Energy Homeostasis: The Roles of Adipose Tissue-Derived Hormones, Peptide YY and Ghrelin

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Key Words

Adipose tissue · Appetite · Diabetes mellitus · Insulin resistance · Obesity

Summary

This review discusses the physiology of the hormones leptin, adiponectin, resistin, peptide YY, and ghrelin and how each of these contributes to energy homeostasis, weight regulation, and the pathogenesis of obesity. The relationship these hormones have with insulin and insulin resistance is also discussed, and the potential therapeutic use of each of these hormones is also considered.

Introduction

Obesity is a growing concern, which the World Health Organisation predicts will affect 700 million people by 2015 [1]. Adipose tissue has been established as a complex endocrine organ, secreting leptin, adiponectin, and resistin, which regulate long-term satiety, appetite, and energy expenditure. Gut hormones play an integral role in short-term appetite control. Ghrelin is the only known gut hormone to stimulate appetite while peptide YY (PYY), glucagon-like peptide-1, cholecystokinin, and oxyntomodulin suppress appetite. Of the hormones that suppress appetite, PYY has attracted particular interest in recent years and will therefore form the basis of our discussion of short-term appetite suppression. This relatively new area of endocrinology is likely to offer better understanding of obesity and potential therapeutic options for obesity and obesity-related morbidities. This review focuses on the specific role these hormones have in energy homeostasis (fig. 1).

Long-Term Control

Leptin

The existence of circulating factors produced in the periphery that changed in relation to body fat stores and that were capable of signalling energy stores in the central nervous system were first postulated by Kennedy [2]. Insulin was the first peripheral hormone to be identified as important in energy homeostasis [3], however, evidence for another hormone came from a series of classic parabiosis studies carried out in mice by Coleman in the early 1970s [4]. A putative factor was discovered 2 decades later by Zhang et al. [5], who first identified leptin through positional cloning of the obese (ob) gene in mice. Leptin, derived from the Greek word 'leptos', meaning 'thin', is a 16 kDa product of the ob gene produced primarily by adipocytes [5]. Lack of a functional gene in ob/ob mice leads to hyperphagia and severe obesity [5]. Diabetic (db) db/db mice which also share a similar phenotype lack a functional leptin receptor, being leptin-resistant [6]. The role of leptin in energy homeostasis (fig. 2) is best emphasised by the observation that the characteristic features of congenital leptin deficiency in humans, which in addition to severe childhood obesity include delayed maturation of the reproductive system and immune dysfunction, are all subsequently ameliorated by daily therapy with recombinant leptin [7, 8], suggesting a role for this adipokine in reproduction and immune function.

Leptin exerts its biological activity by binding to the leptin receptor (LR), of which 6 variants have been identified in mice, classified from a to f [9]. LRb, a type 1 cytokine cell surface receptor, is the only variant that possesses an intracellular domain and is therefore capable of mediating cell signalling (when dimerised) via the recruitment of janus-associated kinase 2 (JAK2), a cytoplasmic tyrosine kinase [10]. Autophosphorylation of JAK and phosphorylation of a select

number of highly conserved tyrosine residues on LRb subsequently leads to the recruitment and phosphorylation of signal transducer and activation of transcription 3 (STAT3), a principal transcription factor [10] which in a homodimerised form regulates most of leptin's bioactivities. In addition, an elegant mechanism exists whereby the intracellular signal is terminated through the up-regulation of the suppressor of cytokine signalling 3 (SOCS3) molecule, which may play a role in mediating leptin resistance [11, 12]. This has been demonstrated in mice that are haploinsufficient for the SOCS3 gene and that have improved leptin and insulin sensitivity compared to wild-type mice [11]. Moreover, a similar effect is observed in mice that are SOCS3-deficient in their brains [12]. Complete absence of the SOCS3 gene is incompatible with life [11]. It must also be noted that both the phosphoinositide-3 kinase (PI-3K) and extracellular signal-regulated protein kinase (ERK) pathways have also been implicated in LRb signalling [13].

Several tissues expressing the LR have been identified and include the liver and pancreas as well as cells of the immune system [14]. However, the most significant site of leptin action occurs in the brain, particularly in the hypothalamus. Cohen et al. [15] elegantly demonstrated that induced loss of LR in the brain leads to obesity. Brain-specific expression of a functional LR ameliorates obesity in the db/db mouse that lacks a functional LR [16]. More specifically, LR-deficient Koletsky rats which have undergone targeted gene therapy and are subsequently able to express functional LRs in the arcuate nu-

cleus (ARC) of the hypothalamus are rescued from the obese phenotype [17].

Leptin mediates its effect on satiety and weight reduction through its ability to regulate levels of hypothalamic neuropeptides within specific neurons of the ARC. Leptin inhibits agouti-related protein (AgRP)- and neuropeptide Y (NPY)-containing neurons, which are orexigenic [9]. It also stimulates pro-opiomelanocortin (POMC)-containing neurons, which are anorexigenic and also promote energy expenditure [9]. POMC neurons may play a pivotal role in energy balance, as selected deletion of LRs on POMC neurons leads to obesity [18]. More recently, other leptin-sensitive regions have been identified within the central nervous system. This largely includes the dopaminergic neuron-rich regions of the ventral tegmental area, which may also form part of the circuitry regulating food intake [19]. Leptin also demonstrates a positive effect on locomotor activity [20].

The melanocortin signalling system is central to energy homeostasis [21]. POMC produced in the ARC undergoes tissue-specific posttranslational modification to produce α -melanocyte stimulating hormone (α -MSH). α -MSH exerts its anorectic effects via the melanocortin-3 receptor (MC3R) and melanocortin-4 receptor (MC4R) which are widely expressed in the hypothalamus and other areas of the brain [21]. Various studies in rodents suggest that MC4R is the key effector in suppressing food intake [21]. AgRP antagonises MC4R whereas leptin stimulates it by indirectly increasing α -MSH

Fig. 2. Leptin – regulation of weight/feeding: A reduction in body weight from an individual ‘set point’ or a period of fasting is accompanied by reduced levels of circulating leptin. This results in increased food intake and a reduction in energy expenditure. This results in a normalisation of leptin concentration and/or body weight.

concentrations (achieved by increasing both POMC gene expression and action potential firing in POMC-containing neurons in the ARC) [21].

Observations from early studies in mice led to postulates placing leptin as the afferent arm of a feedback loop that signalled the brain to suppress feeding and increase energy expenditure leading to weight loss. This was based on experimental data which demonstrated significant reductions in weight and food intake in obese, leptin-deficient rodents after therapy with exogenous leptin [20, 22]. Conversely, these studies demonstrated a less impressive change in weight and energy expenditure in lean rodents in which leptin is not deficient [20]. Moreover, subsequent studies in obese rodents and humans demonstrated markedly high levels of both leptin protein and leptin mRNA [23]. Leptin levels also correlate positively with fat mass [24]. As leptin promotes satiety and weight loss, the hyperleptinaemia of obesity may seem paradoxical but has been attributed to a concept known as ‘leptin resistance’.

Previously, therapy utilising recombinant leptin has reduced weight and suppressed appetite in obese individuals who are deficient of leptin due to a genetic cause [25]. Mutations in the *Ob* gene are rare and tend to occur in the offspring of consanguineous marriages. Previous trials of exogenous leptin therapy in obese individuals without a genetic defect in the leptin signalling system have shown little promise [26,

27], however, more recently, Rosenbaum et al. [28] have demonstrated that leptin may have a role in maintaining reduced body weight after weight loss.

The cause of leptin resistance is still unclear but there are currently two lines of thought. Resistance may involve reduced transport of leptin across the blood-brain barrier (BBB) where it must gain access to the ARC both in animals and humans [29, 30]. Defective signalling at the post-receptor level with aberrant overactivity of SOCS3 [11, 12] has also been implicated. Ageing may also be an independent factor associated with acquired leptin resistance [31]. However, it is unclear as to the exact mechanism(s) responsible for leptin resistance and further work is required.

Adiponectin

Adiponectin, also known as Acrp30, AdipoQ, apM1, and GBP28, was first identified in 1995 [32]. It is a protein, coded for by a gene on chromosome 3q27. It is secreted by brown and white adipose tissue and circulates in the plasma in 3 major forms: a low-molecular weight (LMW), middle-molecular weight (MMW), and a high-molecular weight (HMW) [33–35]. Adiponectin functions at 2 receptors: AdipoR1, primarily in the skeletal muscle, and AdipoR2, primarily in the liver. These receptors contain 7 transmembrane domains but are distinct from G-protein-coupled receptors [36]. The effects of adiponectin have been demonstrated by adiponectin knockout (KO) mice which exhibit severe diet-induced insulin resistance, severe neo-intimal thickening in response to vascular injury, and hypertension induced by salt diet. These effects are reversible by viral-mediated production of adiponectin [37]. Consequently, adiponectin is thought to play a role in glucose and lipid metabolism and in inhibiting inflammation and atherosclerosis. More recently, it has also been suggested that adiponectin has a central effect at the arcuate hypothalamus, stimulating food intake and decreasing energy expenditure during fasting [38].

Decreased adiponectin concentrations have been shown to be associated with obesity [39] and insulin resistance [40] in mice studies, but understanding of the cause of the decrease is limited. However, in obese subjects, an increase in adipose oxidative stress with a consequent increase in reactive oxygen species [41] and cytokines [42] is thought to contribute, at least in part, to decreased adiponectin synthesis. Adiponectin affects insulin resistance by enhancing the action of insulin, with injection of adiponectin into mice triggering a transient decrease in basal glucose but no associated increase in insulin [43]. This led to the proposal that adiponectin may be involved in the pathogenesis of type 2 diabetes mellitus (T2DM). A chromosomal link to T2DM has been found in the region of the adiponectin gene [44], and certain single nucleotide polymorphisms of the adiponectin gene are associated with alterations in plasma adiponectin levels, insulin resistance, and T2DM [45]. Studies have even suggested that high levels of circulating adiponectin may result in a 40% decrease in the

development of T2DM in certain populations [46]. Although the mechanism by which adiponectin increases insulin sensitivity has been investigated, it is not fully understood. Insulin action is initiated by the binding of insulin to its receptor, which then undergoes tyrosine phosphorylation and is activated. Downstream, the phosphatidylinositol-3 kinase (PI3K) pathway stimulates translocation of GLUT-4 transporters into the plasma membrane and therefore increases glucose uptake. Recently, the PI3K pathway has been shown to be inhibited by the target of rapamycin (TOR) complex 1-induced signalling pathway which is mediated by the S6 kinase (S6K)-dependent phosphorylation of the insulin receptor substrate-1 (IRS-1). Adiponectin affects insulin sensitivity by inhibiting the mTOR/S6K signalling pathway, thus disinhibiting the insulin signalling pathway and enhancing insulin action [47]. In addition to its insulin-sensitising properties, recent studies have also indicated that adiponectin may stimulate pancreatic insulin secretion both in vitro and in vivo [48].

Adiponectin KO mice develop neointimal thickening, and consequently adiponectin has been thought to play an important role in the development of atherosclerosis [49]. It has been proposed that in vascular endothelium injury adiponectin accumulates in the subendothelial space [50], suppressing monocyte attachment by inhibition of cell adhesion molecule-1 and E-selectin [51], inhibiting vascular smooth muscle proliferation [52] and suppressing macrophage-to-foam cell formation [53]. High levels of adiponectin have been shown to be protective against the development of coronary artery disease [54]. However, in patients with pre-existing symptomatic coronary artery disease, high adiponectin concentrations are associated with an increase in cardiovascular events. This finding has led to the suggestion that adiponectin concentrations are raised in those with pre-existing disease to try and combat the pro-atherosclerotic state of the patient, although the increase is insufficient to counter the other negative factors present [55].

The HMW form of adiponectin has been found to be the most clinically relevant. Quantity of HMW adiponectin has been found to be more significant in determining insulin sensitivity [56] and coronary artery disease [57] than total adiponectin. Gene mutations that specifically impair HMW adiponectin formation have similarly been shown to be associated with T2DM [58].

The highly significant physiological role of adiponectin has caused speculation over its potential as a therapeutic target. Thiazolidinediones are the most widely recognised medications having a beneficial effect on adiponectin concentrations and have been shown to enhance insulin sensitivity at least in part by increasing circulating adiponectin concentration [59]. More recently, speculation has arisen as to whether other commonly used medication may have an impact on adiponectin concentrations. It seems likely they do, due to evidence suggesting increased adiponectin concentrations with medications such as angiotensin-converting enzyme (ACE) inhibi-

tors, angiotensin II receptor blockers [60], statins, fibrates [61], and niacin (which seems to particularly increase the HMW form) [62]. Further potential therapeutic benefits of manipulating adiponectin concentrations have been speculated, and it seems likely that adiponectin may present a useful therapeutic target for combating and reversing obesity-linked diseases.

Resistin

The last adipose tissue-derived hormone described is resistin. This is a 12.5 kDa protein that was discovered in 2001 [63] and belongs to a family of cysteine-rich proteins [64]. Secreted by adipose tissue, it circulates as a dimeric protein: 2 polypeptides linked by a disulphide bridge [65]. Its physiological role has been subject to much debate and its function remains unclear.

Associations found have suggested a link between increasing resistin and increasing adiposity [63, 66]. Correlations between insulin resistance and resistin concentrations have also been reported in humans [67]. However, the role resistin plays in glucose homeostasis is unclear due to conflicting studies which show decreased levels of the hormone in obesity [68, 69], the ability of thiazolidinediones to exert their antidiabetic effect without any decrease in resistin [68], and only weak correlations between resistin and insulin sensitivity [69]. Genetic variations of the resistin gene have also failed to show an association with obesity [70]. The secretion of the hormone by monocytes and macrophages may help explain the contradictory role of resistin in energy balance [71]. The conflicting data described suggest that resistin is unlikely to be revealed as a major player in the pathogenesis of T2DM and obesity.

Short-Term Control

PYY

PYY is a 36-amino acid gastrointestinal hormone, where Y depicts the abbreviation for tyrosine. It is a member of the pancreatic polypeptide family, which includes pancreatic polypeptide (PP) and neuropeptide Y (NPY), which mediate their effects through G-protein-linked NPY receptors of which there are several subtypes (Y1, Y2, Y4, and Y5 represent fully defined subtypes) [72]. PYY is secreted by L-cells of the distal gut, together with glucagon-like peptide and oxyntomodulin [73]. Peripheral neurons, especially enteric neurons, also express PYY, as does a restricted set of central neurons [74]. Secretion of PYY in the gastrointestinal tract is primarily stimulated by the presence of nutrients (mainly lipids and protein [75]) in the gut lumen and is proportional to the caloric density of the meal ingested [74], but is not altered by gastric distension [76]. Other stimulants of PYY release include intraluminal bile acids, gastric acid, and cholecystokinin [77]. Peak plasma levels of PYY occur in the 2nd hour following food ingestion [78].

PYY exists in 2 forms – the full-length peptide PYY1-36 which is truncated to the biologically active PYY3-36 by

dipeptidyl peptidase-IV [79]. PYY3-36 is the predominant form [79] and has been shown to reduce food intake in rodents and humans [78]. PYY also inhibits transit through the proximal small intestine [80], delays gastric emptying [81], and inhibits gallbladder emptying [82]. PYY secretion stimulates vagal and somatosensory afferent fibres arising in the gastrointestinal tract and terminating at the nucleus tractus solitarius (NTS) of the brain-stem, which transmit information pertaining to recent food intake [83]. Neurons from the NTS can then relay this information to the ARC of the hypothalamus, a key component of the forebrain pathway involved in appetite control [84]. PYY can also act directly in the brain via the blood, entering at areas where the BBB is deficient [85].

PYY3-36 shows high specificity for the Y2 NPY receptor subtype (Y2R) [86] and is thought to work via activation of auto-inhibitory presynaptic Y2Rs present on orexigenic NPY neurons in the ARC [78]. These NPY neurons inhibit anorexigenic POMC neurons and therefore their inhibition by PYY3-36 results in reciprocal activation of POMC neurons to inhibit feeding [87]. PYY3-36 may also work by decreasing plasma ghrelin levels [88] and by inhibition of ghrelin-activated neurons of the ARC [89]. There is conflicting evidence as to whether PYY1-36 has an effect on energy intake in man [90], although data on PYY1-36 is limited.

In obese humans, fasting plasma concentrations of PYY are reduced [88] and PYY responses to a standard meal are attenuated [91], although there does not seem to be associated resistance to the effects of PYY3-36 [88], leading to the hypothesis that PYY deficiency may contribute to the pathogenesis of obesity. Genetic variations in PYY and Y2R genes may contribute to obesity and are associated with the severe obesity of Pima Indian men [92]. Furthermore, a study of a Swedish cohort has shown that the common Y2R variant is less prevalent among obese compared with lean men, indicating the common Y2R variant as protective against obesity [93]. Less is known about potential changes in PYY levels in T2DM, although recently a blunted postprandial PYY response to a high carbohydrate test meal has been found in individuals genetically predisposed to T2DM [94]. The reduced PYY levels seen in obesity and prediabetes may result from abnormalities in its synthesis, release, or clearance, although it is doubtful that increased clearance plays a major role, as the rate of elimination is similar in lean and obese subjects when PYY is exogenously administered [88]. Hypothalamic overexpression of NPY may also have a role in suppressing the anorectic effects of PYY in obese and insulin-resistant patients [95].

In contrast, PYY levels have been found to be increased in disease states characterised by significant weight loss, such as anorexia nervosa [96], coeliac disease, inflammatory bowel disease [97], and cardiac cachexia [98]. It has also been shown that after Roux-en-Y gastric bypass (RYGBP) for the treatment of morbid obesity the postprandial PYY response is exaggerated, which is thought to contribute to the weight loss and maintenance of weight loss after this procedure [99].

The relationship between insulin and PYY is controversial. As exogenous administration of PYY3-36 reinforces insulin's action on glucose uptake in mice [100] and PYY KO mice develop hyperinsulinaemia [101], it has been suggested that lower PYY levels in obesity may contribute to insulin resistance [95]. Furthermore, PYY3-36 infusions increase plasma free fatty acid concentration and subsequently increase postprandial insulin and glucose responses, as compared to saline infusion [102], implying PYY3-36 or PYY3-36-analogue treatment may be beneficial in ameliorating insulin resistance. However, the precise mechanisms by which PYY may affect insulin levels are still unclear, and some studies refute a relationship between the two [88, 103].

The possible use of exogenous PYY3-36 as an anti-obesity agent has provoked much interest. In humans, PYY3-36 administration has been reported to decrease both hunger and single-meal food intake by 36%, without causing illness or compensatory hyperphagia [88]. A synthetic human form of PYY3-36, coupled to 40 kDa polyethylene glycol (PEG), has been created to increase the half-life of PYY3-36 from 3 to 24 h and has been tested in rabbits with no signs of toxicity [104]. An injectable PYY3-36 analogue was tested in phase I studies as an anti-obesity therapy but had limited success due to nausea [105]. It is therefore with further research that PYY3-36 may prove to become a key player in addressing the obesity epidemic.

Ghrelin

Ghrelin is a 28-amino acid peptide produced predominantly by X/A-like cells of the mucosal layer in the fundus of the stomach [106]. Ghrelin exists in either acylated or deacylated form. Acylation of ghrelin with an O-linked octanoyl side group at serine 3 by ghrelin O-acyl transferase (GOAT), a polytopic membrane-bound enzyme, is crucial for its physiological effects [107, 108]. Ghrelin was first discovered in 1999 as a strong stimulant of growth hormone (GH) release, and acylated ghrelin is a natural ligand of the GH secretagogue type 1a receptor (GHS-R1a) [109], which is largely expressed in the ARC of the hypothalamus [110]. In addition to stimulating GH release, ghrelin has also been identified as the only known orexigenic hormone which plays a role in regulating pre-meal hunger and meal initiation as well as long-term energy balance [111, 112]. Both peripheral and intracerebroventricular administrations of ghrelin stimulate food intake, weight gain and adiposity, and decreased energy expenditure [113, 114]. Ghrelin's orexigenic effect is thought to occur via activation of the GHS-R1a receptor in areas such as the hypothalamus [115], although more recently it has been investigated whether des-acyl ghrelin may also induce food intake via a mechanism that is independent of the GHS-R1a [116]. It has also been shown that centrally infused ghrelin effects adipocyte metabolism independently of ghrelin-induced hyperphagia by increasing the rate of glucose utilisation by adipocytes [117]. Ghrelin also has stimulatory effects on lactotroph and corticotroph secretion [118], gastric motility [119], and cardiovascular effects [120].

Ghrelin synthesis and secretion are regulated by nutrient stimulation of the glutathion insulin transhydrogenase (with greater response to carbohydrate or protein ingestion compared to fat [121]) and weight loss [122]. Circulating ghrelin concentrations increase during fasting and decrease after eating [123]. In subjects on fixed feeding schedules, there is a marked surge in ghrelin before each meal, raising the possibility that anticipation of meals, in addition to effects of fasting, may also contribute to ghrelin secretion [124].

The mechanisms by which ghrelin promotes hunger and increased energy intake are not entirely clear. It is possible that the promotion of gastric emptying by ghrelin increases hunger. It has been demonstrated that gastric emptying was positively correlated with fasting plasma ghrelin levels [123]. Ghrelin also acts in the ARC of the hypothalamus to promote hunger by activating the orexigenic NPY/AgRP neurons and by antagonising the satiety effects of leptin via inhibition of POMC neurons [125]. This theory is supported by evidence that blockade of NPY receptors or immunoneutralisation of AgRP attenuates ghrelin-induced feeding. Ghrelin may gain access to the hypothalamus directly by crossing the BBB [126] or indirectly via the vagus nerve, shown by the inhibition of ghrelin's orexigenic effects following vagotomy in rats [127]. The effects of ghrelin also seem to be mediated by the sympathetic nervous system. Convincing studies have found that beta-adrenergic receptors are required for an increase in body weight to occur following central ghrelin administration [118], and a decrease in brown adipose tissue sympathetic nerve activity and lowered brown adipose tissue temperature [128] in addition to lowered core body temperature [129] in response to central ghrelin administration has also been observed.

Plasma levels of ghrelin and leptin are inversely correlated [130]. Ghrelin acts to inhibit leptin's anorexigenic effects on NPY neurons of the ARC [125], and vice versa [131]. This reciprocal relationship between leptin and ghrelin in the hypothalamus is likely to play an important role in the regulation of appetite and feeding, although the precise nature of their interaction is not entirely understood. It has been reported that the acute postprandial rises in insulin may stimulate meal-induced suppression of ghrelin [132]. Furthermore, ghrelin levels are markedly reduced in insulin-resistant subjects compared to insulin-sensitive controls, independent of BMI [133], implicating insulin as a likely candidate for ghrelin regulation.

Ghrelin levels are markedly increased in Prader-Willi syndrome and are thought to mediate the ravenous feeding in this condition [134]. However, in obese subjects fasting, plasma ghrelin levels are lower than in lean controls [135], although appetite is commonly increased, suggesting that higher body mass is associated with increased gastric responsiveness to ghrelin [136]. RYGBP has been found to successfully impair the adaptive response of ghrelin to body weight loss and is thought to contribute to the ability of patients to maintain weight loss after this procedure [137]. There has been considerable interest in the potential therapeutic uses of ghrelin and ghrelin receptor ligands. The use of ghrelin administration and GHS-R1a agonists in aiding weight gain in cachexia and anorexia nervosa has been investigated [138, 139]. Conversely, the use of GHS-R1a antagonists in the treatment of obesity has also been explored, with antagonists such as 2,4-diaminopyrimidine showing promising results in decreasing appetite in animals [140]. However, in order to realise the full potential of ghrelin antagonism as anti-obesity treatment, GHS-R1a antagonists with better selectivity, potency, and pharmacokinetic properties are required [141].

Conclusion

It seems likely that adipokines and gut hormones both have a role to play in the pathogenesis of obesity and obesity-related morbidities, with correlations between them being found. However, clinical applications for these hormones so far remain limited, with only leptin administration showing efficacy in reversing obesity in certain leptin-deficient patients. However, with further clarification of their roles in health it seems likely that adipokines and gut hormones could offer valuable therapeutic approaches to obesity and its related morbidities.

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