catalán V, Gil MJ, Becerril S, Sáinz N, *et al.* Acylated and desacyl ghrelin stimulate lipid accumulation in human visceral adipocytes. *Int J Obes (Lond)* 2009; 33:541-552
46. Rodríguez A, Gómez-Ambrosi J, Catalán V, Becerril S, Sáinz N, Gil MJ, *et al.* Association of plasma acylated ghrelin with blood pressure and left ventricular mass in patients with metabolic syndrome. *J Hypertens* 2010; 28:560-567
47. Baragli A, Lanfranco F, Allasia S, Granata R, Ghigo E. Neuroendocrine and metabolic activities of ghrelin gene products. *Peptides* 2011; 32:2323-2332
48. Pan W, Tu H, Kastin AJ. Differential BBB interactions of three ingestive peptides: obestatin, ghrelin, and adiponectin. *Peptides* 2006; 27:911-916.
49. Knerr I, Herzog D, Rauh M, Rascher W, Horbach T. Leptin and ghrelin expression in adipose tissues and serum levels in gastric banding patients. *Eur J Clin Invest* 2006; 36:389-394.
50. Catalán V, Gómez-Ambrosi J, Rotellar F, Silva C, Gil MJ, Rodríguez A, *et al.* The obestatin receptor (GPR39) is expressed in human adipose tissue and is down-regulated in obesity-associated type 2 diabetes mellitus. *Clin Endocrinol (Oxf)* 2007; 66:598-601.
51. Lim CT, Kola B, Grossman A, Korbonits M. The expression of ghrelin O-acyltransferase (GOAT) in human tissues. *Endocr J* 2011:doi:10.1507/endocrj.K1511E-1117
52. Gurriarán-Rodríguez U, Al-Massadi O, Crujeiras AB, Mosteiro CS, Amil-Diz M, Beiroa D, et al. Preproghrelin expression is a key target for insulin action on adipogenesis. *J Endocrinol* 2011; 210:R1-7
53. Kim MS, Yoon CY, Jang PG, Park YJ, Shin CS, Park HS, *et al.* The mitogenic and antiapoptotic actions of ghrelin in 3T3-L1 adipocytes. *Mol Endocrinol* 2004; 18:2291-2301
54. Theander-Carrillo C, Wiedmer P, Cettour-Rose P, Nogueiras R, Perez-Tilve D, Pfluger P, *et al.* Ghrelin action in the brain controls adipocyte metabolism. *J Clin Invest* 2006; 116:1983-1993
55. Baragli A, Ghe C, Arnoletti E, Granata R, Ghigo E, Muccioli G. Acylated and unacylated ghrelin attenuate isoproterenol-induced lipolysis in isolated rat visceral adipocytes through activation of phosphoinositide 3-kinase gamma and phosphodiesterase 3B. *Biochim Biophys Acta* 2011; 1811:386-396
56. Miegueu P, Pierre DS, Broglio F, Cianflone K. Effect of desacyl ghrelin, obestatin and related peptides on triglyceride storage, metabolism and GHSR signaling in 3T3-L1 adipocytes. *J Cell Biochem* 2011; 112:704-714.
57. Baessler A, Kwitek AE, Fischer M, Koehler M, Reinhard W, Erdmann J, *et al.* Association of the Ghrelin receptor gene

region with left ventricular hypertrophy in the general population: results of the MONICA/KORA Augsburg Echocardiographic Substudy. *Hypertension* 2006; 47:920-927

58. Gurriarán-Rodríguez U, Al-Massadi O, Roca-Rivada A, Crujeiras AB, Gallego R, Pardo M, *et al*. Obestatin as a regulator of adipocyte metabolism and adipogenesis. *J Cell Mol Med* 2011; 15:1927-1940.
59. Katugampola SD, Pallikaros Z, Davenport AP. [125I-His(9)]-ghrelin, a novel radioligand for localizing GHS orphan receptors in human and rat tissue: up-regulation of receptors with athersclerosis. *Br J Pharmacol* 2001; 134:143-149.
60. Iglesias MJ, Pineiro R, Blanco M, Gallego R, Diéguez C, Gualillo O, *et al*. Growth hormone releasing peptide (ghrelin) is synthesized and secreted by cardiomyocytes. *Cardiovasc Res* 2004; 62:481-488.
61. Kleinz MJ, Maguire JJ, Skepper JN, Davenport AP. Functional and immunocytochemical evidence for a role of ghrelin and des-octanoyl ghrelin in the regulation of vascular tone in man. *Cardiovasc Res* 2006; 69:227-235
62. Beiras-Fernández A, Kreth S, Weis F, Ledderose C, Pottinger T, Diéguez C, et al. Altered myocardial expression of ghrelin and its receptor (GHSR-1a) in patients with severe heart failure. *Peptides* 2010; 31:2222-2228
63. Baldanzi G, Filigheddu N, Cutrupi S, Catapano F, Bonissoni S, Fubini A, *et al*. Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. *J Cell Biol* 2002; 159:1029-1037
64. Iglesias MJ, Salgado A, Pineiro R, Rodino BK, Otero MF, Grigorian L, *et al*. Lack of effect of the ghrelin gene-derived peptide obestatin on cardiomyocyte viability and metabolism. *J Endocrinol Invest* 2007; 30:470-476
65. Alloatti G, Arnoletti E, Bassino E, Penna C, Perrelli MG, Ghe C, *et al*. Obestatin affords cardioprotection to the ischemic-reperfused isolated rat heart and inhibits apoptosis in cultures of similarly stressed cardiomyocytes. *Am J Physiol Heart Circ Physiol* 2010; 299:H470-481
66. Bedendi I, Alloatti G, Marcantoni A, Malan D, Catapano F, Ghe C, *et a*l. Cardiac effects of ghrelin and its endogenous derivatives des-octanoyl ghrelin an des-Gln14-ghrelin. *Eur J Pharmacol* 2003; 476:87-95
67. Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, *et al.* Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation* 2001; 104:1430-1435.
68. Xu JP, Wang HX, Wang W, Zhang LK, Tang CS. Ghrelin improves disturbed myocardial energy metabolism in rats with heart failure induced by isoproterenol. *J Pept Sci* 2010; 16:392-402.
69. Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, *et al*. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation* 2001; 104:2034-2038.
70. Xin X, Ren AJ, Zheng X, Qin YW, Zhao XX, Yuan WJ, et al. Disturbance of circulating ghrelin and obestatin in chronic heart failure patients especially in those with cachexia. *Peptides* 2009; 30:2281-2285.
71. Nagaya N, Kojima M, Uematsu M, Yamagishi M, Hosoda H, Oya H, *et al*. Hemodynamic and hormonal effects of human ghrelin in healthy volunteers. *Am J Physiol Regulatory Integrative Comp Physiol* 2001; 280:R1483-R1487.
72. Nagaya N, Miyatake K, Uematsu M, Oya H, Shimizu W, Hosoda H, *et al*. Hemodynamic, renal, and hormonal effects of ghrelin infusion in patients with chronic heart failure. *J Clin Endocrinol Metab* 2001; 86:5854-5859
73. Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, *et al*. Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. *Am J Physiol Heart Circ Physiol* 2008; 294:H426-432.
74. Vestergaard ET, Andersen NH, Hansen TK, Rasmussen LM, Moller N, Sorensen KE, et al. Cardiovascular effects of intravenous ghrelin infusion in healthy young men. *Am J Physiol Heart Circ Physiol* 2007; 293:H3020-3026
75. Ukkola O, Paakko T, Kesaniemi YA. Ghrelin and its promoter variant associated with cardiac hypertrophy. *J Hum Hypertens* 2011:doi:10.1038/jhh.2011.1051.
76. Li ZF, Guo ZF, Cao J, Hu JQ, Zhao XX, Xu RL, *et al.* Plasma ghrelin and obestatin levels are increased in spontaneously hypertensive rats. *Peptides* 2010; 31:297-300.
77. Lin Y, Matsumura K, Fukuhara M, Kagiyama S, Fujii K, Iida M. Ghrelin acts at the nucleus of the solitary tract to decrease arterial pressure in rats. *Hypertension* 2004; 43:977-982.
78. Matsumura K, Tsuchihashi T, Fujii K, Abe I, Iida M. Central ghrelin modulates sympathetic activity in conscious rabbits. *Hypertension* 2002; 40:694-699.
79. Li ZF, Song SW, Qin YW, Zhang JL, Zhao XX, Zhang BL, *et al.* Bolus intravenous injection of obestatin does not change blood pressure level of spontaneously hypertensive rat. *Peptides* 2009; 30:1928-1930

80. Shimizu Y, Nagaya N, Teranishi Y, Imazu M, Yamamoto H, Shokawa T, et al. Ghrelin improves endothelial dysfunction through growth hormone-independent mechanisms in rats. *Biochem Biophys Res Comm* 2003; 310:830-835
81. Iantorno M, Chen H, Kim JA, Tesauro M, Lauro D, Cardillo C, et al. Ghrelin has novel vascular actions that mimic PI 3-kinase-dependent actions of insulin to stimulate production of NO from endothelial cells. *Am J Physiol Endocrinol Metab* 2007; 292:E756-764.
82. Okumura H, Nagaya N, Enomoto M, Nakagawa E, Oya H, Kangawa K. Vasodilatory effect of ghrelin, an endogenous peptide from the stomach. *J Cardiovasc Pharmacol* 2002; 39:779-783.
83. Tesauro M, Schinzari F, Iantorno M, Rizza S, Melina D, Lauro D, et al. Ghrelin improves endothelial function in patients with metabolic syndrome. *Circulation* 2005; 112:2986-2992.
84. Kawczynska-Drozdz A, Olszanecki R, Jawien J, Brzozowski T, Pawlik WW, Korbut R, *et al.* Ghrelin inhibits vascular superoxide production in spontaneously hypertensive rats. *Am J Hypertens* 2006; 19:764-767.
85. Togliatto G, Trombetta A, Dentelli P, Baragli A, Rosso A, Granata R, *et al*. Unacylated ghrelin rescues endothelial progenitor cell function in individuals with type 2 diabetes. *Diabetes* 2010; 59:1016-1025.
86. Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, *et al*. Ghrelin inhibits proinflammatory responses and nuclear factor-κB activation in human endothelial cells. *Circulation* 2004; 109:2221-2226.
87. Rodríguez A, Gómez-Ambrosi J, Catalán V, Becerril S, Sáinz N, Ramírez B, *et al*. Vasodilator effect of ghrelin in the rat aorta. *Endocrinol Nutr* 2008; 55:448-453.
88. Wiley KE, Davenport AP. Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological anatagonist of endothelin-1. *Br J Pharmacol* 2002; 136:1146-1152.
89. Tesauro M, Schinzari F, Rovella V, Di Daniele N, Lauro D, Mores N, *et al*. Ghrelin restores the endothelin 1/nitric oxide balance in patients with obesity-related metabolic syndrome. *Hypertension* 2009; 54:995-1000.