

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Obestatin: is it really doing something?

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/144508> since

Publisher:

Karger

Published version:

DOI:10.1159/000358346

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Questa è la versione dell'autore dell'opera:

*[Current Pharmaceutical Design, VOLUME: 18, ISSUE: 31, DOI:
10.2174/138161212803216988]*

The definitive version is available at:

La versione definitiva è disponibile alla URL:

[<http://www.eurkaselect.com/102425/article>]

Obestatin: is it really doing something?

Letizia Trovato, Davide Gallo, Fabio Settanni, Iacopo Gesmundo, Ezio Ghigo, Riccarda Granata

Division of Endocrinology, Diabetology and Metabolism, Department of Medical Sciences; University of Torino, Torino, Italy

Address all correspondence and requests for reprints to:

Riccarda Granata, PhD

Lab of Molecular and Cellular Endocrinology

Division of Endocrinology, Diabetology and Metabolism

Department of Medical Sciences - University of Torino

Corso Dogliotti, 14 - 10126 Torino, Italy

E-mail: riccarda.granata@unito.it

Phone: (+39) 011 670 9558

Fax: (+39) 011 664 74 21

Abstract

Obestatin was identified in 2005 by Zhang and colleagues as a ghrelin-associated peptide, derived from post-translational processing of the prepro-ghrelin gene. Initially, obestatin was reported to activate the G-protein-coupled receptor GPR39 and to reduce food intake and gastric emptying. However, obestatin remains a controversial peptide, as these findings have been questioned and its receptor is still a matter of debate, as well as its effects on feeding behavior. Recently, interaction with the glucagon-like peptide 1 receptor has been suggested, in line with obestatin positive effects on glucose and lipid metabolism. In addition, obestatin displays a variety of cellular effects, by regulating metabolic cell functions, increasing cell survival and proliferation, and inhibiting apoptosis and inflammation in different cell types. Finally, like ghrelin, obestatin is produced in the gastrointestinal tract, including the pancreas and adipose tissue, and exerts both local actions in peripheral tissues, and distant effects at the central level. Therefore, obestatin may indeed be considered a hormone, although additional studies are required to clarify its physiopathological role and to definitely identify its receptor.

Introduction

Ghrelin, a 28-amino acid octanoylated peptide, was isolated from the stomach as the endogenous ligand of the type 1 growth hormone (GH) secretagogue receptor (GHS-R1a) [1]. Ghrelin acylation on serine 3, by ghrelin O-acyltransferase (GOAT) is essential for receptor binding and for ghrelin endocrine activities, including stimulation of GH release and food intake [2]. Des-acyl ghrelin, the major circulating form of ghrelin, although unable to bind to GHS-R1a and devoid of GH-releasing activity, displays many peripheral functions, which are either equal or opposed to those of acylated ghrelin, through binding to a yet unknown receptor [3].

In 2005, Zhang and colleagues, on the basis of bioinformatic searches, identified obestatin, a 23 amino acid amidated peptides encoded by the ghrelin precursor (preproghrelin) [4]. The term obestatin originated from the Latin verb 'obedere', meaning 'to devour', and 'statin', denoting suppression. Indeed, obestatin was initially claimed to behave as a physiological opponent of ghrelin, inhibiting either ghrelin orexigenic action or the stimulatory effect on GH secretion [4]. Interestingly, ghrelin, as well as des-acyl ghrelin and obestatin, have been shown to play a role in glucose and lipid homeostasis and, whereas ghrelin effects are mainly diabetogenic, both des-acyl ghrelin and obestatin behave as antidiabetogenic peptides by positively influencing glucose and lipid metabolism [5].

Obestatin was initially discovered as the cognate ligand for the orphan G protein-coupled receptor GPR39 [4,6]. However, these findings were later challenged by several independent groups, that were unable to confirm that obestatin has agonist properties on GPR39 [5,7,8]. More recently, obestatin binding to the glucagon-like peptide 1 receptor (GLP-1R) in pancreatic β -cells and adipocytes has been proposed, in line with the effects of GLP-1 and its analogs in these cells [9,10]. Furthermore, different cell types have shown specific binding sites for obestatin, suggesting biological responses of the peptide at different levels [6,10,11]. At present, the identification of obestatin receptor is still an open issue, although the many existing studies seem to exclude GPR39 from the possible candidates.

With regards to its distribution, obestatin, like ghrelin, was found to be mainly produced by the rat stomach [4], in cells of rat and human gastric mucosa, as well as in gastric myenteric cholinergic neurons [12]. However, other studies found little or no immunoreactivity in the stomach [13,14], therefore additional

studies will be required to confirm obestatin presence at the gastric level. Obestatin expression has been also shown in the endocrine pancreas, adipose tissue, skeletal muscle, liver, lung, thyroid, mammary gland and male reproductive system, suggesting autocrine/paracrine activities in peripheral tissues and organs [5,10,12,15-19].

Like ghrelin, plasma obestatin is increased in anorexia nervosa and reduced in obesity; therefore, its levels may reflect body adiposity and insulin resistance, and obestatin may be a good nutritional marker and a potential target for the diagnosis and treatment of anorexia nervosa [20]. Furthermore and differently from ghrelin, whose levels were found unchanged, plasma obestatin and autonomic function were increased in orexin-deficient narcolepsy, suggesting a role for obestatin in the disrupted sleep-state control in narcolepsy [21].

Serum obestatin also decreased with metabolic syndrome, and further decreased with TRIB3 Q84R polymorphism, which has been associated with carotid atherosclerosis [22]. Therefore, it was proposed that obestatin would exert protective effects against insulin resistance and carotid atherosclerosis. Obestatin, like ghrelin, is expressed in human neuroendocrine tumors (NETs) and exerts antiproliferative effects in neuroendocrine cell lines [16,23]. However, in healthy controls obestatin levels were reduced by food intake, and no variation was observed in patients with pancreatic NETs [23]. Moreover, the ghrelin/obestatin ratio is reduced in inflammatory bowel disease [24], chronic atrophic gastritis [25] and following *Helicobacter pylori* eradication [26], suggesting obestatin involvement in the pathogenesis of these diseases.

With regard to its central activities, besides counteracting ghrelin effects on food intake and GH secretion, obestatin was found to exert its own effects, by suppressing food-intake, slowing gastric emptying and jejunal motility and reducing body weight gain in rodents [4,6,27]. Despite these findings, obestatin central effects are still controversial, as studies in rats and mice also showed no inhibitory effect on food intake and body weight gain, in both absence or presence of ghrelin [7,8]. In addition, obestatin inhibits thirst and influences memory, anxiety, and sleep likely through indirect action on vagal neurons or by regulating secretion of other hormones that may reach the target cells [28-30]. In peripheral cells and tissues, obestatin promotes cell proliferation [10,31,32], prevents apoptosis, regulates cell function and differentiation, reduces inflammation, promotes cardioprotection and myogenesis and regulates atherogenesis and immune cell

function [5,8-11,33-41]. These effects were found to either involve GPR39 [18,36], GLP-1R [9,10] or specific but yet unknown receptors [11].

This is an overview of obestatin biological effects, particularly at the peripheral level and with major attention on its metabolic functions. Whether obestatin may or not be considered a hormone, it is certainly a biologically active peptide, showing unexpected effects which makes it appealing both from a scientific point of view and as a possible therapeutic candidate in conditions such as metabolic dysfunctions.

Obestatin effects in adipocyte function

The role of obestatin in adipocyte function has been addressed by different groups and the findings, which in most cases go in the same direction, have evidenced novel and unexpected biological effects of the peptide.

Indeed, obestatin plays a relevant role in adipocytes, regulating cell survival, adipogenesis, glucose and lipid metabolism and adipokine release.

Obestatin was found to increase c-fos staining in adipocytes from mouse white adipose tissue, and to increase c-fos protein expression in 3T3-L1 preadipocytes [6]. These effects involved activation of GPR39, as demonstrated by binding studies and by lack of obestatin-induced c-fos upregulation in GPR39 null mice [6]. In 3T3-L1 cells and human adipocytes, obestatin even increased extracellular signal-related kinase (ERK1/2) and phosphatidylinositol 3-kinase (PI3K)/Akt phosphorylation [6,9], in line with the survival and metabolic effects observed in these cells, and the survival and proliferative effects in other cell types [9,10,31,32,37]. Apart from GPR39, obestatin has demonstrated binding specificity in 3T3-L1 preadipocytes, human subcutaneous (SC) preadipocytes and differentiated adipocytes [9]. Interestingly, obestatin binding is displaced by the GLP-1R agonist Ex-4 and by the antagonist Ex-9, and prevented by small interfering RNA (siRNA) against GLP-1R, suggesting possible interaction with GLP-1R [9]. Besides the debated role of GPR39, additional studies in these and other cell types, are needed to clarify whether GLP-1R may be or not a putative obestatin receptor [7].

With regard to adipogenic signaling, obestatin has been shown to increase the expression of key regulators of adipogenesis, such as the CCAAT-enhancer-binding proteins (C-EBP) C/EBP α , C/EBP β , C/EBP δ and peroxisome proliferator-activated receptor gamma (PPAR γ) [36]. The adipogenic role of obestatin was further confirmed *in vivo*, in white adipose tissues of rats infused with obestatin and in obestatin-treated mice

fed with either a low fat diet (LFD) or a high fat diet (HFD) [9,36]. Interestingly, obestatin also regulates lipid metabolism by inhibiting lipolysis, as observed in 3T3-L1 and human SC and omental (OM) adipocytes isolated from both lean and obese individuals, and from HFD-treated mice [9,37]. Moreover, AMP kinase (AMPK) phosphorylation, whose increase has been associated with inhibition of lipolysis, was found enhanced by obestatin in both 3T3-L1 and human adipocytes [9]. *In vivo* studies also showed that in rat, plasma triglyceride levels were significantly reduced by a chronic 14-day treatment with a stable obestatin analog, N-terminally PEGylated obestatin [42], suggestive of a possible role of obestatin in lipid homeostasis. Furthermore, in cow WAT, obestatin infusion decreased the expression levels of ABCA1 (ATP-binding cassette A1), a key cholesterol transporter [43].

Besides lipid metabolism, obestatin also regulates glucose homeostasis in adipocytes. In fact, obestatin promotes glucose uptake in both 3T3-L1 and human SC adipocytes, and induces the translocation of glucose transporter GLUT4 [9,36]. The NAD-dependent deacetylase sirtuin 1 (SIRT1) [44], a positive regulator of glucose transport and insulin signaling in adipocytes, was found involved in these effects as its down-regulation, by means of specific small interfering RNA (siRNA), blocked obestatin-induced glucose uptake in 3T3-L1 adipocytes [9]. PI3K/Akt plays a major role in insulin-stimulated glucose transport and GLUT4 translocation to the cell membrane, and obestatin was found to promote Akt phosphorylation in 3T3-L1 and human SC adipocytes and to activate Akt downstream pathways such as GSK-3 β , mTOR and S6K1 [9,36].

Akt activation was also shown *in vivo*, in fat, muscle and liver of obestatin-treated mice fed a HFD, and in WAT of obestatin-infused rats [9,36]. In 3T3-L1 and human SC adipocytes, obestatin even increased the secretion of adiponectin and inhibited that of leptin, which exert either positive or negative role on glucose metabolism, respectively [9,45,46].

In vivo studies showed positive effects of obestatin in regulating glucose and lipid homeostasis. Indeed, obestatin reduces insulin resistance and inflammation in mice fed a HFD, by improving glucose levels and increasing both plasma and pancreatic insulin levels, likely because of its insulinotropic action [9]. Accordingly, obestatin was found to preserve islet area and to increase glucose-induced insulin secretion in islets from mice fed with both LFD and HFD. Furthermore, obestatin counteracted the effect of HFD on lipolysis, apoptosis and reduction of glucose uptake in epididymal fat. Interestingly, obestatin-treated mice showed increased number of small, likely insulin-sensitive adipocytes in epididymal and particularly SC fat,

as compared to untreated animals, suggesting WAT expansion and increased protection against diet-induced insulin resistance [9]. In addition, and in agreement with the results obtained in cultured adipocytes, mice treated with obestatin showed increased plasma adiponectin and reduced leptin levels, in both epididymal and SC adipose tissue of HFD mice. In these mice, obestatin even reduced inflammation, an hallmark of insulin resistance and diabetes, by inhibiting proinflammatory cytokine release in fat, muscle and liver, through activation of signaling pathways involved in glucose metabolism and insulin signaling [9].

Obestatin and pancreatic functions

Obestatin expression has been demonstrated in the endocrine pancreas, where it colocalizes with ghrelin in both fetal and adult human pancreas, suggesting that these hormones may act together as local regulators of β -cell fate and function [10,15-17,47,48]. Obestatin is also secreted by pancreatic β -cell lines and human pancreatic islets, and incubation of both INS-1E β -cells and human islets with an anti-obestatin antibody was found to reduce cell viability, suggesting autocrine/paracrine survival effects of the peptide [10].

Obestatin has been shown to reduce apoptosis and to promote proliferation of β -cells and human pancreatic islets cultured in either serum starved conditions or with inflammatory cytokines [10]. Indeed, identifying molecules improving β -cell survival and function is of major importance for designing new therapeutic strategies in diabetes, and for improving islet transplantation, which are both characterized by increased inflammation and β -cell loss [49]. Obestatin survival effects in β -cells involved cAMP increase and activation of survival pathways such as PI3K/Akt and extracellular signal-related kinase (ERK)1/2. In addition, obestatin upregulated the expression of genes which play a key role in insulin signaling, glucose homeostasis and β -cell survival and differentiation, such as insulin receptor substrate 2 (IRS-2), cAMP response element-binding protein (CREB), pancreatic and duodenal homeobox 1 (PDX-1) and glucokinase [10]. Obestatin effects in β -cells and underlying signaling pathways were found to be very similar to those of GLP-1; therefore, it was hypothesized that obestatin would interact with GLP-1R. In fact, obestatin-induced survival in β -cells was lost in the presence of the GLP-1R antagonist exendin-9 and, besides recognizing specific binding sites in β -cells, obestatin showed specific binding to GLP-1R and upregulated GLP-1R mRNA [10]. Apart from GLP-1R, obestatin also interacted with ghrelin and des-acyl ghrelin binding sites,

and obestatin survival effects in α -cells were blunted by the ghrelin receptor antagonist [D-Lys3]-GHRP-6, suggesting cross-talk between the different ghrelin gene-derived peptides [10].

Obestatin, like ghrelin and des-acyl ghrelin, was shown to display antiapoptotic effects in human pancreatic islet microendothelial cells (MECs) exposed to chronic hyperglycemia. These effects were similar to those induced by the GLP-1R agonist exendin-4 (Ex-4); moreover, the signaling pathways involved were the same as those elicited in pancreatic β -cells [34]. On the basis of these findings, it was suggested that all the ghrelin gene peptides may improve islet vascularization and, by consequence, islet cell function [34].

Obestatin ability to promote *in vitro* β -cell generation from mouse pancreatic islet-derived precursors has been recently investigated [35]. In cultured mouse pancreatic islets, obestatin induced the generation of islet-like cell clusters (ICCs), that showed increased insulin gene expression and C-peptide secretion, as compared to untreated ICCs. This effect was likely due to obestatin-induced regulation of developmental pathways, such as down-regulation of fibroblast growth factor receptors (FGFRs), modulation of Notch receptors and induction of neurogenin 3 (Ngn3) [35]. These findings, together with its early expression in the developing pancreas [50], suggest a role of obestatin in pancreas regeneration and formation and its potential implication for cell-based replacement therapy in diabetes.

In newborn rats treated with streptozotocin (STZ), obestatin, like ghrelin and des-acyl ghrelin, reduced diabetes at adult age, by preventing β -cell loss, reducing glucose levels and increasing insulin expression and secretion in pancreatic islets [33]. Obestatin has been also shown to inhibit the development of cerulein-induced pancreatitis in rats, by reducing pancreatitis-evoked activity of digestive enzymes, improving pancreatic blood flow and decreasing serum levels of proinflammatory interleukin-1 β [41]. Moreover, intravenous administration of obestatin stimulates pancreatic protein output in anaesthetized rats, via a cholecystokinin- and vagal-dependent mechanism [39, 40].

The role of obestatin on insulin secretion is still controversial, as both stimulatory and inhibitory effects have been described. Obestatin was found to inhibit insulin secretion even more effectively than ghrelin, to inhibit somatostatin and pancreatic polypeptide (PP) secretion, and to stimulate glucagon secretion in isolated mouse islets [51]. Obestatin was also shown to either inhibit insulin release *in vivo* in rats and in isolated rat pancreatic islets [52], or to have no effect on glucose and insulin levels, in both basal or fasting conditions in rats and mice [27]. Conversely, in perfused rat pancreas obestatin either potentiated or inhibited insulin

secretion, when used at low or at high concentrations, respectively [53], and increased the stimulatory effects of arginine and tolbutamide [53]. Obestatin-induced insulin release in response to glucose has been also described *in vitro*, in β -cell lines and human pancreatic islets [10], as well as *in vivo* and in pancreatic islets of mice fed with both LFD and HFD [9]. Obestatin insulinotropic action is further supported by studies showing GLP-1R involvement in obestatin survival and metabolic effects in β -cells and adipocytes, as well as obestatin ability to interact with GLP-1R [5,9,10].

Obestatin effects in cardiovascular system and skeletal muscle

Besides the effects on cell survival and glucose/lipid metabolism in the endocrine pancreas and adipocytes, different groups have investigated the cardiovascular effects of obestatin and its role on myogenesis. In humans, fasting plasma obestatin levels have been found to negatively correlate with systolic blood pressure [54]; however, obestatin levels were increased in hypertensive rats [55]. Moreover, obestatin may have a role in blood pressure regulation, as its concentrations have been shown to positively correlate with systemic blood pressure in normal pregnant women and in those with pregnancy-induced hypertension [56].

In the isolated heart, the addition of rat obestatin before ischemia reduced infarct size and contractile dysfunction in a concentration-dependent manner. Moreover, in rat H9c2 cardiac cells or isolated ventricular myocytes subjected to ischemia/reperfusion, obestatin reduced cardiomyocyte apoptosis and reduced caspase-3 activation [11]. Obestatin also preserved papillary muscle contractility, β -adrenergic response, as well as β 1-adrenoreceptors and alpha-myosin heavy chain (α -MHC) levels in rat diabetic myocardial tissue [39]. However, obestatin was also found unable to prevent arabinoside-induced apoptosis or to modify the cell cycle or viability of HL-1 cardiomyocytes [57].

A role for obestatin/GPR39 has been also demonstrated in muscle regeneration, in which obestatin was found to exert an autocrine function to control the myogenic differentiation program, through involvement of GPR39 [18]. Obestatin infusion in rats also increased the expression of myogenic genes, further supporting its role in muscle regeneration [18].

Conclusions

Obestatin is still a debated peptide, particularly because of its controversial effects at the central level and the yet unknown identity of the receptor involved in its activities. Although GLP-1R has been suggested as a possible candidate receptor, this possibility needs to be sustained by additional studies, and the role of GPR39 cannot be completely excluded. Notably, obestatin exerts survival effects in different cell types, positively regulates glucose and lipid metabolism, reduces inflammation and promotes cardioprotection and muscle regeneration. Obestatin secretion has been also demonstrated in different tissues and cells, where autocrine/paracrine effects were demonstrated. Therefore, on these basis, obestatin may be considered a hormone and hopefully, future studies and results will help to convince the more skeptical groups on the potential biological and therapeutic importance of this peptide.

Figure legends

Figure 1. Simplified representation of obestatin biological effects. Question marks indicate controversial data. (GLP-1R, glucagon-like peptide-1 receptor; GPR39, G-protein-coupled receptor 39; CNS, central nervous system; GI, gastro-intestinal).

References

1. 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
2. Mohan H, Unniappan S, Discovery of ghrelin o-acyltransferase. *Endocr Dev* 2013; 25:16-24
3. Delhanty PJ, Neggers SJ, van der Lely AJ, Des-acyl ghrelin: a metabolically active peptide. *Endocr Dev* 2013; 25:112-121
4. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ, Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 2005; 310:996-999
5. Granata R, Baragli A, Settanni F, Scarlatti F, Ghigo E, Unraveling the role of the ghrelin gene peptides in the endocrine pancreas. *J Mol Endocrinol* 2010; 45:107-118
6. Zhang JV, Jahr H, Luo CW, Klein C, Van Kolen K, Ver Donck L, De A, Baart E, Li J, Moechars D, Hsueh AJ, Obestatin induction of early-response gene expression in gastrointestinal and adipose tissues and the mediatory role of G protein-coupled receptor, GPR39. *Mol Endocrinol* 2008; 22:1464-1475
7. Zhang JV, Li L, Huang Q, Ren PG, Obestatin receptor in energy homeostasis and obesity pathogenesis. *Prog Mol Biol Transl Sci* 2013; 114:89-107
8. Ren G, He Z, Cong P, Chen H, Guo Y, Yu J, Liu Z, Ji Q, Song Z, Chen Y, Peripheral administration of TAT-obestatin can influence the expression of liporegulatory genes but fails to affect food intake in mice. *Peptides* 2013; 42C:8-14
9. Granata R, Gallo D, Luque RM, Baragli A, Scarlatti F, Grande C, Gesmundo I, Cordoba-Chacon J, Bergandi L, Settanni F, Togliatto G, Volante M, Garetto S, Annunziata M, Chanclon B, Gargantini E, Rocchietto S, Matera L, Datta G, Morino M, Brizzi MF, Ong H, Camussi G, Castano JP, Papotti M, Ghigo E, Obestatin regulates adipocyte function and protects against diet-induced insulin resistance and inflammation. *FASEB J* 2012; 26:3393-3411
10. Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V, Nano R, Annunziata M, Campiglia P, Arnoletti E, Ghe C, Volante M, Papotti M, Muccioli G, Ghigo E, Obestatin promotes survival of pancreatic beta-cells and human islets and induces expression of genes involved in the regulation of beta-cell mass and function. *Diabetes* 2008; 57:967-979
11. Alloatti G, Arnoletti E, Bassino E, Penna C, Perrelli MG, Ghe C, Muccioli G, 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
12. Dun SL, Brailoiu GC, Brailoiu E, Yang J, Chang JK, Dun NJ, Distribution and biological activity of obestatin in the rat. *J Endocrinol* 2006; 191:481-489
13. Chanoine JP, Wong AC, Barrios V, Obestatin, acylated and total ghrelin concentrations in the perinatal rat pancreas. *Horm Res* 2006; 66:81-88

14. Bang AS, Soule SG, Yandle TG, Richards AM, Pemberton CJ, Characterisation of proghrelin peptides in mammalian tissue and plasma. *J Endocrinol* 2007; 192:313-323
15. Gronberg M, Tsolakis AV, Magnusson L, Janson ET, Saras J, Distribution of obestatin and ghrelin in human tissues: immunoreactive cells in the gastrointestinal tract, pancreas, and mammary glands. *J Histochem Cytochem* 2008; 56:793-801
16. Volante M, Rosas R, Ceppi P, Rapa I, Cassoni P, Wiedenmann B, Settanni F, Granata R, Papotti M, Obestatin in human neuroendocrine tissues and tumours: expression and effect on tumour growth. *J Pathol* 2009; 218:458-466
17. Zhao CM, Furnes MW, Stenstrom B, Kulseng B, Chen D, Characterization of obestatin- and ghrelin-producing cells in the gastrointestinal tract and pancreas of rats: an immunohistochemical and electron-microscopic study. *Cell Tissue Res* 2008; 331:575-587
18. Gurriaran-Rodriguez U, Santos-Zas I, Al-Massadi O, Mosteiro CS, Beiroa D, Nogueiras R, Crujeiras AB, Seoane LM, Senaris J, Garcia-Caballero T, Gallego R, Casanueva FF, Pazos Y, Camina JP, The obestatin/GPR39 system is up-regulated by muscle injury and functions as an autocrine regenerative system. *J Biol Chem* 2012; 287:38379-38389
19. Moretti E, Vindigni C, Tripodi SA, Mazzi L, Nuti R, Figura N, Collodel G, Immunolocalisation of ghrelin and obestatin in human testis, seminal vesicles, prostate and spermatozoa. *Andrologia* 2013;
20. Seim I, Walpole C, Amorim L, Josh P, Herington A, Chopin L, The expanding roles of the ghrelin-gene derived peptide obestatin in health and disease. *Mol Cell Endocrinol* 2012; 340:111
21. Huda MS, Mani H, Durham BH, Dovey TM, Halford JC, Aditya BS, Pinkney JH, Wilding JP, Hart IK, Plasma obestatin and autonomic function are altered in orexin-deficient narcolepsy, but ghrelin is unchanged. *Endocrine* 2013; 43:696-704
22. Cui AD, Gai NN, Zhang XH, Jia KZ, Yang YL, Song ZJ, Decreased serum obestatin consequent upon TRIB3 Q84R polymorphism exacerbates carotid atherosclerosis in subjects with metabolic syndrome. *Diabetol Metab Syndr* 2012; 4:52
23. Gronberg M, Tsolakis AV, Holmback U, Stridsberg M, Grimelius L, Janson ET, Ghrelin and obestatin in human neuroendocrine tumors: expression and effect on obestatin levels after food intake. *Neuroendocrinology* 2013; 97:291-299
24. Alexandridis E, Zisimopoulos A, Liratzopoulos N, Katsos I, Manolas K, Kouklakis G, Obestatin/ghrelin ratio: a new activity index in inflammatory bowel diseases. *Inflamm Bowel Dis* 2009; 15:1557-1561
25. Gao XY, Kuang HY, Liu XM, Ma ZB, Nie HJ, Guo H, Plasma obestatin levels in men with chronic atrophic gastritis. *Peptides* 2008; 29:1749-1754
26. Ulasoglu C, Isbilen B, Doganay L, Ozen F, Kiziltas S, Tuncer I, Effect of *Helicobacter pylori* eradication on serum ghrelin and obestatin levels. *World J Gastroenterol* 2013; 19:2388-2394
27. Green BD, Irwin N, Flatt PR, Direct and indirect effects of obestatin peptides on food intake and the regulation of glucose homeostasis and insulin secretion in mice. *Peptides* 2007; 28:981-987

28. Samson WK, White MM, Price C, Ferguson AV, Obestatin acts in brain to inhibit thirst. *Am J Physiol Regul Integr Comp Physiol* 2007; 292:R637-643
29. Carlini VP, Schioth HB, Debarioglio SR, Obestatin improves memory performance and causes anxiolytic effects in rats. *Biochem Biophys Res Commun* 2007; 352:907-912
30. Szentirmai E, Krueger JM, Obestatin alters sleep in rats. *Neurosci Lett* 2006; 404:222-226
31. Camina JP, Campos JF, Caminos JE, Dieguez C, Casanueva FF, Obestatin-mediated proliferation of human retinal pigment epithelial cells: regulatory mechanisms. *J Cell Physiol* 2007; 211:1-9
32. Pazos Y, Alvarez CJ, Camina JP, Casanueva FF, Stimulation of extracellular signal-regulated kinases and proliferation in the human gastric cancer cells KATO-III by obestatin. *Growth Factors* 2007; 25:373-381
33. Granata R, Volante M, Settanni F, Gauna C, Ghe C, Annunziata M, Deidda B, Gesmundo I, Aribat T, van der Lely AJ, Muccioli G, Ghigo E, Papotti M, Unacylated ghrelin and obestatin increase islet cell mass and prevent diabetes in streptozotocin-treated newborn rats. *J Mol Endocrinol* 2010; 45:9-17
34. Favaro E, Granata R, Miceli I, Baragli A, Settanni F, Cavallo Perin P, Ghigo E, Camussi G, Zanone MM, The ghrelin gene products and exendin-4 promote survival of human pancreatic islet endothelial cells in hyperglycaemic conditions, through phosphoinositide 3-kinase/Akt, extracellular signal-related kinase (ERK)1/2 and cAMP/protein kinase A (PKA) signalling pathways. *Diabetologia* 2012; 55:1058-1070
35. Baragli A, Grande C, Gesmundo I, Settanni F, Taliano M, Gallo D, Gargantini E, Ghigo E, Granata R, Obestatin enhances in vitro generation of pancreatic islets through regulation of developmental pathways. *PLoS ONE* 2013; 8:e64374
36. Gurriaran-Rodriguez U, Al-Massadi O, Roca-Rivada A, Crujeiras AB, Gallego R, Pardo M, Seoane LM, Pazos Y, Casanueva FF, Camina JP, Obestatin as a regulator of adipocyte metabolism and adipogenesis. *J Cell Mol Med* 2011; 15:1927-1940
37. Miegueu P, St Pierre D, 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
38. Pruszyńska-Oszmalek E, Szczepankiewicz D, Hertig I, Skrzypski M, Sassek M, Kaczmarek P, Kolodziejcki PA, Mackowiak P, Nowak KW, Strowski MZ, Wojciechowicz T, Obestatin inhibits lipogenesis and glucose uptake in isolated primary rat adipocytes. *J Biol Regul Homeost Agents* 2013; 27:23-33
39. Aragno M, Mastrocola R, Ghe C, Arnoletti E, Bassino E, Alloatti G, Muccioli G, Obestatin induced recovery of myocardial dysfunction in type 1 diabetic rats: underlying mechanisms. *Cardiovasc Diabetol* 2012; 11:129

40. Kellokoski E, Kunnari A, Jokela M, Makela S, Kesaniemi YA, Horkko S, Ghrelin and obestatin modulate early atherogenic processes on cells: enhancement of monocyte adhesion and oxidized low-density lipoprotein binding. *Metabolism* 2009; 58:1572-1580
41. Ceranowicz P, Warzecha Z, Dembinski A, Cieszkowski J, Dembinski M, Sendur R, Kusnierz-Cabala B, Tomaszewska R, Kuwahara A, Kato I, Pretreatment with obestatin inhibits the development of cerulein-induced pancreatitis. *J Physiol Pharmacol* 2009; 60:95-101
42. Agnew A, Calderwood D, Chevallier OP, Greer B, Grieve DJ, Green BD, Chronic treatment with a stable obestatin analog significantly alters plasma triglyceride levels but fails to influence food intake; fluid intake; body weight; or body composition in rats. *Peptides* 2011; 32:755-762
43. Grala TM, Kay JK, Walker CG, Sheahan AJ, Littlejohn MD, Lucy MC, Roche JR, Expression analysis of key somatotrophic axis and liporegulatory genes in ghrelin- and obestatin-infused dairy cows. *Domest Anim Endocrinol* 2010; 39:76-83
44. Yoshizaki T, Milne JC, Imamura T, Schenk S, Sonoda N, Babendure JL, Lu JC, Smith JJ, Jirousek MR, Olefsky JM, SIRT1 exerts anti-inflammatory effects and improves insulin sensitivity in adipocytes. *Mol Cell Biol* 2009; 29:1363-1374
45. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K, Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; 116:1784-1792
46. Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, Boss O, Pernin A, Chin WW, Cusin I, Rohner-Jeanrenaud F, Burger AG, Zapf J, Meier CA, Direct effects of leptin on brown and white adipose tissue. *J Clin Invest* 1997; 100:2858-2864
47. Walia P, Asadi A, Kieffer TJ, Johnson JD, Chanoine JP, Ontogeny of ghrelin, obestatin, proghrelin, and prohormone convertases in rat pancreas and stomach. *Pediatr Res* 2009; 65:39-44
48. Turk N, Dagistanli FK, Sacan O, Yanardag R, Bolkent S, Obestatin and insulin in pancreas of newborn diabetic rats treated with exogenous ghrelin. *Acta Histochem* 2012; 114:349-357
49. Donath MY, Halban PA, Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia* 2004; 47:581-589
50. Wierup N, Svensson H, Mulder H, Sundler F, The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regul Pept* 2002; 107:63-69
51. Qader SS, Hakanson R, Rehfeld JF, Lundquist I, Salehi A, Proghrelin-derived peptides influence the secretion of insulin, glucagon, pancreatic polypeptide and somatostatin: a study on isolated islets from mouse and rat pancreas. *Regul Pept* 2008; 146:230-237
52. Ren AJ, Guo ZF, Wang YK, Wang LG, Wang WZ, Lin L, Zheng X, Yuan WJ, Inhibitory effect of obestatin on glucose-induced insulin secretion in rats. *Biochem Biophys Res Commun* 2008; 369:969-972
53. Egido EM, Hernandez R, Marco J, Silvestre RA, Effect of obestatin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas. *Regul Pept* 2009;

54. Anderwald-Stadler M, Krebs M, Promintzer M, Mandl M, Bischof MG, Nowotny P, Kastenbauer T, Luger A, Prager R, Anderwald C, Plasma obestatin is lower at fasting and not suppressed by insulin in insulin-resistant humans. *Am J Physiol Endocrinol Metab* 2007; 293:E1393-1398
55. Li ZF, Guo ZF, Cao J, Hu JQ, Zhao XX, Xu RL, Huang XM, Qin YW, Zheng X, Plasma ghrelin and obestatin levels are increased in spontaneously hypertensive rats. *Peptides* 2010; 31:297-300
56. Ren AJ, He Q, Shi JS, Guo ZF, Zheng X, Lin L, Wang YK, Xia SY, Sun LL, Du X, Sun Y, Zhang LM, Yuan WJ, Association of obestatin with blood pressure in the third trimesters of pregnancy. *Peptides* 2009; 30:1742-1745
57. Iglesias MJ, Salgado A, Pineiro R, Rodino BK, Otero MF, Grigorian L, Gallego R, Dieguez C, Gualillo O, Gonzalez-Juanatey JR, Lago F, Lack of effect of the ghrelin gene-derived peptide obestatin on cardiomyocyte viability and metabolism. *J Endocrinol Invest* 2007; 30:470-476

Figure 1

