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REVIEW ARTICLE

Obestatin as a key regulator of metabolism and cardiovascular function with emerging therapeutic potential for diabetes

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Obestatin is a 23-amino acid C-terminally amidated gastrointestinal peptide derived from preproghrelin and which forms an α helix. Although obestatin has a short biological half-life and is rapidly degraded, it is proposed to exert wide-ranging pathophysiological actions. Whilst the precise nature of many of its effects is unclear, accumulating evidence supports positive actions on both metabolism and cardiovascular function. For example, obestatin has been reported to inhibit food and water intake, body weight gain and gastrointestinal motility and also to mediate promotion of cell survival and prevention of apoptosis. Obestatin-induced increases in beta cell mass, enhanced adipogenesis and improved lipid metabolism have been noted along with up-regulation of genes associated with beta cell regeneration, insulin production and adipogenesis. Furthermore, human circulating obestatin levels generally demonstrate an inverse association with obesity and diabetes, whilst the peptide has been shown to confer protective metabolic effects in experimental diabetes, suggesting that it may hold therapeutic potential in this setting. Obestatin also appears to be involved in blood pressure regulation and to exert beneficial effects on endothelial function, with experimental studies indicating that it may also promote cardioprotective actions against, for example, ischaemia–reperfusion injury. This review will present a critical appraisal of the expanding obestatin research area and discuss the emerging therapeutic potential of this peptide for both metabolic and cardiovascular complications of diabetes.

Abbreviations

BK_{Ca}, large conductance calcium-activated potassium channel; CART, cocaine- and amphetamine-related transcript; CCK, cholecystokinin; CRF, corticotrophin-releasing factor; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; GLUT-4, glucose transporter type 4; HOMA-IR, homeostatic model assessment of insulin resistance; NPY, neuropeptide Y; PEG, polyethylene glycol; PI3K, phosphoinositide 3-kinase; POMC, proopiomelanocortin; SK6l, ribosomal protein s6 kinase; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; WAT, white adipose tissue.

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Tables of Links

TARGETS		
GPCRs[b]	**Enzymes**[c]	
GLP-1 receptor	Adenylate cyclase	eNOS
GPR39	Akt (PKB)	ERK1
Nuclear hormone receptors[a]	Caspase 3	ERK2
PPARγ	Caspase 7	PI3K
Transporters[d]	Caspase 9	PKCδ
GLUT-4	GSK3β	PKCε

LIGANDS	
Adiponectin	Insulin
cAMP	Leptin
Cholecystokinin	Neuropeptide Y
CRF	Nitric oxide (NO)
Exendin(9-39)	Obestatin
Ghrelin	Vasopressin
GLP-1	VEGF
Glucagon	YIL781

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d]Alexander *et al.*, 2015a,b,c,d).

Introduction

Currently, 415 million adults are thought to have diabetes worldwide, with an increasing number developing obesity-related type 2 diabetes mellitus (T2DM) at a typically younger age. More worryingly, this figure is rapidly rising to epidemic proportions and is estimated to reach 642 million by 2040 (International Diabetes Federation, 2015). The disease itself is a leading cause of global mortality, accounting for 5 million adult deaths in 2015, with the major underlying factor being cardiovascular disease due to common complications, such as atherosclerosis, nephropathy and stroke, which confer a four-fold increased risk of death (Grundy *et al.*, 1999). This is despite optimal management with established metabolic and cardiovascular therapies, including metformin and angiotensin-converting enzyme inhibitors. Although there have been some recent advances in the development of novel anti-diabetic agents, such as drugs targeting the glucagon-like peptide-1 (GLP-1) receptor, the potential cardiovascular benefits of such therapies remain controversial (Tate *et al.*, 2015). The need for improved treatment strategies that improve both the metabolic profile and cardiovascular risk in diabetic patients is therefore clear. In this regard, this article will focus on obestatin, a recently discovered endogenous peptide with emerging metabolic and cardiovascular actions that may be relevant to T2DM. Whilst previous reviews have tended to highlight the pathophysiological actions of obestatin in relation to its well-characterized sister hormone, ghrelin, we will specifically focus on the somewhat controversial metabolic effects of obestatin and discuss these together with its emerging cardiovascular actions in order to provide a balanced up-to-date critical appraisal of this developing research area with a view towards potential therapeutic applications.

Biology of obestatin

Obestatin discovery

First discovered in 2005 using bioinformatics, obestatin is a 23-amino acid peptide that is derived from the same 117-residue prepropeptide as ghrelin (Zhang *et al.*, 2005). It displays a post-translational amide modification of the C-terminal, which was initially suggested to be essential for binding of obestatin (Zhang *et al.*, 2005) and later demonstrated to be essential for stabilization of the peptide into its regular conformation (Scrima *et al.*, 2007), which has now been determined. Detailed analysis using nuclear magnetic resonance and circular dichroism spectroscopy found that both human and mouse obestatin, as well as fragments of human obestatin: (6–23), (11–23) and (16–23), adopted an α-helical secondary structure despite their different sequences (Alen *et al.*, 2012). It seems likely that this characteristic structure is required for binding of obestatin to its receptor, although the specific domains involved remain to be determined. Subsequent to its initial discovery, the receptor for obestatin was reported to be the GPCR, GPR39 (Zhang *et al.*, 2005), although this has been highly disputed (Lauwers *et al.*, 2006), with zinc ions appearing to be the endogenous ligand for this receptor (Holst *et al.*, 2007; Popovics and Stewart, 2011). In support of this, gene-modified mice lacking GPR39 displayed a similar metabolic profile, for example, food intake, body weight, adiposity and fasting glucose/insulin (which are modified by obestatin; see below), compared with wild-type controls, and an intact metabolic response to obestatin, providing strong evidence that GPR39 is not the native receptor for obestatin, at least in the gastrointestinal (GI) tract (Tremblay *et al.*, 2007). However, another group reported increased gastric emptying in the same GPR39$^{-/-}$ mice (Moechars *et al.*, 2006), thereby supporting the initial findings (Zhang *et al.*, 2005). Indeed, more recent studies have indicated that obestatin up-regulates GPR39 in isolated rat adipocytes and mouse white adipose tissue (WAT), where it may mediate at least some of its reported effects via induction of c-Fos and ERK1/2 signalling (Zhang *et al.*, 2008a; Pruszynska-Oszmalek *et al.*, 2013; Ren *et al.*, 2013a). Further to several similarities between the emerging actions of obestatin (see below) and those of GLP-1, particularly in relation to pancreatic beta cells, the GLP-1 receptor was suggested as a candidate for the obestatin receptor. Indeed, obestatin was shown to bind to and up-regulate the GLP-1 receptor, and its effects on beta cell

survival were attenuated by the GLP-1 receptor antagonist, exendin(9–39) (Granata *et al.*, 2008). Furthermore, in mouse 3T3-L1 and human adipocytes, both activation and blockade of the GLP-1 receptor inhibited obestatin binding (Granata *et al.*, 2012). In contrast, obestatin was unable to bind to the GLP-1 receptor or to displace GLP-1 binding in INS-1 pancreatic beta cells and HEK293 cells overexpressing GLP-1 receptors (Unniappan *et al.*, 2008). Taken together, these data are generally supportive of the suggestion that obestatin may signal through the GLP-1 receptor, although there is currently insufficient independently verified evidence to allow definite conclusions to be drawn. Nonetheless, the prospect of involvement of the GLP-1 receptor in obestatin signalling is particularly intriguing further to our recent reports of direct cardioprotective actions of GLP-1 receptor activation (Robinson *et al.*, 2015; Tate *et al.*, 2016). In this regard, we have suggested that obestatin may signal via an adenylate cyclase-linked GPCR in the cardiovascular system (Agnew *et al.*, 2012), although the precise identity of the cognate receptor(s) for obestatin remains to be determined. Indeed, it is likely that the obestatin receptor may vary between tissues, and detailed biochemical analysis using, for example, binding studies with putative orphan receptors or ligand-based affinity chromatography (Pattnaik, 2005), will be required in order to gain a clearer understanding of its signalling.

Tissue distribution

Obestatin and ghrelin are largely produced throughout the GI tract (e.g. stomach, pancreas and duodenum) with predominant expression in the gastric mucosa (Zhao *et al.*, 2008), although their distribution is somewhat species specific. For example, in the rat, obestatin is found in the GI tract, within the A-like cells and oxyntic glands of the gastric mucosa and cholinergic neurons of the myenteric plexus, and in the Leydig cells of the testis where it is co-localized with its precursor peptide, preproghrelin (Zhao *et al.*, 2008; Mizutani *et al.*, 2009). Obestatin is also expressed in the brain where it promotes calcium signalling via stimulation of intracellular calcium store release (Ku *et al.*, 2015), which may mediate some of its proposed central actions (see below). In rodents, ghrelin is reported to be present in the GI mucosa (Dun *et al.*, 2006) and is also expressed by cholinergic neurons of the myenteric plexus (Xu *et al.*, 2005). Similarly, in humans, the majority of obestatin production is localized to the GI tract, with predominance in the stomach versus the duodenum, jejunum and ileum (where it is specifically found in the crypts of Lieberkuhn and Brunner's glands), and absence from the colon, whilst obestatin is also expressed in both the periphery of the pancreatic islets and the exocrine pancreatic ducts (Grönberg *et al.*, 2008). Furthermore, both obestatin and ghrelin have been identified in epithelial ducts of the human mammary gland (Grönberg *et al.*, 2008), with ghrelin-positive cells found in some human breast cancers and cell lines (Cassoni *et al.*, 2004). In contrast, there are conflicting reports with regard to obestatin/ghrelin co-expression, with one study reporting a high degree of co-localization in human cells (Grönberg *et al.*, 2008), whereas another found that only 60% of obestatin immunoreactive cells were also immunoreactive for ghrelin (Zhao *et al.*, 2008). However, it should be noted that these differences may be explained by variations in detection or sensitivity between the two studies.

Stability of obestatin and circulating levels

Once obestatin enters the circulation, it is rapidly degraded by a number of proteases, such as aminopeptidase and post-prolyl endopeptidase, which are largely located in the blood, liver and kidney (Vergote *et al.*, 2008). Its half-life in the plasma is a critical determinant of whether obestatin is able to reach and act upon its target tissues, and published figures in rodents are highly variable. For example, the half-life of native mouse obestatin in mouse plasma is reported to be 42.2 min, compared with 12.6 min in liver and 138 min in kidney membranes (Vergote *et al.*, 2008), whilst the half-life of rodent obestatin in rat liver homogenate was found to be 21.7 min and increased over threefold by the addition of a polyethylene glycol (PEG) group to the N-terminus (Agnew *et al.*, 2011). A large number of groups have investigated the circulating physiological levels of obestatin in both rodents and humans, with a wide range of values reported (rodents: 1.34 to 2560; humans: 8.4 to 22 057 $pg{\cdot}mL^{-1}$; see Table 1). The most likely explanation for these markedly different results is due to variations in the sensitivity of the employed detection methods and their specificity for obestatin versus proghrelin (Seim *et al.*, 2011). Interestingly, one group reported human plasma obestatin levels of 267 ± 10 $pg{\cdot}mL^{-1}$ (Zamrazilová *et al.*, 2008), whilst another published values of 68.3 ± 14.8 $pg{\cdot}mL^{-1}$ (Monteleone *et al.*, 2008b), that is, fourfold lower, despite using the apparently same detection method. However, these differences may also be due to diurnal variations in obestatin production, which has been reported to follow a pulsatile pattern comparable with that of ghrelin (Zizzari *et al.*, 2007). Such observations highlight the importance of following rigorous sampling and analysis protocols in order to achieve reliable estimates of circulating obestatin levels, which to date have been both conflicting and largely uninformative.

Metabolic actions of obestatin

Obestatin and the GI system

Further to its original discovery, obestatin was first reported to inhibit jejunal contraction, food intake and body weight gain in rats, in addition to antagonising ghrelin-induced contraction of isolated jejunum muscle (Zhang *et al.*, 2005), actions that are clearly relevant to T2DM. These initial findings with regard to GI transit have since been confirmed by the same authors (Zhang *et al.*, 2007) and others, who have reported obestatin to reduce antral and duodenal motility in the fed state and to impede restoration of normal fasted-state duodenal activity (Ataka *et al.*, 2008; Fujimiya *et al.*, 2008; Fujimiya *et al.*, 2012). Decreased duodenal and jejunal motility in adult rats have also been confirmed by a recent study, although increased GI contractility was demonstrated in suckling and adolescent rats in response to obestatin in this same investigation (Słupecka *et al.*, 2014). Furthermore, a clinical investigation reported increased preprandial obestatin levels in children with unexplained delayed gastric emptying (Saliakelis *et al.*, 2014). However, a significant number of investigators have failed to reproduce such effects of obestatin on GI motility (Bassil *et al.*, 2007; De Smet *et al.*, 2007; Gourcerol and Taché, 2007; Gourcerol *et al.*, 2007a;

Table 1

Circulating levels of obestatin in normal physiology

Obestatin level (pg·mL^{-1})	Study details		
	Tissue	Detection	Reference
24.9 ± 3[a]	Plasma (human ♂/♀)	Unknown	Huda *et al.*, 2007
139.3 ± 46.8[b]	Plasma (human ♂/♀)	RIA	Lippl *et al.*, 2008
438.9 ± 350.7[b]	Serum (human ♂/♀)	RIA	Koca *et al.*, 2008
181 ± 15.3[a]	Plasma (human ♀)	RIA	Sedlácková *et al.*, 2008
270.3 ± 28.21[b]	Blood (human ♀)	RIA	Aydin *et al.*, 2008
63.4 ± 9.5[b]	Plasma (human ♀)	RIA	Ren *et al.*, 2009
227.8 ± 116.9[b]	Serum (human ♂/♀)	RIA	Aygen *et al.*, 2009
148.2 ± 96.8[b]	Plasma (human ♂/♀)	RIA	Kukuvitis *et al.*, 2010
4600 ± 1600[b]	Serum (human ♂/♀)	EIA	Mafra *et al.*, 2010
364.9 ± 101.4[c]	Plasma (human child ♂/♀)	Unknown	Buescher *et al.*, 2010
1156.1 ± 1361.8[b]	Serum (human ♂/♀)	RIA	Gutierrez-Grobe *et al.*, 2010
32.5 ± 5[b]	Plasma (human ♂/♀)	RIA	Kosowicz *et al.*, 2011
243.5 ± 65.37[b]	Serum (human ♂)	RIA	Moretti *et al.*, 2011
3600[d]	Serum (human ♂/♀)	EIA	Aktas *et al.*, 2011
844.87 (805.14)[e]	Serum (human infants ♂/♀)	RIA	Savino *et al.*, 2012
205 ± 48[b]	Plasma (human ♀)	EIA	Hedayati *et al.*, 2012
2674 (2343–4890)[f]	Plasma (human ♂/♀)	RIA	Grönberg *et al.*, 2013
3663.90 ± 2313.95[b]	Plasma (human ♂/♀)	EIA	Lei *et al.*, 2014
58.5 ± 10.3[b]	Serum (human ♂/♀)	EIA	Emami *et al.*, 2014
410.72 ± 115.44[b]	Plasma (human ♂/♀)	EIA	Liu *et al.*, 2014
69.7 ± 7.5[b]	Plasma (human ♂/♀)	RIA	Gao *et al.*, 2014
21.68 ± 1.42[b]	Serum (human child ♂/♀)	EIA	Taskin *et al.*, 2014
325.3 ± 163.6[b]	Serum (human child ♂/♀)	RIA	Saliakelis *et al.*, 2014
200 ± 20[b]	Plasma (human infants ♂/♀)	RIA	Zhang *et al.*, 2014
8.4 (1.9–13.0)[g]	Serum (human ♀)	EIA	Taskin *et al.*, 2015
22 057 ± 873[a]	Serum (human ♂/♀)	EIA	Ayada *et al.*, 2015
805 ± 30[a]	Plasma (mouse/rat ♂)	RIA	Zizzari *et al.*, 2007
Below detection limit	Plasma (rat ♂)	RIA	Mondal *et al.*, 2008
1680 ± 100[a]	Plasma (rat ♂)	EIA	Guo *et al.*, 2008
2560 ± 120[a]	Plasma (rat ♂)	EIA	Ghanbari-Niaki, 2010
1800 ± 180[a]	Plasma (rat ♂)	EIA	Huang *et al.*, 2012
1.34 ± 0.1[c]	Serum (rat ♂)	RIA	Kong *et al.*, 2010

EIA, enzyme immunoassay.
[a]Mean ± SEM.
[b]Mean ± SD.
[c]Mean ± SEM or mean ± SD.
[d]Median.
[e]Median (interquartile range).
[f]Median (first to fourth quartile).
[g]Median (min–max).

Yamamoto *et al.*, 2007; Chen *et al.*, 2008, 2010, 2012a,b; Depoortere *et al.*, 2008). Furthermore, obestatin is incapable of preventing ghrelin-mediated acceleration of gastric emptying or intestinal motility (Bassil *et al.*, 2007; Ataka *et al.*, 2008), and obestatin levels and the ghrelin/obestatin ratio are unchanged in patients with gastroparesis, a condition associated with delayed gastric emptying (Harsch *et al.*, 2009), thereby challenging the proposed actions of obestatin on GI motility. Obestatin immunoreactivity in the stomach has also been questioned (Bang *et al.*, 2007). Similarly, the originally reported beneficial effects of obestatin on food intake and body weight have also been questioned, with more studies disputing (Seoane *et al.*, 2006; Sibilia *et al.*, 2006; Gourcerol *et al.*, 2006, 2007a,b; Nogueiras *et al.*, 2007; Tremblay *et al.*,

2007; Yamamoto *et al.*, 2007; Zizzari *et al.*, 2007; Gourcerol and Taché, 2007; Holst *et al.*, 2007; Kobelt *et al.*, 2008; Mondal *et al.*, 2008; Unniappan *et al.*, 2008; Depoortere *et al.*, 2008; Van Dijck *et al.*, 2009; Agnew *et al.*, 2011; Ren *et al.*, 2013a; Yuan *et al.*, 2015) rather than confirming the initial findings (Bresciani *et al.*, 2006; Green *et al.*, 2007; Nagaraj *et al.*, 2008, 2009; Brunetti *et al.*, 2009, 2010; Hassouna *et al.*, 2012) on feeding behaviour. Notably, within these negative studies, obestatin was found not to influence cholecystokinin (CCK)-mediated satiety signalling (Gourcerol *et al.*, 2006) and to inhibit water more potently than food intake, leading the authors to suggest that previously reported effects of obestatin on food intake may occur secondary to those on water intake (Samson *et al.*, 2007), although these data have not been reproduced by other groups (Van Dijck *et al.*, 2009; Agnew *et al.*, 2011). Similarly, despite demonstrating significant effects of obestatin administration on food intake in rats in response to 24 h food and water deprivation, a recent study reported no effects on water intake (Motorykina *et al.*, 2015).

Further to its apparent, albeit controversial, effects on GI motility, food intake and body weight, obestatin has also been reported to modulate the actions of its sister hormone, ghrelin. For example, obestatin was shown to inhibit the orexigenic actions of ghrelin in rodents and fish (Zizzari *et al.*, 2007; Yuan *et al.*, 2015), although some groups found no effect (Seoane *et al.*, 2006; Nogueiras *et al.*, 2007). Furthermore, although obestatin did not affect brain expression of neuropeptide Y (NPY) and its receptors, agouti-related peptide, proopiomelanocortin (POMC), cocaine- and amphetamine-related transcript (CART) and CCK in rodents, which are all involved in the regulation of food intake (Nogueiras *et al.*, 2007; Yuan *et al.*, 2015), it was able to inhibit ghrelin-induced expression of NPY and NPY receptors, but not POMC, CART or CCK (Yuan *et al.*, 2015). Notably, both native obestatin and a natural obestatin variant (preproghrelin polymorphism Gln90Leu) decreased ghrelin-induced food intake in mice, together with growth hormone secretion and c-Fos activation in the brain (Hassouna *et al.*, 2012). Conversely, obestatin-mediated decreases in GI motility were prevented by injection of corticotrophin-releasing factor (CRF) receptor antagonists, whilst c-Fos expression was induced by obestatin administration, indicating that potential actions on food intake and GI motility may occur, at least in part, via the vagal afferent pathway and central CRF receptors (Ataka *et al.*, 2008; Fujimiya *et al.*, 2008; Zhang *et al.*, 2008a; Fujimiya *et al.*, 2012).

In addition to its proposed physiological actions, it appears that obestatin may also confer some benefits in GI disease. For example, in rats, obestatin protects against experimental ulcerative colitis via acute attenuation of lipid peroxidation and TH_1-mediated inflammation, chronic suppression of polymorphonuclear leukocyte infiltration, induction of glutathione synthesis, improved mucosal blood flow and stimulation of cell proliferation in colonic mucosa, effects that may be mediated by activation of anti-inflammatory cytokines (Pamukcu *et al.*, 2013; Matuszyk *et al.*, 2015). Furthermore, obestatin administration has been shown to confer protective effects against ischaemia–reperfusion injury in rat ileum (Şen *et al.*, 2015), whilst the ghrelin/obestatin ratio (but not obestatin levels) is reported to be elevated in patients with active inflammatory bowel diseases (Crohn's disease and colitis) compared with those in remission (Jung *et al.*, 2015; Alexandridis *et al.*, 2009), suggesting that obestatin signalling may play a role in this setting.

Obestatin and the pancreas

Pancreatic beta cell loss, reduced beta cell function and inflammation are characteristic of both type 1 diabetes mellitus (T1DM) and T2DM and so are a major focus of research aimed at development of novel metabolic therapies (Donath and Halban, 2004). Indeed, obestatin and ghrelin are co-expressed in both fetal and adult endocrine pancreas with co-localization at the islet periphery, thereby suggesting a synergistic relationship that may be connected with pancreatic beta cell function (Granata *et al.*, 2010a). In 2008, obestatin was reported to be secreted by human pancreatic islets and pancreatic beta cell lines, to enhance their viability in response to both serum starvation and cytokines and to inhibit apoptosis (Granata *et al.*, 2008; Favaro *et al.*, 2012). In addition, survival of these cells was compromised upon incubation with an anti-obestatin antibody, whilst genes associated with insulin production, beta cell survival, mass, growth and differentiation (insulin receptor substrate 2, cAMP response element binding protein, pancreatic and duodenal homeobox-1, and glucokinase) were up-regulated by obestatin, together with activation of phosphoinositide 3-kinase (PI3K)/Akt, ERK1/2 and cAMP (Granata *et al.*, 2008), thus highlighting a potential autocrine/paracrine role. Obestatin also enhances generation of pancreatic islet-like clusters together with increased insulin gene expression during endocrine pancreatic precursor cell selection and differentiation, which appears to occur via pathways involving fibroblast growth factor receptors, notch receptors and neurogenin 3, suggesting a role in pancreatic development and regeneration (Baragli *et al.*, 2013). Notably, the reported anti-apoptotic actions of obestatin in the pancreas appear to extend to its microvascular endothelial cells, indicating that such protection may be mediated indirectly via support of islet vascularization (Favaro *et al.*, 2012). Similarly, obestatin has been shown to protect against acute pancreatitis in rats, induced by either cerulein or ischaemia/reperfusion, via increasing pancreatic blood supply in parallel with reduced inflammation and digestive enzyme activity, and also to promote pancreatic repair and regeneration in these animals (Ceranowicz *et al.*, 2009; Bukowczan *et al.*, 2015). Indeed, circulating obestatin levels are increased in patients with acute pancreatitis (Kanat *et al.*, 2014), supporting a protective function in this setting.

Although obestatin appears to activate pancreatic insulin gene expression, at least *in vitro*, its effects on insulin secretion are unclear due to highly variable reports (Green *et al.*, 2007; Granata *et al.*, 2008; Qader *et al.*, 2008; Ren *et al.*, 2008). For example, several studies have shown obestatin to have no effect on circulating glucose or insulin in normoglycaemic mice and rats (Green *et al.*, 2007; Kiewiet *et al.*, 2008; Unniappan *et al.*, 2008; Agnew *et al.*, 2011), although glucose-induced insulin secretion in rats *in vivo* and in mouse and rat isolated islets was inhibited by obestatin (Qader *et al.*, 2008; Ren *et al.*, 2008), which is consistent with reports of an inverse relationship between obestatin and insulin levels in humans (Gao *et al.*, 2008; Lippl *et al.*, 2008).

In contrast, other studies have shown obestatin to stimulate insulin secretion in human islets in both the presence and absence of glucose and to potentiate the insulinotropic actions of arginine and tolbutamide (Granata *et al.*, 2008; Egido *et al.*, 2009). Interestingly, obestatin is capable of regulating secretion of other pancreatic hormones (glucagon, pancreatic polypeptide and somatostatin) in isolated rodent islets (Qader *et al.*, 2008) and increases pancreatic protein output in rats via vagal activation (Kapica *et al.*, 2007). Although the precise pancreatic actions of obestatin remain unclear, the presented evidence highlighting beneficial effects on beta cell metabolism and survival coupled with its ability to modulate insulin levels and inflammation clearly supports further investigation of this peptide as a potential therapeutic target in diabetes.

Obestatin and adipose tissue

Similar to the GI and pancreatic actions of obestatin, its reported effects on adipose tissue function, production and survival are also subject to some debate. Several groups have demonstrated obestatin secretion from rat WAT and adipocytes from both mice and humans (Gurriarán-Rodríguez *et al.*, 2011b; Granata *et al.*, 2012), although one study implied that adipose tissue does not secrete obestatin (Zhang *et al.*, 2008b). Expression of the obestatin precursor, preproghrelin, has also been reported in mouse epididymal and subcutaneous adipose tissue, whilst both neutralization of preproghrelin protein products (including obestatin) and inhibition of preproghrelin gene expression decrease adipocyte differentiation (Granata *et al.*, 2012). In addition to its secretion, obestatin may mediate important actions on adipose tissue (see below), pointing towards a potential autocrine/paracrine role (Gurriarán-Rodríguez *et al.*, 2011a). Indeed, adipose tissue is considered to be endocrine in nature, further to adipokine-mediated regulation of glucose, lipid and energy homeostasis, as well as inflammation. Notably, obesity and deregulation of these processes, which appear to be modulated by obestatin, are frequently associated with insulin resistance and diabetes (Hotamisligil, 2006; Xin *et al.*, 2009; Galic *et al.*, 2010; Guilherme *et al.*, 2010).

Specifically, obestatin is reported to improve survival and inhibit apoptosis of 3T3-L1 pre-adipocytes via stimulation of ERK1/2 and PI3K/Akt, which are established mediators of adipocyte proliferation and survival (Miegueu *et al.*, 2011; Granata *et al.*, 2012), and to increase adipogenesis of these cells as well as that of human omental and subcutaneous adipocytes, in parallel with induction of adipogenic gene expression (Gurriarán-Rodríguez *et al.*, 2011b; Ren *et al.*, 2013a). However, obestatin-induced proliferation of 3T3-L1 pre-adipocytes was not associated with adipogenesis (Ren *et al.*, 2013b). A similar investigation in porcine pre-adipocytes found obestatin to stimulate proliferation and differentiation and to inhibit apoptosis via promotion of PPARγ and CCAAT–enhancer-binding protein α and inhibition of caspase-3/7/9 (Tang *et al.*, 2014). Consistent with these findings, isoprenaline-induced lipolysis in both 3T3-L1 pre-adipocytes and human subcutaneous and omental adipocytes was reduced by obestatin, with cells from obese subjects also demonstrating this obestatin response under basal conditions (Granata *et al.*, 2012). In contrast, in isolated rat adipocytes, obestatin has been shown to inhibit lipogenesis and potentiate adrenaline-induced lipolysis (Pruszynska-Oszmalek *et al.*, 2013), although it also had no effect on 3T3-L1 preadipocyte glycerol release (Ren *et al.*, 2013b). Recently, obestatin has been demonstrated to promote pre-adipocyte differentiation, lipid accumulation and leptin secretion, whilst decreasing and increasing lipolysis during differentiation and adipogenesis, respectively (Wojciechowicz *et al.*, 2015), indicating that the actions of obestatin in these settings may be complex.

Effects of obestatin on both tissue and circulating lipid levels have also been widely investigated. For example, acute obestatin treatment in 3T3-L1 differentiating mouse adipocytes increased triglyceride levels (Miegueu *et al.*, 2011), although circulating concentrations were reduced in rats or mice subjected to chronic treatment with native or modified obestatin, with activation of glycerolipid metabolism and PPAR signalling proposed as a potential mechanism (Agnew *et al.*, 2011; Nagaraj *et al.*, 2014). Although circulating cholesterol levels remained unaltered in obestatin-injected rats, decreased expression of cholesterol transporter ABCA1 was demonstrated in bovine WAT further to obestatin treatment (Grala *et al.*, 2010; Agnew *et al.*, 2011). Consistent with beneficial actions of obestatin on lipid metabolism, phosphorylation of AMP activated protein kinase (AMPK) is reported to be increased by obestatin in 3T3-L1 adipocytes and human adipose tissue, whilst in human subcutaneous adipocytes, this effect occurs in parallel with modulation of adiponectin and leptin expression (Granata *et al.*, 2012).

With regard to glucose metabolism, obestatin has been shown to inhibit glucose transport in isolated rat adipocytes and to down-regulate glucose transporter type 4 (GLUT-4) in adipose tissue (Pruszynska-Oszmalek *et al.*, 2013; Ren *et al.*, 2013a). In contrast, glucose uptake is reported to be enhanced by obestatin in both 3T3-L1 and human subcutaneous adipocytes, together with increased translocation of GLUT-4 to the plasma membrane increased via up-regulation of sirtuin 1, which is important in mediating the insulin response, and activation of key signalling pathways, including Akt, glycogen synthase kinase-3β (GSK3β), mechanistic target of rapamycin (mTOR), and ribosomal protein S6 kinase 1 (SK61; Granata *et al.*, 2012). Similar data have been generated by other groups upon investigation of WAT from obestatin-treated animals (Gurriarán-Rodríguez *et al.*, 2011b), suggesting that obestatin is likely to activate rather than inhibit glucose metabolism in adipose tissue.

Obestatin in obesity and diabetes

Although the precise metabolic actions of obestatin are still to be defined, it appears to play an important role with clear potential relevance to obesity and diabetes. Indeed, circulating levels of obestatin have been widely measured in this setting in both animals and humans (summarized in Table 2). Similar to the physiological situation, the data have been somewhat inconsistent (likely due to the reasons previously discussed), although it seems that obestatin levels are generally altered in diabetes and obesity. For example, decreased circulating obestatin has been documented in overweight/obese patients and those with impaired glucose control, metabolic syndrome, T2DM and insulin resistance (Anderwald-Stadler *et al.*, 2007; Qi *et al.*, 2007; Fontenot *et al.*, 2007; Guo *et al.*, 2007; Gao *et al.*, 2008, 2010;

Table 2

Effect of disease pathology on circulating levels of obestatin

Obestatin Level (pg·mL^{-1})			Study details		
Normal physiology	Disease pathology	Ghrelin/ obestatin ↑↓	Tissue	Detection	Reference
160	100 (OB)	—	Serum (human ♀)	RIA	Fontenot *et al.*, 2007
70.5 ± 6.4[b]	42.6 ± 9.8[b] (OB)	OB ↑	Plasma (human ♂/♀)	RIA	Guo *et al.*, 2007
325 ± 109[b]	398 ± 102[b] (OB, PWS)	—	Plasma (human ♂/♀)	RIA	Butler and Bittel, 2007
—	—	OB ↓	Plasma (human ♀)	RIA	Vicennati *et al.*, 2007
27.8 ± 4.0[a]	17.2 ± 2.0[a] (OB)	—	Plasma (human ♂/♀)	RIA	Huda *et al.*, 2008
72.3 ± 8.9[a]	55.6 ± 6.4[a] (OB)	—	Plasma (human ♂)	RIA	Gao *et al.*, 2008
267.9 ± 10.8[a]	201 ± 12, 298 ± 17[a] (OB, AXN)	OB, AXN ↑	Plasma (human ♀)	RIA	Zamrazilová *et al.*, 2008
0.15 ± 0.01[a]	0.12 ± 0.01, 0.20 ± 0.01[a] (OB, AXN)	—	Plasma (human ♀)	RIA	Nakahara *et al.*, 2008
69.7 ± 7.5[b]	52.9 ± 7.9[b] (OB)	—	Plasma (human ♂/♀)	RIA	Gao *et al.*, 2010
228 ± 60[b]	212 ± 44[b] (OB)	OB ↓	Plasma (human child ♂/♀)	RIA	Zou *et al.*, 2009
—	288 ± 104[b] (OB)	OB ↓	Plasma (human child ♂/♀)	RIA	Reinehr *et al.*, 2008
—	—	OB, AXN ↑	Blood (human child ♂/♀)	EIA	Shen *et al.*, 2013
2803 ± 939[c]	3670 ± 1336[c] (OB)	OB ↓	Plasma (human child ♂/♀)	EIA	Wali *et al.*, 2014
1870 ± 590[b]	2030 ± 510[b] (OB)	OB ↓	Serum (human elderly ♀)	EIA	Mora *et al.*, 2013
49.2 ± 2.2[a]	64.5 ± 2.2[a] (AXN)	—	Plasma (human ♀)	RIA	Harada *et al.*, 2008
82.5 ± 29.3[b]	130 ± 17[b] (AXN)	—	Plasma (human ♀)	EIA	Monteleone *et al.*, 2008a
68.3 ± 14.8[b]	86.2 ± 24.4, 74.9 ± 22.4[b] (AXN, BMN)	AXN ↑	Plasma (human ♀)	EIA	Monteleone *et al.*, 2008b
288 ± 26[a]	393 ± 25[a] (AXN)	—	Plasma (human ♀)	RIA	Germain *et al.*, 2009
48.4 ± 11.2[a]	72.6 ± 7.0[a] (AXN)	—	Plasma (human ♀)	RIA	Uehara *et al.*, 2011
325 ± 26[a]	276 ± 15[a] (HTN)	HTN ↓	Plasma (human ♂)	RIA	Li *et al.*, 2010b
4720 ± 820[c]	5060 ± 680[c] (HTN)	—	Plasma (human ♂)	EIA	Shao *et al.*, 2014
474 ± 43[b]	338 ± 67, 283 ± 75[b] (OB, HTN)	OB, HTN ↑	Plasma (human ♂/♀)	RIA	Wang *et al.*, 2014
38.6 ± 1.5[a]	44.6 ± 2.3[a] (HTN)	HTN ↑	Plasma (rat ♂)	RIA	Li *et al.*, 2010a
436.4 ± 114[b]	435 ± 127[b] (IHD)	—	Serum (human ♂/♀)	RIA	Ozbay *et al.*, 2008
162 ± 12[b]	163 ± 9[b] (CHF)	CHF ↓	Plasma (human ♂/♀)	RIA	Xin *et al.*, 2009
212 ± 38[b]	356 ± 85[b] (CRS)	—	Plasma (human ♂/♀)	EIA	Shi *et al.*, 2012
224 ± 19[b]	276 ± 15[b] (PE)	PE ↓	Serum (human pregnant ♀)	RIA	Wu *et al.*, 2015
469 ± 23[b]	383 ± 26[b] (IR)	—	Plasma (human ♂/♀)	RIA	Anderwald-Stadler *et al.*, 2007
43.8 ± 1.4[b]	37·4 ± 1.3[b] (DB)	—	Plasma (human ♂/♀)	RIA	Qi *et al.*, 2007
—	257 ± 10[a] (DB)	—	Plasma (human ♂/♀)	RIA	Harsch *et al.*, 2009
301 ± 35[a]	267 ± 17[a] (DB)	—	Blood (human ♂)	RIA	St-Pierre *et al.*, 2010
5072 ± 608[a]	7203 ± 615[a] (DB)	—	Plasma (human child ♂/♀)	EIA	Prodam *et al.*, 2014

AXN, anorexia nervosa; BMN, bulimia nervosa; CHF, chronic heart failure; CRS, cardiorenal syndrome; DB, diabetes, EIA, enzyme immunoassay; HTN, hypertension; IHD, ischaemic heart disease; IR, insulin resistance; OB, obesity; PE, pre-eclampsia; PWS, Prader–Willi Syndrome.
[a]Mean ± SEM.
[b]Mean ± SD.
[c]Mean ± SEM or mean ± SD.

Huda*et al.*, 2008; Nakahara *et al.*, 2008; Zou *et al.*, 2009; Beasley *et al.*, 2009; Cui *et al.*, 2012; Shen *et al.*, 2013; Gu *et al.*, 2013; Wang *et al.*, 2014). Inverse correlations between circulating obestatin and body mass index, insulin, glucose, leptin, homeostatic model assessment of insulin resistance and glycated haemoglobin have also been reported (Lippl *et al.*, 2008; Nakahara *et al.*, 2008; Gu *et al.*, 2013; Shen *et al.*, 2013; Wang *et al.*, 2014), with reduced numbers of obestatin-positive cells evident in the gastric mucosa of overweight/obese subjects with abdominal obesity (Gao *et al.*, 2010, 2014). Similarly, in the experimental setting, obestatin is reported to decrease with insulin administration in normoglycaemic rats (Huang *et al.*, 2012). Consistent with these data, obestatin levels increased with body weight reduction following gastric banding and sleeve gastrectomy surgery in obese and T2DM patients, respectively, and with

standard weight loss in obese children (Haider *et al.*, 2007; Arrigo *et al.*, 2012; Lee *et al.*, 2013). Obestatin levels were also higher in individuals with anorexia nervosa (Harada *et al.*, 2008; Monteleone *et al.*, 2008a,b; Germain *et al.*, 2009, 2010; Sedlácková *et al.*, 2011; Uehara *et al.*, 2011; Sedlackova *et al.*, 2012; Shen *et al.*, 2013), and whilst they were decreased with hypothyroidism (associated with weight gain), they were increased with hyperthyroidism (associated with weight loss) (Emami *et al.*, 2014). Interestingly, the combination of preproghrelin polymorphisms $Leu^{72}Met$ and $Gln^{90}Leu$ have been associated with increased risk of anorexia nervosa (Dardennes *et al.*, 2007).

Although the majority of studies appear to support an inverse relationship between circulating obestatin and obesity/diabetes, increased obestatin levels have also been reported in patients with obesity, metabolic syndrome, impaired glucose control, T1DM, Prader–Willi syndrome (which is linked with obesity) and bulimia nervosa (Butler and Bittel, 2007; Vicennati *et al.*, 2007; Reinehr *et al.*, 2008; Sedlácková *et al.*, 2011; Arrigo *et al.*, 2012; Sedlackova *et al.*, 2012; Mora *et al.*, 2013; Prodam *et al.*, 2014; Wali *et al.*, 2014), whilst levels have been shown to be decreased in hyperthyroidism and in pregnant women 24 h post-partum (which typically increases insulin sensitivity) (Baykus *et al.*, 2012; Gurgul *et al.*, 2012). Other studies have found obestatin levels to be unaltered following gastric surgery-induced weight loss in both obese and T2DM patients (Roth *et al.*, 2009; Lee *et al.*, 2013; Siejka *et al.*, 2013) and in bulimia nervosa (Monteleone *et al.*, 2008b).

Of direct relevance to diabetes, obestatin levels were recently reported to be negatively correlated with the presence of c-peptide and anti-insulin antibodies in children at T1DM disease onset, which may therefore be indicative of islet dysfunction (Prodam *et al.*, 2014). Consistent with a link between obestatin and the pancreas in diabetes, a study using rodent islets incubated in high glucose demonstrated differential effects of obestatin on insulin release, with low concentrations exerting a stimulating effect, whilst high concentrations were inhibitory, thereby suggesting that beta cells may be less responsive to obestatin in diabetes (Egido *et al.*, 2009). Obestatin treatment has also been shown to confer protective actions in experimental streptozotocin-induced diabetes, specifically preservation of islet size and beta cell mass together with stimulation of insulin secretion, improved glucose tolerance and reduced blood glucose (Granata *et al.*, 2010b). Similarly, insulin sensitivity and glucose tolerance were improved in obestatin-treated mice fed either a standard or high-fat diet, with comparable effects on glucose-induced insulin secretion observed in islets isolated from these animals (Granata *et al.*, 2012). Furthermore, *ex vivo* adipose tissue analysis revealed enhanced glucose uptake, reduced lipolysis and apoptosis, in addition to increased abundance of smaller adipocytes (likely to be insulin sensitive), particularly in subcutaneous adipose tissue. The observed beneficial effects of obestatin in this setting were associated with reduced production of pro-inflammatory cytokines, for example, TNF-α, highlighting apparent anti-inflammatory actions, at least in experimental diabetes (Granata *et al.*, 2012).

Considering the reasonably consistent alteration of circulating levels of obestatin in patients with metabolic disease (the majority of which display reduced concentrations), together with its established actions on the GI system, pancreas and adipose tissue, and emerging evidence supporting beneficial effects of obestatin treatment in experimental T1DM and T2DM, it is clear that this peptide demonstrates vast potential as a novel therapeutic target that is worthy of further investigation in the context of metabolic dysfunction linked with obesity and diabetes.

Cardiovascular actions of obestatin

In addition to the ascribed metabolic actions of obestatin, it is becoming increasingly evident that it may also exert important effects on the cardiovascular system. This is perhaps not surprising given the established cardiovascular actions of its sister hormone, ghrelin (Tokudome *et al.*, 2014). Here, we highlight the emerging effects of obestatin on the cardiovascular system, with clear relevance to its more widely studied metabolic actions in the context of diabetes, which often leads to cardiovascular complications.

Obestatin and blood pressure regulation

Accumulating data support a relationship between circulating obestatin levels and blood pressure. However, the nature of this interaction has been differentially reported, similar to the previously discussed findings in regard to metabolic disease, which is frequently associated with hypertension. Fasting plasma obestatin levels were first reported to be negatively correlated with systolic blood pressure in insulin-resistant patients (Anderwald-Stadler *et al.*, 2007), findings that were later corroborated in patients with mild-to-moderate untreated essential hypertension in association with reduced ghrelin and ghrelin/obestatin ratio (Li *et al.*, 2010b) and in hypertensive versus normotensive obese patients (Wang *et al.*, 2014). However, a study conducted in patients with pulmonary arterial hypertension found that circulating obestatin levels tended to increase, whilst the ghrelin/obestatin ratio was decreased compared with controls, and identified as an independent disease predictor (Li *et al.*, 2013). Similarly, spontaneously hypertensive rats demonstrated increased fasting obestatin levels, although in this case, ghrelin and the ghrelin/obestatin ratio were also elevated (Li *et al.*, 2010a). Furthermore, in both normal pregnancy and those associated with hypertension (which is linked with hyperinsulinaemia and insulin resistance), plasma obestatin was positively correlated with mean arterial blood pressure, with the hypertensive group showing markedly higher levels versus normotensive controls, with these differences resolving within 3–5 days post-delivery (Ren *et al.*, 2009). Indeed, the same study reported no correlation between mean arterial blood pressure and circulating obestatin in non-pregnant women. Other studies investigating the relationship between obestatin levels and blood pressure in men over 80 years of age and effects of bolus obestatin administration in spontaneously hypertensive rats have failed to produce positive findings (Li *et al.*, 2009; Shao *et al.*, 2014). As previously highlighted, there appear to be fundamental issues with measurement of obestatin levels, which may relate to differences in detection or sensitivity, but are also likely to be influenced by physiological factors such as feeding state

and diurnal variation, which consequently make the available data difficult to interpret. Nonetheless, the clinical and experimental studies to date would generally suggest that obestatin plays some role in blood pressure regulation, although standardization and refinement of the employed plasma analysis techniques are clearly required in order to define the precise nature of any interaction.

Obestatin and endothelial function in health and disease

Although the specific relationship between obestatin and blood pressure remains to be determined, more definitive evidence is emerging in support of beneficial actions on the endothelium, which plays a major role in both blood pressure regulation and protection against the development of diabetic cardiovascular complications, suggesting that it may represent a viable therapeutic target in this setting. Obestatin was first reported to exert direct anti-inflammatory effects on human EA.hy926 endothelial cells, by decreasing TNF-α-induced vascular cell adhesion molecule-1 (VCAM-1) expression, whilst not influencing associated monocyte adhesion or monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) expression (Kellokoski *et al.*, 2009). However, the same study found obestatin to also promote binding of oxidized LDL to thioglycollate-stimulated mouse peritoneal macrophages, thereby suggesting that it may mediate differential modulation of early atherogenic processes. Obestatin can also bind to microvascular endothelial cells in pancreatic islets and promote survival and proliferation of these cells under high-glucose conditions by inhibiting caspase-3-, Akt- and ERK1/2-dependent apoptosis pathways, effects that were interestingly prevented by the GLP-1 receptor antagonist, exendin(9–39) (Favaro *et al.*, 2012). Recently, several groups have reported that obestatin induces vascular relaxation, both *ex vivo* and *in vivo* in an NO-dependent manner (Agnew *et al.*, 2012; Ku *et al.*, 2015; Schinzari *et al.*, 2015). First, obestatin was shown to induce dose-dependent relaxation of isolated rat aorta and superior artery, which was inhibited by both endothelial denudation and the NO inhibitor, NG-monomethyl-L-arginine (Agnew *et al.*, 2012). Comprehensive *ex vivo* analysis identified a pathway involving an adenylate cyclase-linked GPCR, PI3K/Akt and Ca^{2+}-dependent endothelial NO synthase activation, coupled to downstream vascular smooth muscle soluble guanylate cyclase and large conductance calcium-activated potassium channel (BK_{Ca}) activation (Agnew *et al.*, 2012; see manuscript for a detailed signalling schematic). Similar findings have since been reported in mouse cerebral artery, in which obestatin-induced vasodilation was shown to be endothelial NO synthase-dependent and maintained in both the presence of the ghrelin receptor antagonist YIL-781 and vessels from ghrelin receptor-deficient mice (Ku *et al.*, 2015). Interestingly, basal NO bioactivity was markedly reduced in mice lacking the ghrelin receptor together with elevated superoxide generation, highlighting potential protective actions of obestatin in the cerebral circulation. Importantly, the reported *ex vivo* vascular effects of obestatin appear to translate to humans. A recent study reported induction of NO-dependent vasodilatation (as assessed by increased forearm blood flow) in both obese and non-obese subjects, which was associated with inhibition of endothelin-1 signalling (Schinzari *et al.*, 2015). Furthermore, it seems that obestatin may also exert notable actions on the microvasculature, which is a major regulator of blood pressure. Specifically, hyperglycaemia-induced generation of nitrite (stable oxidation product of NO), VEGF and pro-inflammatory IL-1β, in pancreatic microvascular endothelial cells were attenuated by obestatin, whilst obestatin improved mouse skeletal muscle regeneration via stimulation of microvascularization secondary to induction of satellite stem cell expansion and VEGF/VEGFR-2 expression (Favaro *et al.*, 2012; Gurriarán-Rodríguez *et al.*, 2015). Taken together, these data clearly indicate that obestatin may play a role in both normal regulation of blood pressure and vascular function and in the setting of diabetes, which is characterized by endothelial dysfunction and reduced NO production, and frequently associated with cardiovascular complications.

Obestatin and the heart

In addition to its emerging vascular effects, it appears that obestatin may exert both direct and indirect actions on the heart. Shortly after its discovery, obestatin was shown to bind specifically to GPR39 on HL-1 cardiomyocytes, although no parallel acute effects on cell viability, cell cycle or fatty acid/glucose uptake were observed (Iglesias *et al.*, 2007). Obestatin was later reported to reduce infarct size and contractile dysfunction in isolated rat hearts subjected to ischaemia–reperfusion by conferring dose-dependent protection against cell death via activation of PI3K, PKC-ε, PKC-δ and ERK1/2 pathways (Alloatti *et al.*, 2010). Notably, this study also employed radioreceptor binding assays to highlight the presence of specific high-affinity obestatin-binding sites localized on the membranes of both the ventricular myocardium and cardiomyocytes, supporting the assertion that obestatin receptors are expressed in the heart. Similarly, obestatin improved basal papillary muscle contractility and responsiveness to β-adrenoceptor stimulation in streptozotocin-induced T1DM rats, but not in non-diabetic controls, via protection against loss of β-adrenoceptors and rescue of myosin heavy chain isoforms (Aragno *et al.*, 2012). These findings are consistent with a previous observation that topical obestatin administration induces positive inotropic effects in frog hearts *ex vivo* (Sazdova *et al.*, 2009). In the clinical setting, there appears to be no correlation between ischaemic heart disease and plasma obestatin (Ozbay *et al.*, 2008). However, a clear tendency towards increased plasma obestatin levels in chronic heart failure patients is observed, which becomes significant in those with cachexia, whilst elevated circulating concentrations of both obestatin and vasopressin are associated with cardiorenal syndrome (Xin *et al.*, 2009; Shi *et al.*, 2012). Indeed, obestatin is reported to inhibit experimental angiotensin II and dehydration-induced release of vasopressin (Samson *et al.*, 2007, 2008), which is a key regulator of physiological fluid/electrolyte balance implicated in heart failure progression (Goldsmith and Gheorghiade, 2005; Wasilewski *et al.*, 2015). Although current data supporting direct cardiac effects of obestatin may be limited, such actions are likely to be significant given the established structural and functional changes that occur in diabetes and which are linked to markedly increased susceptibility to hypertension and ischaemia (Bugger and Abel, 2014).

Summary and future perspective

It is becoming increasingly evident that obestatin exerts wide-ranging metabolic and cardiovascular actions with clear relevance to the pathophysiology of diabetes and obvious therapeutic potential (summarized in Figure 1). Whilst the precise effects of both endogenous and exogenous obestatin in this setting remain to be determined, the attraction of a dual-action therapeutic targeting both the metabolic and cardiovascular complications of diabetes is clear, particularly in light of the recent large-scale clinical trial data suggesting that the cardiovascular actions of the established T2DM therapy, GLP-1, which showed vast cardiovascular potential, may not be clinically significant (Scirica *et al.*, 2013; White *et al.*, 2013). In recognition of this fact and the emerging actions of obestatin, several groups have focussed on characterising and maximising its biological activity.

Interestingly, it appears that differential domains of obestatin may preferentially mediate its metabolic and cardiovascular effects. For example, obestatin(1–4) is reported to decrease food intake, body weight and plasma total antioxidant capacity in rats and to modulate blood glucose (Khirazova *et al.*, 2013, 2015; Motorykina *et al.*, 2015), whilst in mice, obestatin(1–13) reduced food intake, body weight gain and circulating lipids and obestatin(6–18) decreased epididymal fat and triglycerides to a greater extent versus native obestatin (Nagaraj *et al.*, 2008). Conversely, administration of the C-terminal fragment, obestatin(11–23), to high fat-fed mice resulted in equivalent reductions in food intake and postprandial glucose levels compared with the full-length peptide, whilst the N-terminal fragment, obestatin(1–10), failed to induce metabolic changes in this setting (Green *et al.*, 2007; Subasinghage *et al.*, 2010). With regard to its cardiovascular effects, although both obestatin(1–10) and obestatin(11–23) induced dose-dependent *ex vivo* vasodilatation, this was significantly reduced compared with obestatin (1–23) (Agnew *et al.*, 2012).

Similar to the approach taken with regard to therapeutic advancement of GLP-1, a major focus of recent obestatin research has been directed towards development of stable obestatin peptides that are resistant to endogenous degradation. Indeed, several such analogues based on N-terminal PEGylation, amino acid substitution and iodination strategies demonstrate significantly improved stability and bioactivity (Nagaraj *et al.*, 2009; Agnew *et al.*, 2011; De Spiegeleer *et al.*, 2012). For example, chronic treatment of normal rats with N-terminally PEGylated obestatin, but not native obestatin, markedly reduced triglyceride levels (Agnew *et al.*, 2011), and amino acid substitutions of obestatin(1–13) and obestatin(6–18) conferred variable favourable actions on food intake, body weight, epididymal fat and total cholesterol in mice, together with the activation of key metabolic signalling pathways (Nagaraj *et al.*, 2009, 2014). Interestingly, an alternate obestatin modification approach involving trans-activating transcriptional activator peptide fusion to promote cell permeability has reported greater inhibition of *in vitro* apoptosis and increased glycerol/free fatty acid release in 3T3-L1 human preadipocytes compared with native obestatin, whilst chronic treatment in mice decreased abdominal fat mass, together with modulation of key metabolic genes, such as adiponectin and GLUT-4, in liver and WAT (Ren *et al.*, 2013a). Taken together, these preliminary studies provide some confidence that, at least in principle, it may be possible to effectively target obestatin signalling in

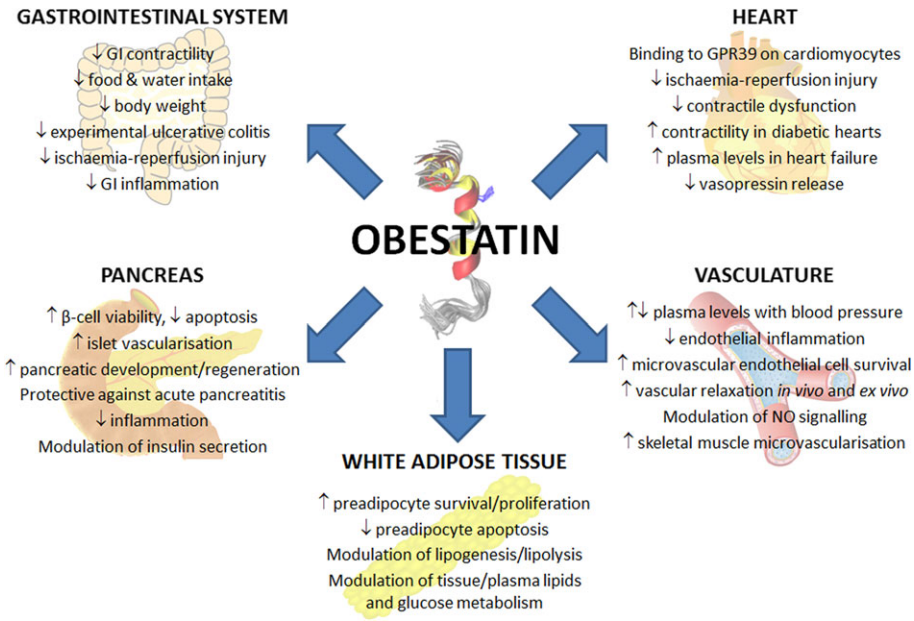

Figure 1

Summary of the reported pathophysiological effects of obestatin. Obestatin targets several tissues, including the GI system, pancreas, WAT, the heart and vasculature, where it exerts diverse biological actions relevant to the metabolic and cardiovascular complications of diabetes.

humans. Given its increasingly evident metabolic and cardiovascular actions, it is clear that obestatin holds potential as a viable and novel dual treatment strategy for diabetes patients.

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Author contributions

E.C. and K.J.B. drafted the manuscript; B.D.G. planned and critically reviewed the manuscript; D.J.G. prepared the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

References

Agnew A, Calderwood D, Chevallier OP, Greer B, Grieve DJ, Green BD (2011). 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 32: 755–762.

Agnew AJ, Robinson E, McVicar CM, Harvey AP, Ali IH, Lindsay JE *et al.* (2012). The gastrointestinal peptide obestatin induces vascular relaxation via specific activation of endothelium-dependent NO signalling. Br J Pharmacol 166: 327–338.

Aktas B, Yilmaz Y, Eren F, Yonal O, Kurt R, Alahdab YO *et al.* (2011). Serum levels of vaspin, obestatin, and apelin-36 in patients with nonalcoholic fatty liver disease. Metabolism 60: 544–549.

Alen BO, Nieto L, Gurriaran-Rodriguez U, Mosteiro CS, Alvarez-Perez JC, Otero-Alen M *et al.* (2012). The NMR structure of human obestatin in membrane-like environments: insights into the structure–bioactivity relationship of obestatin. PLoS One 7: e45434.

Alexander SP, Cidlowski JA, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015a). The Concise Guide to PHARMACOLOGY 2015/16: nuclear hormone receptors. Br J Pharmacol 172: 5956–5978.

Alexander SP, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015b). The Concise Guide to PHARMACOLOGY 2015/16: G protein-coupled receptors. Br J Pharmacol 172: 5744–5869.

Alexander SP, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015c). The Concise Guide to PHARMACOLOGY 2015/16: enzymes. Br J Pharmacol 172: 6024–6109.

Alexander SP, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E *et al.* (2015d). The Concise Guide to PHARMACOLOGY 2015/16: transporters. Br J Pharmacol 172: 6110–6202.

Alexandridis E, Zisimopoulos A, Liratzopoulos N, Katsos I, Manolas K, Kouklakis G (2009). Obestatin/ghrelin ratio: a new activity index in inflammatory bowel diseases. Inflamm Bowel Dis 15: 1557–1561.

Alloatti G, Arnoletti E, Bassino E, Penna C, Perrelli MG, Ghé C *et al.* (2010). 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 299: H470–H481.

Anderwald-Stadler M, Krebs M, Promintzer M, Mandl M, Bischof MG, Nowotny P *et al.* (2007). Plasma obestatin is lower at fasting and not suppressed by insulin in insulin-resistant humans. Am J Physiol Endocrinol Metab 293: E1393–E1398.

Aragno M, Mastrocola R, Ghé C, Arnoletti E, Bassino E, Alloatti G *et al.* (2012). Obestatin induced recovery of myocardial dysfunction in type 1 diabetic rats: underlying mechanisms. Cardiovasc Diabetol 11: 129.

Arrigo T, Gitto E, Ferraù V, Munafò C, Alibrandi A, Marseglia GL *et al.* (2012). Effect of weight reduction on leptin, total ghrelin and obestatin concentrations in prepubertal children. J Biol Regul Homeost Agents 26: S95–S103.

Ataka K, Inui A, Asakawa A, Kato I, Fujimiya M (2008). Obestatin inhibits motor activity in the antrum and duodenum in the fed state of conscious rats. Am J Physiol Gastrointest Liver Physiol 294: G1210–G1218.

Ayada C, Toru U, Genc O, Simsek H, Sahin S, Admis O *et al.* (2015). Serum levels of obestatin and adiponectin in patients with obstructive sleep apnea syndrome. Acta Physiol (Oxf) 215 : 90.Suppl S705

Aydin S, Ozkan Y, Erman F, Gurates B, Kilic N, Colak R *et al.* (2008). Presence of obestatin in breast milk: relationship among obestatin, ghrelin, and leptin in lactating women. Nutrition 24: 689–693.

Aygen B, Dogukan A, Dursun FE, Aydin S, Kilic N, Sahpaz F *et al.* (2009). Ghrelin and obestatin levels in end-stage renal disease. J Int Med Res 37: 757–765.

Bang AS, Soule SG, Yandle TG, Richards AM, Pemberton CJ (2007). Characterisation of proghrelin peptides in mammalian tissue and plasma. J Endocrinol 192: 313–323.

Baragli L, Grande C, Gesmundo I, Settanni F, Taliano M, Gallo D *et al.* (2013). Obestatin enhances in vitro generation of pancreatic islets through regulation of developmental pathways. PLoS One 8: e64374.

Bassil AK, Häglund Y, Brown J, Rudholm T, Hellström PM, Näslund E *et al.* (2007). Little or no ability of obestatin to interact with ghrelin or modify motility in the rat gastrointestinal tract. Br J Pharmacol 150: 58–64.

Baykus Y, Gurates B, Aydin S, Celik H, Kavak B, Aksoy A *et al.* (2012). Changes in serum obestatin, preptin and ghrelins in patients with gestational diabetes mellitus. Clin Biochem 45: 198–202.

Beasley JM, Ange BA, Anderson CA, Miller Iii ER, Holbrook JT, Appel LJ (2009). Characteristics associated with fasting appetite hormones (obestatin, ghrelin, and leptin). Obesity (Silver Spring) 17: 349–354.

Bresciani E, Rapetti D, Donà F, Bulgarelli I, Tamiazzo L, Locatelli V *et al.* (2006). Obestatin inhibits feeding but does not modulate GH and corticosterone secretion in the rat. J Endocrinol Invest 29: RC16–RC18.

Brunetti L, Leone S, Orlando G, Recinella L, Ferrante C, Chiavaroli A *et al.* (2009). Effects of obestatin on feeding and body weight after standard or cafeteria diet in the rat. Peptides 30: 1323–1327.

Brunetti L, Di Nisio C, Recinella L, Orlando G, Ferrante C, Chiavaroli A *et al.* (2010). Obestatin inhibits dopamine release in rat hypothalamus. Eur J Pharmacol 641: 142–147.

Buescher AK, Buescher R, Hoyer PF (2010). Regulation of ghrelin, obestatin and adiponectin in pediatric patients with chronic renal insufficiency and after renal transplantation. Endocr Rev 31: P3–697.

Bugger H, Abel ED (2014). Molecular mechanisms of diabetic cardiomyopathy. Diabetologia 57: 660–671.

Bukowczan J, Warzecha Z, Ceranowicz P, Kuśnierz-Cabala B, Tomaszewska R, Dembinski A (2015). Pretreatment with obestatin reduces the severity of ischemia/reperfusion-induced acute pancreatitis in rats. Eur J Pharmacol 760: 113–121.

Butler MG, Bittel DC (2007). Plasma obestatin and ghrelin levels in subjects with Prader–Willi syndrome. Am J Med Genet A 143: 415–421.

Cassoni P, Ghé C, Marrocco T, Tarabra E, Allia E, Catapano F *et al.* (2004). Expression of ghrelin and biological activity of specific receptors for ghrelin and des-acyl ghrelin in human prostate neoplasms and related cell lines. Eur J Endocrinol 150: 173–184.

Ceranowicz P, Warzecha Z, Dembinski A, Cieszkowski J, Dembinski M, Sendur R *et al.* (2009). Pretreatment with obestatin inhibits the development of cerulein-induced pancreatitis. J Physiol Pharmacol 60: 95–101.

Chen CY, Lee WJ, Chong K, Lee SD, Liao YD (2012a). Impact of intracerebroventricular obestatin on plasma acyl ghrelin, des-acyl ghrelin and nesfatin-1 levels, and on gastric emptying in rats. Mol Med Rep 6: 191–196.

Chen CY, Chien EJ, Chang FY, Lu CL, Luo JC, Lee SD (2008). Impacts of peripheral obestatin on colonic motility and secretion in conscious fed rats. Peptides 29: 1603–1608.

Chen CY, Doong ML, Li CP, Liaw WJ, Lee HF, Chang FY*et al.* (2010). A novel simultaneous measurement method to assess the influence of intracerebroventricular obestatin on colonic motility and secretion in conscious rats. Peptides 31: 1113–1117.

Chen CY, Tsai CY, Lee WJ, Liaw WJ, Chiang CH, Ho ST*et al.* (2012b). Intracerebroventricular O-n-octanoylated ghrelin and its splice variant-induced feeding is blocked by insulin, independent of obestatin or CRF receptor, in satiated rats. Nutrition 28: 812–820.

Cui AD, Gai NN, Zhang XH, Jia KZ, Yang YL, Song ZJ (2012). Decreased serum obestatin consequent upon TRIB3 Q84R polymorphism exacerbates carotid atherosclerosis in subjects with metabolic syndrome. Diabetol Metab Syndr 4: 52.

Dardennes RM, Zizzari P, Tolle V, Foulon C, Kipman A, Romo L *et al.* (2007). Family trios analysis of common polymorphisms in the obestatin/ghrelin, BDNF and AGRP genes in patients with anorexia nervosa: association with subtype, body-mass index, severity and age of onset. Psychoneuroendocrinology 32: 106–113.

Depoortere I, Thijs T, Moechars D, De Smet B, Ver Donck L, Peeters TL (2008). Effect of peripheral obestatin on food intake and gastric emptying in ghrelin-knockout mice. Br J Pharmacol 153: 1550–1557.

De Smet B, Thijs T, Peeters TL, Depoortere I (2007). Effect of peripheral obestatin on gastric emptying and intestinal contractility in rodents. Neurogastroenterol Motil 19: 211–217.

De Spiegeleer B, Van Dorpe S, Vergote V, Wynendaele E, Pauwels E, Van De Wiele C *et al.* (2012). In vitro metabolic stability of iodinated obestatin peptides. Peptides 33: 231–237.

Donath MY, Halban PA (2004). Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. Diabetologia 47: 581–589.

Dun SL, Brailoiu GC, Brailoiu E, Yang J, Chang JK, Dun NJ (2006). Distribution and biological activity of obestatin in the rat. J Endocrinol 191: 481–489.

Egido EM, Hernández R, Marco J, Silvestre RA (2009). Effect of obestatin on insulin, glucagon and somatostatin secretion in the perfused rat pancreas. Regul Pept 152: 61–66.

Emami A, Nazem R, Hedayati M (2014). Is association between thyroid hormones and gut peptides, ghrelin and obestatin, able to suggest new regulatory relation between the HPT axis and gut? Regul Pept 189: 17–21.

Favaro E, Granata R, Miceli I, Baragli A, Settanni F, Cavallo Perin P *et al.* (2012). 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 55: 1058–1070.

Fontenot E, DeVente JE, Seidel ER (2007). Obestatin and ghrelin in obese and in pregnant women. Peptides 28: 1937–1944.

Fujimiya M, Asakawa A, Ataka K, Kato I, Inui A (2008). Different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility in conscious rats. World J Gastroenterol 14: 6318–6326.

Fujimiya M, Ataka K, Asakawa A, Chen CY, Kato I, Inui A (2012). Regulation of gastroduodenal motility: acyl ghrelin, des-acyl ghrelin and obestatin and hypothalamic peptides. Digestion 85: 90–94.

Galic S, Oakhill JS, Steinberg GR (2010). Adipose tissue as an endocrine organ. Mol Cell Endocrinol 316: 129–139.

Gao XY, Kuang HY, Liu XM, Ma ZB (2010). Decreased gastric body mucosa obestatin expression in overweight and obese patients. Peptides 31: 291–296.

Gao XY, Kuang HY, Liu XM, Ma ZB (2014). Decreased gastric body mucosa obestatin expression in abdominal obesity patients with normal body mass index. Biomed Environ Sci 27: 385–387.

Gao XY, Kuang HY, Liu XM, Wang XY, Pan YH, Ma XX (2008). Decreased obestatin in plasma in metabolically obese, normal-weight men with normal glucose tolerance. Diabetes Res Clin Pract 79: e5–e6.

Germain N, Galusca B, Grouselle D, Frere D, Billard S, Epelbaum J *et al.* (2010). Ghrelin and obestatin circadian levels differentiate bingeing–purging from restrictive anorexia nervosa. J Clin Endocrinol Metab 95: 3057–3062.

Germain N, Galusca B, Grouselle D, Frere D, Tolle V, Zizzari P *et al.* (2009). Ghrelin/obestatin ratio in two populations with low bodyweight: constitutional thinness and anorexia nervosa. Psychoneuroendocrinology 34: 413–419.

Ghanbari-Niaki A (2010). Plasma obestatin, estradiol, and liver ATP concentrations in response to endurance exercise training at different durations in male rats. Int J Endocrinol Metab 8: 147–152.

Goldsmith SR, Gheorghiade M (2005). Vasopressin antagonism in heart failure. J Am Coll Cardiol 46: 1785–1791.

Gourcerol G, Coskun T, Craft LS, Mayer JP, Heiman ML, Wang L *et al.* (2007a). Preproghrelin-derived peptide, obestatin, fails to influence food intake in lean or obese rodents. Obesity (Silver Spring) 15: 2643–2652.

Gourcerol G, Million M, Adelson DW, Wang Y, Wang L, Rivier J *et al.* (2006). Lack of interaction between peripheral injection of CCK and obestatin in the regulation of gastric satiety signaling in rodents. Peptides 27: 2811–2819.

Gourcerol G, St-Pierre DH, Tache Y (2007b). Lack of obestatin effects on food intake: should obestatin be renamed ghrelin-associated peptide (GAP)? Regul Pept 141: 1–7.

Gourcerol G, Taché Y (2007). Obestatin – a ghrelin-associated peptide that does not hold its promise to suppress food intake and motility. Neurogastroenterol Motil 19: 161–165.

Grala TM, Kay JK, Walker CG, Sheahan AJ, Littlejohn MD, Lucy MC *et al.* (2010). Expression analysis of key somatotropic axis and liporegulatory genes in ghrelin- and obestatin-infused dairy cows. Domest Anim Endocrinol 39: 76–83.

Granata R, Baragli A, Settanni F, Scarlatti F, Ghigo E (2010a). Unraveling the role of the ghrelin gene peptides in the endocrine pancreas. J Mol Endocrinol 45: 107–118.

Granata R, Gallo D, Luque RM, Baragli A, Scarlatti F, Grande C *et al.* (2012). Obestatin regulates adipocyte function and protects against diet-induced insulin resistance and inflammation. FASEB J 26: 3393–3411.

Granata R, Settanni F, Gallo D, Trovato L, Biancone L, Cantaluppi V *et al.* (2008). 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 57: 967–979.

Granata R, Volante M, Settanni F, Gauna C, Ghé C, Annunziata M *et al.* (2010b). Unacylated ghrelin and obestatin increase islet cell mass and prevent diabetes in streptozotocin-treated newborn rats. J Mol Endocrinol 45: 9–17.

Green BD, Irwin N, Flatt PR (2007). Direct and indirect effects of obestatin peptides on food intake and the regulation of glucose homeostasis and insulin secretion in mice. Peptides 28: 981–987.

Grönberg M, Tsolakis AV, Holmbäck U, Stridsberg M, Grimelius L, Janson ET (2013). Ghrelin and obestatin in human neuroendocrine tumors: expression and effect on obestatin levels after food intake. Neuroendocrinology 97: 291–299.

Grönberg M, Tsolakis AV, Magnusson L, Janson ET, Saras J (2008). Distribution of obestatin and ghrelin in human tissues: immunoreactive cells in the gastrointestinal tract, pancreas, and mammary glands. J Histochem Cytochem 56: 793–801.

Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV *et al.* (1999). Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 100: 1134–1146.

Gu PY, Kang DM, Wang WD, Chen Y, Zhao ZH, Zheng H *et al.* (2013). Relevance of plasma obestatin and early arteriosclerosis in patients with type 2 diabetes mellitus. J Diabetes Res 2013: 563919.

Guilherme A, Virbasius JV, Puri V, Czech MP (2010). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 9: 367–377.

Guo ZF, Ren AJ, Zheng X, Qin YW, Cheng F, Zhang J *et al.* (2008). Different responses of circulating ghrelin, obestatin levels to fasting, re-feeding and different food compositions, and their local expressions in rats. Peptides 29: 1247–1254.

Guo Z, Zheng X, Qin Y, Hu J, Chen S, Zhang Z (2007). Circulating preprandial ghrelin to obestatin ratio is increased in human obesity. J Clin Endocrinol Metab 92: 1875–1880.

Gurgul E, Ruchala M, Kosowicz J, Zamyslowska H, Wrotkowska E, Moczko J *et al.* (2012). Ghrelin and obestatin in thyroid dysfunction. Endokrynol Pol 63: 456–462.

Gurriarán-Rodríguez U, Al-Massadi O, Crujeiras AB, Mosteiro CS, Amil-Diz M, Beiroa D *et al.* (2011a). Preproghrelin expression is a key target for insulin action on adipogenesis. J Endocrinol 210: R1–R7.

Gurriarán-Rodríguez U, Al-Massadi O, Roca-Rivada A, Crujeiras AB, Gallego R, Pardo M *et al.* (2011b). Obestatin as a regulator of adipocyte metabolism and adipogenesis. J Cell Mol Med 15: 1927–1940.

Gurriarán-Rodríguez U, Santos-Zas I, González-Sánchez J, Beiroa D, Moresi V, Mosteiro CS *et al.* (2015). Action of obestatin in skeletal muscle repair: stem cell expansion, muscle growth, and microenvironment remodeling. Mol Ther 6: 1003–1021.

Gutierrez-Grobe Y, Villalobos-Blasquez I, Sánchez-Lara K, Villa AR, Ponciano-Rodríguez G, Ramos MH *et al.* (2010). High ghrelin and obestatin levels and low risk of developing fatty liver. Ann Hepatol 9: 52–57.

Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M *et al.* (2007). Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. J Clin Endocrinol Metab 92: 1168–1171.

Harada T, Nakahara T, Yasuhara D, Kojima S, Sagiyama K, Amitani H *et al.* (2008). Obestatin, acyl ghrelin, and des-acyl ghrelin responses to an oral glucose tolerance test in the restricting type of anorexia nervosa. Biol Psychiatry 63: 245–247.

Harsch IA, Koebnick C, Tasi AM, Hahn EG, Konturek PC (2009). Ghrelin and obestatin levels in type 2 diabetic patients with and without delayed gastric emptying. Dig Dis Sci 54: 2161–2166.

Hassouna R, Zizzari P, Viltart O, Yang SK, Gardette R, Videau C *et al.* (2012). A natural variant of obestatin, Q90L, inhibits ghrelin's action on food intake and GH secretion and targets NPY and GHRH neurons in mice. PLoS One 7: e51135.

Hedayati M, Saghebjoo M, Ghanbari-Niaki A (2012). Effects of circuit resistance training intensity on the plasma ghrelin to obestatin ratios in healthy young women. Int J Endocrinol Metab 10: 475–479.

Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO *et al.* (2007). GPR39 signaling is stimulated by zinc ions but not by obestatin. Endocrinology 148: 13–20.

Hotamisligil GS (2006). Inflammation and metabolic disorders. Nature 444: 860–867.

Huang J, Zhang Y, Yu S, Gan X, Su Y, Yuan J *et al.* (2012). Circulating obestatin concentration is lowered by insulin in rats. Exp Clin Endocrinol Diabetes 120: 56–58.

Huda MS, Durham BH, Wong SP, Deepak D, Kerrigan D, McCulloch P *et al.* (2008). Plasma obestatin levels are lower in obese and post-gastrectomy subjects, but do not change in response to a meal. Int J Obes (Lond) 32: 129–135.

Huda M, Mani H, Durham B, Dovey T, Halford J, Aditya S *et al.* (2007). Changes in circulating plasma ghrelin and obestatin in narcolepsy–cataplexy. Sleep 30 (Suppl): A218–A219.

Iglesias MJ, Salgado A, Piñeiro R, Rodiño BK, Otero MF, Grigorian L *et al.* (2007). Lack of effect of the ghrelin gene-derived peptide obestatin on cardiomyocyte viability and metabolism. J Endocrinol Invest 30: 470–476.

International Diabetes Federation (2015). IDF Diabetes Atlas Seventh Edition http://www.idf.org/diabetesatlas.

Jung JY, Jeong JB, Kim JW, Kim SH, Koh SJ, Kim BG *et al.* (2015). Circulating ghrelin levels and obestatin/ghrelin ratio as a marker of activity in ulcerative colitis. Intest Res 13: 68–73.

Kanat BH, Ayten R, Aydin S, Girgin M, Çetinkaya Z, Ilhan YS *et al.* (2014). Significance of appetite hormone ghrelin and obestatin levels in the assessment of the severity of acute pancreatitis. Turkish J Gastroenterol 25: 309–313.

Kapica M, Zabielska M, Puzio I, Jankowska A, Kato I, Kuwahara A *et al.* (2007). Obestatin stimulated the secretion of pancreatic juice enzymes through a vagal pathway in anaesthetized rats – preliminary results. J Physiol Pharmacol 58: 123–130.

Kellokoski E, Kunnari A, Jokela M, Makela S, Kesaniemi YA, Horkko S (2009). Ghrelin and obestatin modulate early atherogenic processes

on cells: enhancement of monocyte adhesion and oxidized low-density lipoprotein binding. Metabolism 58: 1572–1580.

Khirazova EE, Bayzhumanov AA, Motorykina ES, Devyatov AA, Maslova MV, Graf AV*et al.* (2015). Antioxidant defense system after single and chronic administration of obestatin and its fragment (1–4) to normal and overweight male rats. Bull Exp Biol Med 159: 38–40.

Khirazova EE, Maslova MV, Motorykina ES, Frid DA, Graf AV, Maklakova AS *et al.* (2013). Effects of single intranasal administration of obestatin fragments on the body weight and feeding and drinking behaviors. Dokl Biol Sci 453: 336–337.

Kiewiet RM, Gauna C, van Aken MO, van de Zande B, van der Lely AJ (2008). Bolus administration of obestatin does not change glucose and insulin levels neither in the systemic nor in the portal circulation of the rat. Peptides 29: 2144–2149.

Kobelt P, Wisser AS, Stengel A, Goebel M, Bannert N, Gourcerol G *et al.* (2008). Peripheral obestatin has no effect on feeding behavior and brain Fos expression in rodents. Peptides 29: 1018–1027.

Koca SS, Ozgen M, Aydin S, Dag S, Evren B, Isik A (2008). Ghrelin and obestatin levels in rheumatoid arthritis. Inflammation 31: 329–335.

Kong XJ, Gao L, Peng H, Shi X (2010). Effects of electro-acupuncture on expression of obestatin in hypothalamus of rats with simple obesity. Zhong Xi Yi Jie He Xue Bao 8: 480–485.

Kosowicz J, Baumann-Antczak A, Ruchała M, Gryczyñska M, Gurgul E, Sowiñski J (2011). Thyroid hormones affect plasma ghrelin and obestatin levels. Horm Metab Res 43: 121–125.

Ku JM, Andrews ZB, Barsby T, Reichenbach A, Lemus MB, Drummond GR *et al.* (2015). Ghrelin-related peptides exert protective effects in the cerebral circulation of male mice through a nonclassical ghrelin receptor(s). Endocrinology 156: 280–290.

Kukuvitis A, Froudarakis M, Tryfon S, Tzouvelekis A, Saroglou M, Karkavitsas N *et al.* (2010). Acute effect of smoking on plasma obestatin levels. Tob Induc Dis 8: 2.

Lauwers E, Landuyt B, Arckens L, Schoofs L, Luyten W (2006). Obestatin does not activate orphan G protein-coupled receptor GPR39. Biochem Biophys Res Commun 351: 21–25.

Lee WJ, Chen CY, Ser KH, Chong K, Chen SC, Lee PC *et al.* (2013). Differential influences of gastric bypass and sleeve gastrectomy on plasma nesfatin-1 and obestatin levels in patients with type 2 diabetes mellitus. Curr Pharm Des 19: 5830–5835.

Lei Y, Liang Y, Chen Y, Liu X, Liao X, Luo F (2014). Increased circulating obestatin in patients with chronic obstructive pulmonary disease. Multidiscip Respir Med 9: 5.

Li ZF, Guo ZF, Cao J, Hu JQ, Zhao XX, Xu RL *et al.* (2010a). Plasma ghrelin and obestatin levels are increased in spontaneously hypertensive rats. Peptides 31: 297–300.

Li ZF, Guo ZF, Yang SG, Zheng X, Cao J, Qin YW (2010b). Circulating ghrelin and ghrelin to obestatin ratio are low in patients with untreated mild-to-moderate hypertension. Regul Pept 165: 206–209.

Li ZF, Song SW, Qin YW, Zhang JL, Zhao XX, Zhang BL *et al.* (2009). Bolus intravenous injection of obestatin does not change blood pressure level of spontaneously hypertensive rat. Peptides 30: 1928–1930.

Li ZF, Zhou DX, Pan WZ, Zhang L, Ge JB (2013). Circulating ghrelin was negatively correlated with pulmonary arterial pressure in atrial septal defect patients. Chin Med J (Engl) 126: 3936–3939.

Lippl F, Erdmann J, Lichter N, Tholl S, Wagenpfell S, Adam O *et al.* (2008). Relation of plasma obestatin levels to BMI, gender, age and insulin. Horm Metab Res 40: 806–812.

Liu W, Yue H, Zhang J, Pu J, Yu Q (2014). Effects of plasma ghrelin, obestatin, and ghrelin/obestatin ratio on blood pressure circadian rhythms in patients with obstructive sleep apnea syndrome. Chin Med J (Engl) 127: 850–855.

Mafra D, Guebre-Egziabher F, Cleaud C, Arkouche W, Mialon A, Drai J *et al.* (2010). Obestatin and ghrelin interplay in hemodialysis patients. Nutrition 26: 1100–1104.

Matuszyk A, Ceranowicz P, Warzecha Z, Cieszkowski J, Bonior J, Jaworek J *et al.* (2015). Obestatin accelerates the healing of acetic acid-induced colitis in rats. Oxid Med Cell Longev 2016 .2834386

Miegueu P, St Pierre D, Broglio F, Cianflone K (2011). Effect of desacyl ghrelin, obestatin and related peptides on triglyceride storage, metabolism and GHSR signaling in 3T3-L1 adipocytes. J Cell Biochem 112: 704–714.

Mizutani M, Atsuchi K, Asakawa A, Matsuda N, Fujimura M, Inui A *et al.* (2009). Localization of acyl ghrelin- and des-acyl ghrelin-immunoreactive cells in the rat stomach and their responses to intragastric pH. Am J Physiol Gastrointest Liver Physiol 297: G974–G980.

Moechars D, Depoortere I, Moreaux B, de Smet B, Goris I, Hoskens L *et al.* (2006). Altered gastrointestinal and metabolic function in the GPR39-obestatin receptor-knockout mouse. Gastroenterology 131: 1131–1141.

Mondal MS, Toshinai K, Ueno H, Koshinaka K, Nakazato M (2008). Characterization of obestatin in rat and human stomach and plasma, and its lack of acute effect on feeding behavior in rodents. J Endocrinol 198: 339–346.

Monteleone P, Serritella C, Martiadis V, Maj M (2008a). Deranged secretion of ghrelin and obestatin in the cephalic phase of vagal stimulation in women with anorexia nervosa. Biol Psychiatry 64: 1005–1008.

Monteleone P, Serritella C, Martiadis V, Scognamiglio P, Maj M (2008b). Plasma obestatin, ghrelin, and ghrelin/obestatin ratio are increased in underweight patients with anorexia nervosa but not in symptomatic patients with bulimia nervosa. J Clin Endocrinol Metab 93: 4418–4421.

Mora M, Granada ML, Roca M, Palomera E, Puig R, Serra-Prat M *et al.* (2013). Obestatin does not modify weight and nutritional behaviour but is associated with metabolic syndrome in old women. Clin Endocrinol (Oxf) 78: 882–890.

Moretti E, Collodel G, Iacoponi F, Geminiani M, Pascarelli NA, Campagna S *et al.* (2011). Detection of obestatin in seminal plasma and its relationship with ghrelin and semen parameters. Fertil Steril 95: 2303–2309.

Motorykina ES, Khirazova EE, Maslova MV, Maklakova AS, Graf AV, Bayzhymanov AA *et al.* (2015). Changes in feeding and drinking motivations and glucose content in male rats after single or chronic administration of obestatin or its fragment (1–4). Dokl Biol Sci 460: 1–4.

Nagaraj S, Peddha MS, Manjappara UV (2008). Fragments of obestatin as modulators of feed intake, circulating lipids, and stored fat. Biochem Biophys Res Commun 366: 731–737.

Nagaraj S, Peddha MS, Manjappara UV (2009). Fragment analogs as better mimics of obestatin. Regul Pept 158: 143–148.

Nagaraj S, Raghavan AV, Rao SN, Manjappara UV (2014). Obestatin and Nt8U influence glycerolipid metabolism and PPAR gamma signaling in mice. Int J Biochem Cell Biol 53: 414–422.

Nakahara T, Harada T, Yasuhara D, Shimada N, Amitani H, Sakoguchi T*et al.* (2008). Plasma obestatin concentrations are negatively correlated with body mass index, insulin resistance index, and

plasma leptin concentrations in obesity and anorexia nervosa. Biol Psychiatry 64: 252–255.

Nogueiras R, Pfluger P, Tovar S, Arnold M, Mitchell S, Morris A *et al.* (2007). Effects of obestatin on energy balance and growth hormone secretion in rodents. Endocrinology 148: 21–26.

Ozbay Y, Aydin S, Dagli AF, Akbulut M, Dagli N, Kilic N *et al.* (2008). Obestatin is present in saliva: alterations in obestatin and ghrelin levels of saliva and serum in ischemic heart disease. BMB Rep 41: 55–61.

Pamukcu O, Kumral ZN, Ercan F, Yegen BÇ, Ertem D (2013). Anti-inflammatory effect of obestatin and ghrelin in dextran sulfate sodium–induced colitis in rats. J Pediatr Gastroenterol Nutr 57: 211–218.

Pattnaik P (2005). Surface plasmon resonance: applications in understanding receptor–ligand interaction. Appl Biochem Biotechnol 126: 79–92.

Popovics P, Stewart AJ (2011). GPR39: a Zn^{2+}-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. Cell Mol Life Sci 68: 85–95.

Prodam F, Cadario F, Bellone S, Trovato L, Moia S, Pozzi E *et al.* (2014). Obestatin levels are associated with C-peptide and anti-insulin antibodies at the onset whereas unacylated and acylated ghrelin levels are not predictive of long-term metabolic control in children with type 1 diabetes. J Clin Endocrinol Metab 99: E599–E607.

Pruszynska-Oszmalek E, Szczepankiewicz D, Hertig I, Skrzypski M, Sassek M, Kaczmarek P *et al.* (2013). Obestatin inhibits lipogenesis and glucose uptake in isolated primary rat adipocytes. J Biol Regul Homeost Agents 27: 23–33.

Qader SS, Håkanson R, Rehfeld JF, Lundquist I, Salehi A (2008). 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 146: 230–237.

Qi X, Li L, Yang G, Liu J, Li K, Tang Y*et al.* (2007). Circulating obestatin levels in normal subjects and in patients with impaired glucose regulation and type 2 diabetes mellitus. Clin Endocrinol (Oxf) 66: 593–597.

Reinehr T, De ousa G, Roth CL (2008). Obestatin and ghrelin levels in obese children and adolescents before and after reduction of overweight. Clin Endocrinol (Oxf) 68: 304–310.

Ren A, Guo Z, Wang YK, Wang LG, Wang W, Lin L *et al.* (2008). Inhibitory effect of obestatin on glucose-induced insulin secretion in rats. Biochem Biophys Res Commun 369: 969–972.

Ren AJ, He Q, Shi JS, Guo ZF, Zheng X, Lin L *et al.* (2009). Association of obestatin with blood pressure in the third trimesters of pregnancy. Peptides 30: 1742–1745.

Ren G, He Z, Cong P, Chen H, Guo Y, Yu J *et al.* (2013a). Peripheral administration of TAT-obestatin can influence the expression of liporegulatory genes but fails to affect food intake in mice. Peptides 42: 8–14.

Ren G, He Z, Cong P, Yu J, Qin Y, Chen Y*et al.* (2013b). Effect of TAT-obestatin on proliferation, differentiation, apoptosis and lipolysis in 3 T3-L1 preadipocytes. J Pept Sci 19: 684–691.

Robinson E, Cassidy RS, Tate M, Zhao Y, Lockhart S, Calderwood D *et al.* (2015). Exendin-4 protects against post-myocardial infarction remodelling via specific actions on inflammation and the extracellular matrix. Basic Res Cardiol 110: 20.

Roth C, Reinehr T, Schernthaner G, Kopp H, Kriwanek S, Schernthaner G (2009). Ghrelin and obestatin levels in severely obese women before and after weight loss after Roux-en-Y gastric bypass surgery. Obes Surg 19: 29–35.

Saliakelis E, Iakovou I, Varlamis G, Karatzas N, Garstioni S, Fotoulaki M (2014). Serum obestatin, ghrelin, and ghrelin/obestatin ratio are increased in children with symptoms suggestive of delayed gastric emptying of unclear etiology. Eur J Nucl Med Mol Imaging 41: S579–S580.

Samson WK, White MM, Price C, Ferguson AV (2007). Obestatin acts in brain to inhibit thirst. Am J Physiol Regul Integr Comp Physiol 292: R637–R643.

Samson WK, Yosten GL, Chang JK, Ferguson AV, White MM (2008). Obestatin inhibits vasopressin secretion: evidence for a physiological action in the control of fluid homeostasis. J Endocrinol 196: 559–564.

Savino F, Benetti S, Lupica MM, Petrucci E, Palumeri E, Cordero di Montezemolo L (2012). Ghrelin and obestatin in infants, lactating mothers and breast milk. Horm Res Paediatr 78: 297–303.

Sazdova IV, Ilieva BM, Minkov IB, Schubert R, Gagov HS (2009). Obestatin as contractile mediator of excised frog heart. Cent Eur J Biol 4: 327–334.

Schinzari F, Iantorno M, Campia U, Mores N, Rovella V, Tesauro M *et al.* (2015). Vasodilator responses and endothelin-dependant vasoconstriction in metabolically healthy obesity and the metabolic syndrome. Am J Physiol Endocrinol Metab 309: E787–E792.

Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B *et al.* (2013). Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med 369: 1317–1326.

Scrima M, Campiglia P, Esposito C, Gomez-Monterrey I, Novellino E, D'Ursi AM (2007). Obestatin conformational features: a strategy to unveil obestatin's biological role? Biochem Biophys Res Commun 363: 500–505.

Sedlácková D, Dostálová I, Hainer V, Beranová L, Kvasnicková H, Hill M *et al.* (2008). Simultaneous decrease of plasma obestatin and ghrelin levels after a high-carbohydrate breakfast in healthy women. Physiol Res 57 (Suppl 1): S29–S37.

Sedlackova D, Kopeckova J, Papezova H, Hainer V, Kvasnickova H, Hill M *et al.* (2012). Comparison of a high-carbohydrate and high-protein breakfast effect on plasma ghrelin, obestatin, NPY and PYY levels in women with anorexia and bulimia nervosa. Nutr Metab (Lond) 9: 52.

Sedlácková D, Kopečková J, Papežová H, Vybíral S, Kvasničková H, Hill M *et al.* (2011). Changes of plasma obestatin, ghrelin and NPY in anorexia and bulimia nervosa patients before and after a high-carbohydrate breakfast. Physiol Res 60: 165–173.

Seim I, Walpole C, Amorim L, Josh P, Herington A, Chopin L (2011). The expanding roles of the ghrelin-gene derived peptide obestatin in health and disease. Mol Cell Endocrinol 340: 111–117.

Şen LS, Karakoyun B, Yeğen C, Akkiprik M, Yüksel M, Ercan F *et al.* (2015). Treatment with either obestatin or ghrelin attenuates mesenteric ischemia–reperfusion-induced oxidative injury of the ileum and the remote organ lung. Peptides 71: 8–19.

Seoane LM, Al-Massadi O, Pazos Y, Pagotto U, Casanueva FF (2006). Central obestatin administration does not modify either spontaneous or ghrelin-induced food intake in rats. J Endocrinol Invest 29: RC13–RC15.

Shao L, Zhao YT, Teng LL, Li MZ, Jiang H (2014). Circulating obestatin levels correlate with fasting insulin and HOMA-IR but not with hypertension in elderly men. Cell Biochem Biophys 69: 89–92.

Shen C, Yu T, Tang ZH, Wu KM (2013). Changes in ghrelin and obestatin levels before and after a meal in children with simple obesity and anorexia. Horm Res Paediatr 79: 341–346.

Shi JB, Guo ZF, Zheng X, Wang ZB, Ma YJ (2012). Circulating obestatin is increased in patients with cardiorenal syndrome and positively correlated with vasopressin. Peptides 38: 377–380.

Sibilia V, Bresciani E, Lattuada N, Rapetti D, Locatelli V, De Luca V *et al.* (2006). Intracerebroventricular acute and chronic administration of obestatin minimally affect food intake but not weight gain in the rat. J Endocrinol Invest 29: RC31–RC34.

Siejka A, Jankiewicz-Wika J, Kołomecki K, Cywiński J, Piestrzeniewicz K, Swiętosławski J *et al.* (2013). Long-term impact of vertical banded gastroplasty (VBG) on plasma concentration of leptin, soluble leptin receptor, ghrelin, omentin-1, obestatin, and retinol binding protein 4 (RBP4) in patients with severe obesity. Cytokine 64: 490–493.

Słupecka M, Pierzynowski SG, Kuwahara A, Kato I, Woliński J (2014). Age-dependent effect of obestatin on intestinal contractility in Wistar rats. Gen Comp Endocrinol 208: 109–115.

Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SP *et al.* (2016). The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. Nucl. Acids Res. 44: D1054–D1068.

St-Pierre DH, Settanni F, Olivetti I, Gramaglia E, Tomelini M, Granata R *et al.* (2010). Circulating obestatin levels in normal and type 2 diabetic subjects. J Endocrinol Invest 33: 211–214.

Subasinghage AP, Green BD, Flatt PR, Irwin N, Hewage CM (2010). Metabolic and structural properties of human obestatin {1–23} and two fragment peptides. Peptides 31: 1697–1705.

Tang S, Dong X, Zhang W (2014). Obestatin changes proliferation, differentiation and apoptosis of porcine preadipocytes. Ann Endocrinol (Paris) 75: 1–9.

Taskin MI, Bulbul E, Adali E, Hismiogulları AA, Inceboz U (2015). Circulating levels of obestatin and copeptin in obese and nonobese women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 189: 19–23.

Taskin E, Atli B, Kiliç M, Sari Y, Aydin S (2014). Serum, urine, and saliva levels of ghrelin and obestatin pre-and post-treatment in pediatric epilepsy. Pediatr Neurol 51: 365–369.

Tate M, Chong A, Robinson E, Green BD, Grieve DJ (2015). Selective targeting of glucagon-like peptide-1 signalling as a novel therapeutic approach for cardiovascular disease in diabetes. Br J Pharmacol 172: 721–736.

Tate M, Robinson E, Green BD, McDermott BJ, Grieve DJ (2016). Exendin-4 attenuates adverse cardiac remodelling in streptozotocin-induced diabetes via specific actions on infiltrating macrophages. Basic Res Cardiol 111: 1.

Tokudome T, Kishimoto I, Myazato M, Kangawa K (2014). Ghrelin and the cardiovascular system. Cardiovasc Issues Endocrinol 43: 125–133.

Tremblay F, Perreault M, Klaman LD, Tobin JF, Smith E, Gimeno RE (2007). Normal food intake and body weight in mice lacking the G protein-coupled receptor GPR39. Endocrinology 148: 501–506.

Uehara M, Yasuhara D, Nakahara T, Harada T, Ushikai M, Asakawa A *et al.* (2011). Increase in energy intake leads to a decrease in obestatin in restricting-type of anorexia nervosa. Exp Clin Endocrinol Diabetes 119: 536–539.

Unniappan S, Speck M, Kieffer TJ (2008). Metabolic effects of chronic obestatin infusion in rats. Peptides 29: 1354–1361.

Van Dijck A, Annemie VD, Van Dam D, Debby VD, Vergote V, Valentijn V*et al.* (2009). Central administration of obestatin fails to show inhibitory effects on food and water intake in mice. Regul Pept 156: 77–82.

Vergote V, Van Dorpe S, Peremans K, Burvenich C, De Spiegeleer B (2008). In vitro metabolic stability of obestatin: kinetics and identification of cleavage products. Peptides 29: 1740–1748.

Vicennati V, Genghini S, De Iasio R, Pasqui F, Pagotto U, Pasquali R (2007). Circulating obestatin levels and the ghrelin/obestatin ratio in obese women. Eur J Endocrinol 157: 295–301.

Wali P, King J, He Z, Tonb D, Horvath K (2014). Ghrelin and obestatin levels in children with failure to thrive and obesity. J Pediatr Gastroenterol Nutr 58: 376–381.

Wang WM, Li SM, Du FM, Zhu ZC, Zhang JC, Li YX (2014). Ghrelin and obestatin levels in hypertensive obese patients. J Int Med Res 42: 1202–1208.

Wasilewski MA, Myers VD, Recchia FA, Feldman AM, Tilley DG (2015). Arginine vasopressin receptor signaling and functional outcomes in heart failure. Cell Signal 28: 224–233.

White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL *et al.* (2013). Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med 369: 1327–1335.

Wojciechowicz T, Skrzypski M, Kołodziejski PA, Szczepankiewicz D, Pruszyńska-Oszmałek E, Kaczmarek P *et al.* (2015). Obestatin stimulates differentiation and regulates lipolysis and leptin secretion in rat preadipocytes. Mol Med Rep 12: 8169–8175.

Wu W, Fan X, Yu Y, Wang Y (2015). Maternal serum ratio of ghrelin to obestatin decreased in preeclampsia. Pregnancy Hypertens 5: 263–266.

Xin X, Ren AJ, Zheng X, Qin YW, Zhao XX, Yuan WJ *et al.* (2009). Disturbance of circulating ghrelin and obestatin in chronic heart failure patients especially in those with cachexia. Peptides 30: 2281–2285.

Xu L, Depoortere I, Tomasetto C, Zandecki M, Tang M, Timmermans JP *et al.* (2005). Evidence for the presence of motilin, ghrelin, and the motilin and ghrelin receptor in neurons of the myenteric plexus. Regul Pept 124: 119–125.

Yamamoto D, Ikeshita N, Daito R, Herningtyas EH, Toda K, Takahashi K *et al.* (2007). Neither intravenous nor intracerebroventricular administration of obestatin affects the secretion of GH, PRL, TSH and ACTH in rats. Regul Pept 138: 141–144.

Yuan X, Cai W, Liang XF, Su H, Yuan Y, Li A *et al.* (2015). Obestatin partially suppresses ghrelin stimulation of appetite in 'high-responders' grass carp, *Ctenopharyngodon idellus*. Comp Biochem Physiol A Mol Integr Physiol 184: 144–149.

Zamrazilová H, Hainer V, Ková DS, Papežová H, Kunešová M, Bellisle F *et al.* (2008). Plasma obestatin levels in normal weight, obese and anorectic women. Physiol Res 57: S49–S55.

Zhang JV, Jahr H, Luo CW, Klein C, Van Kolen K, Ver Donck L *et al.* (2008a). Obestatin induction of early-response gene expression in gastrointestinal and adipose tissues and the mediatory role of G protein-coupled receptor, GPR39. Mol Endocrinol 22: 1464–1475.

Zhang JV, Klein C, Ren PG, Kass S, Ver Donck L, Moechars D *et al.* (2007). Response to Comment on "Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake". Science 315: 766.

Zhang JV, Ren P, Avsian-Kretchmer O (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 310: 996–999.

Zhang S, Zhai G, Zhang J, Zhou J, Chen C (2014). Ghrelin and obestatin plasma levels and ghrelin/obestatin prepropeptide gene polymorphisms in small for gestational age infants. J Int Med Res 42: 1232–1242.

Zhang W, Chai B, Li JY, Wang H, Mulholland MW (2008b). Effect of des-acyl ghrelin on adiposity and glucose metabolism. Endocrinology 149: 4710–4716.

Zhao CM, Furnes MW, Stenström B, Kulseng B, Chen D (2008). Characterization of obestatin- and ghrelin-producing cells in the gastrointestinal tract and pancreas of rats: an immunohistochemical and electron-microscopic study. Cell Tissue Res 331: 575–587.

Zizzari P, Longchamps R, Epelbaum J, Bluet-Pajot MT (2007). Obestatin partially affects ghrelin stimulation of food intake and growth hormone secretion in rodents. Endocrinology 148: 1648–1653.

Zou CC, Liang L, Wang CL, Fu JF, Zhao ZY (2009). The change in ghrelin and obestatin levels in obese children after weight reduction. Acta Paediatr Int J Paediatr 98: 159–165.