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**OBESTATIN AS A KEY REGULATOR OF METABOLISM AND
CARDIOVASCULAR FUNCTION WITH EMERGING THERAPEUTIC
POTENTIAL FOR DIABETES**

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Short running title: Metabolic and cardiovascular actions of obestatin

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SUMMARY

Obestatin is a 23-amino acid C-terminally amidated gastrointestinal peptide derived from proghrelin and which forms an alpha 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 to also mediate promotion of cell survival and prevention of apoptosis. Obestatin-induced increases in β -cell mass, enhanced adipogenesis and improved lipid metabolism have been noted along with upregulation of genes associated with β -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.

KEY WORDS: Obestatin; diabetes; metabolism; cardiovascular system

ABBREVIATIONS:

BK_{Ca}, large conductance calcium-activated potassium channel; cAMP, cyclic adenosine monophosphate; CART, cocaine and amphetamine-related transcript; C/EBP, CCAAT-enhancer-binding protein; CCK, cholecystokinin; CRF, corticotrophin releasing factor; EIA, enzyme immunoassay; ERK, extracellular signal-regulated kinase; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; GLUT-4, glucose transporter type 4; GPR39, G-protein coupled receptor 39; GSK-3 β , glycogen synthase kinase-3 β ; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; mTOR, mechanistic target of rapamycin; NO, nitric oxide; NPY, neuropeptide Y; PEG, polyethylene glycol; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; POMC, proopiomelanocortin; PPAR, peroxisome proliferator-activated receptor; RIA, radioimmunoassay; SK61, ribosomal protein S6 kinase 1; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TNF- α , tumour necrosis factor- α ; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; WAT, white adipose tissue.

INTRODUCTION

Currently 415M 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 642M by 2040 (International Diabetes Federation, 2015). The disease itself is a leading cause of global mortality, accounting for 5M 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 4-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 which 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 which may be relevant to T2DM. Whilst previous reviews have tended to highlight the pathophysiological actions of obestatin in relation to its well characterised 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 which 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 stabilisation of the peptide in to 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 G-protein coupled receptor 39 (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 e.g. food intake, body weight, adiposity, fasting glucose/insulin (which are modified by obestatin; see below), compared to wild-type controls, and an intact metabolic response to obestatin, providing strong evidence that GRP39 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 upregulates 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 extracellular signal-

regulated kinase (ERK) 1/2 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 β -cells, the GLP-1 receptor (GLP-1R) was suggested as a candidate for the obestatin receptor. Indeed, obestatin was shown to bind and upregulate the GLP-1R and its effects on β -cell survival were attenuated by the GLP-1R antagonist, exendin(9-39) (Granata et al., 2008). Furthermore, in mouse 3T3-L1 and human adipocytes both activation and blockade of the GLP-1R inhibited obestatin binding (Granata et al., 2012). In contrast, obestatin was unable to bind to the GLP-1R or to displace GLP-1 binding in INS-1 pancreatic β -cells and HEK293 cells overexpressing GLP-1R (Unniappan et al., 2008). Taken together, these data are generally supportive of the suggestion that obestatin may signal through the GLP-1R, although there is currently insufficient independently-verified evidence to allow definite conclusions to be drawn. Nonetheless, the prospect of involvement of the GLP-1R in obestatin signalling is particularly intriguing further to our recent reports of direct cardioprotective actions of GLP-1R activation (Robinson et al. 2015; Tate et al. 2016). In this regard, we have suggested that obestatin may signal via an adenylate cyclase-linked G protein-coupled receptor 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, 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 myentric plexus, and in the Leydig cells of the testis where it is co-localised 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 localised 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-localisation 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 minutes, compared with 12.6 minutes in liver and 138 minutes in kidney membranes (Vergote et al., 2008), whilst the half-life of rodent obestatin in rat liver homogenate was found to be 21.7 minutes, and increased over 3-fold by the addition of a polyethylene glycol (PEG) group to the N-terminus (Agnew et al., 2011). A large number of groups have investigated circulating physiological levels of obestatin in both rodents and humans, with a wide range of values reported (rodents: 1.34 to 2,560; humans: 8.4 to 22,057 pg/ml; 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/mL (Zamrazilová et al., 2008), whilst another published values of 68.3 ± 14.8 pg/mL (Monteleone et al., 2008b), i.e. 4-fold 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 to 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 gastrointestinal 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 which 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; Yamamoto et al., 2007; Chen et al., 2008, 2010, 2012a, 2012b; 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; Sibilias et al., 2006; Gourcerol et al., 2006, 2007a, 2007b; 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-hour 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 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 TH₁-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 which 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) are reported to be elevated in patients with active inflammatory bowel diseases (Crohn's disease and colitis) compared to 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 β -cell loss, reduced β -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 foetal and adult endocrine pancreas with co-localisation at the islet periphery, thereby suggesting a synergistic relationship which may be connected with

pancreatic β -cell function (Granata et al., 2010a). In 2008, obestatin was reported to be secreted by human pancreatic islets and pancreatic β -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, β -cell survival, mass, growth and differentiation (insulin receptor substrate 2, cyclic adenosine monophosphate (cAMP) response element binding protein, pancreatic and duodenal homeobox-1, glucokinase) were upregulated by obestatin, together with activation of phosphoinositide 3-kinase (PI3K)/Akt, ERK1/2 and cAMP production (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 vascularisation (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, 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 β -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 gastrointestinal 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 neutralisation 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 preadipocytes via stimulation of ERK1/2 and P13K/Akt, which are established mediators of adipocyte proliferation and survival (Miegeue 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 preadipocytes was not associated with adipogenesis (Ren et al., 2013b). A similar investigation in porcine preadipocytes found obestatin to stimulate proliferation and differentiation and to inhibit apoptosis via promotion of peroxisome proliferator-activated receptor (PPAR) γ and CCAAT-enhancer-binding protein (C/EBP) α and inhibition of caspase-3/7/9 (Tang et al., 2014). Consistent with these findings, isoproterenol-induced lipolysis in both 3T3-L1 preadipocytes 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 (Pruszyńska-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 preadipocyte 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 (Miegeu 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 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 downregulate glucose transporter type 4 (GLUT-4) in adipose tissue (Pruszyńska-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 upregulation of sirtuin 1, which is important in mediating the insulin response, and activation of key signalling pathways, including Akt, glycogen synthase kinase-3 β (GSK-3 β), 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 (summarised 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; 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 (HOMA-IR) 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, 2008b; Germain et al., 2009, 2010; Sedláčková 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 Leu72Met and Gln90Leu 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; Sedláčková 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 hours postpartum (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 stimulatory effect whilst high concentrations were inhibitory, thereby suggesting that β -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 β -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 e.g. tumour necrosis factor- α (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 which 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 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 which 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 administered 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 standardisation and refinement of the employed plasma analysis techniques is 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) expression (Kellokoski et al., 2009). However, the same study found obestatin to also promote binding of oxidised low-density lipoprotein (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 which were interestingly prevented by the GLP-1R 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 a nitric oxide (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, L-NMMA (Agnew et al., 2012). Comprehensive *ex vivo* analysis identified a pathway involving an adenylate cyclase-linked G protein-coupled receptor, PI3K/Akt and Ca²⁺-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 vasodilation (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), vascular endothelial growth factor (VEGF), and pro-inflammatory interleukin-1 β , in pancreatic microvascular endothelial cells were attenuated by obestatin, whilst obestatin improved mouse skeletal muscle regeneration via stimulation of microvascularisation secondary to induction of satellite stem cell expansion and VEGF/VEGF receptor 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 characterised 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 GPR-39 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, protein kinase C (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 localised 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 -adrenergic stimulation in streptozotocin-induced T1DM rats, but not in non-diabetic controls, via protection against loss of β -adrenoreceptors 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 cardio-renal 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 Gheorghide, 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 which occur in diabetes and 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 (summarised 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 to the full-length peptide, while 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 which 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 activation of key metabolic signalling pathways (Nagaraj et al., 2009, 2014). Interestingly, an alternate obestatin modification approach involving TAT 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 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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STATEMENT OF CONFLICTS OF INTEREST

None.

AUTHORSHIP CONTRIBUTION

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.

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FIGURE LEGEND

Figure 1 Summary of the reported pathophysiological effects of obestatin. Obestatin targets several tissues, including the gastrointestinal system, pancreas, white adipose tissue, the heart and vasculature, where it exerts diverse biological actions relevant to the metabolic and cardiovascular complications of diabetes.

TABLE 1: Circulating levels of obestatin in normal physiology

Obestatin Level (pg/ml)	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	Sedláčková 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) ^c	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
22057 ± 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 et al., 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; RIA, radioimmunoassay. ^aMean ± SEM, ^bMean ± SD, ^cMean ± SEM or Mean ± SD, ^dMedian, ^eMedian (Interquartile range), ^fMedian (1st -4th quartile), ^gMedian (min-max).

TABLE 2: Effect of disease pathology on circulating levels of obestatin

Obestatin Level (pg/ml)		Ghrelin/Obestatin ↑↓	Tissue	Study Details	
Normal Physiology	Disease Pathology			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; PWS, Prader-Willi Syndrome; PE, preeclampsia; RIA, radioimmunoassay.

^aMean ± SEM, ^bMean ± SD, ^cMean ± SEM or Mean ± SD.

FIGURE 1



