

Gut Hormones and Energy Balance, The Future for Obesity Therapy?

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Abstract

BACKGROUND: The prevalence of obesity is increasing in both developed and developing countries along with associated diseases such as type 2 diabetes and coronary heart disease. The recent discovery of a number of gut hormones that play a role in appetite regulation and are released or suppressed in response to a meal may offer new targets for the treatments of obesity.

CONTENT: In addition to the obvious role of the gut in the digestion and absorption of nutrients, the intestine and associated visceral organs, including the pancreas, liver, and visceral adipose depots, have important sensing and signaling roles in the regulation of energy homeostasis. Signals reflecting energy stores, recent nutritional state, and other parameters are integrated in the central nervous system, particularly in the hypothalamus, to coordinate energy intake and expenditure.

SUMMARY: Our understanding of the role of the gut in energy balance and insights into gut-derived signals will stimulate previously unexplored therapeutics for obesity and other disorders of energy balance.

KEYWORDS: Obesity, Energy Balance, Gut Hormones, Satiation, Satiety

Introduction

Obesity has reached epidemic proportions throughout the world and poses significant health and economic burdens to both developed and developing societies. Globally, the World Health Organization estimates that a billion adults have a body mass index greater than 25 kg/m² and 300 million are obese with BMI over 30 kg/m². Obesity is a state of excess adiposity with significant consequences due to perturbation of a complex system of energy regulation.

The neurohormonal control of appetite, body composition, and glucose homeostasis is mediated by hormones secreted from adipose tissue, endocrine glands, and enteroendocrine cells, which converge at the vagus nerve, brainstem and hypothalamus to modulate complex interactions of neurotransmitters and central appetite-regulating peptides. These hormonal signals are tightly regulated to maintain body weight/adiposity within a narrow, individually defined range that may be further impacted by variables such as ingested calories, meal composition, and lifestyle (1).

Energy balance is a homeostatic system. Although malfunctions of this system can cause obesity (2), the relatively recent increase in the incidence of obesity is not thought to be the result of specific defects, but of a regulatory system unable to cope with the current context of cheap, high-energy foodstuffs, mechanized transport and non-manual labour. Commandeering elements of this regulatory system might provide the best opportunity for us to combat obesity (3).

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The gastrointestinal tract is the body's largest endocrine organ and releases more than 30 different regulatory peptide hormones that influence a number of physiological processes and act on tissues including exocrine glands, smooth muscle and the peripheral nervous system. Most of these hormones are sensitive to gut nutrient content, and short-term feelings of hunger and satiety are believed to be mediated, in part, by coordinated changes in circulating gut hormone levels (4).

Current pharmacologic agents licensed to treat obesity result in a very modest weight loss in clinical trials. Their long-term safety and efficacy are unclear, and none are approved for treatment periods longer than 2 years. More efficacious drugs are therefore required to battle the obesity epidemic (5).

The gut hormones are attractive targets for obesity therapy, because unlike indiscriminate chemical agents, they target only the relevant appetite control systems in the brain. In order to maintain permanent weight loss, patients may require life-long treatment and side-effects may be less likely to occur during prolonged treatment with gut hormones, which are endogenously produced daily in normal individuals, compared with non-specific neurotransmitter mimetics or antagonists. Furthermore, inappropriate gut hormone release or signalling may itself contribute to the pathophysiology of obesity. If this is the case, then correcting it by the administration of exogenous gut hormones or their antagonists would be appropriate (6).

Elucidating the physiological circuits involved in appetite regulation will allow us to understand how individual gut hormones work in concert to control food intake. By furthering our knowledge it is hoped it will open up novel therapeutic avenues for the treatment of obesity (6).

Satiation & Satiety Signals

Despite substantial fluctuations in daily food intake, animals maintain a remarkably stable body weight, because overall caloric ingestion and expenditure are exquisitely matched over long periods of time, through the process of energy homeostasis. The brain receives hormonal, neural, and metabolic signals pertaining to body-energy status and, in response to these inputs, coordinates adaptive alterations of energy intake and expenditure. To regulate food consumption, the brain must modulate appetite, and the core of appetite regulation lies in the gut-brain axis (8).

“Satiety” refers to processes that promote meal termination, thereby limiting meal size (9,10). “Satiety” refers to postprandial events that affect the interval to the next meal, thereby regulating meal frequency, which is also influenced by learned habits (11). Satiety results from a coordinated series of neural and humoral signals that emanate from the gut in response to mechanical and chemical properties of ingested food. Although the relevant signals are commonly dubbed “satiety signals,” this term is usually a misnomer, because most of them promote termination of ongoing meals and do not delay subsequent meal initiation or affect intake if delivered between meals (12).

A primary function of the gut is to achieve efficient nutrient digestion and absorption; many satiety signals optimize these processes by influencing gastrointestinal (GI) motility and secretion. Their additional capacity to limit meal size enhances this control by restricting the rate at which nutrients reach the gut (13). Meals are typically stopped long before gastric capacity is reached, and when food is diluted with noncaloric bulking agents, the volume ingested increases to maintain constant caloric intake (14). Therefore, animals can consume much larger meals than they typically do. A major function of satiety is to prevent overconsumption during individual meals, thereby averting deleterious consequences from incomplete digestion as well as excessive disturbances in circulating levels of glucose and other nutrients (15).

Satiation signals arise from multiple sites in the GI system, including the stomach, proximal small intestine, distal small intestine, colon, and pancreas. Ingested food evokes satiety by two primary effects on the GI tract — gastric distention and release of peptides from enteroendocrine cells. The hindbrain is the principal central site receiving input from short-acting satiety signals, which are transmitted both neurally. Although the perception of fullness clearly involves higher forebrain centers, conscious awareness of GI feedback signals is not required for satiety. Even animals whose hindbrain is surgically disconnected from the forebrain exhibit satiety and respond to GI satiety peptides (16,17). Therefore, gut-hindbrain communication is sufficient for satiety, although this normally interacts with higher cognitive centers to regulate feeding.

Pathways relaying short-acting satiety signals from the gut to the hindbrain also interact at several levels with long-acting adiposity hormones involved in body-weight regulation, such as leptin and insulin. Through multifaceted mechanisms, adiposity hormones function as gain-setters to modulate the sensitivity of vagal and hindbrain responses to GI satiety signals. Adiposity hormones thereby regulate

short-term food intake to achieve longterm energy balance (18,19).

Gastric satiation signals. Oral and gastric stimuli happen concurrently during eating, and up to 40% of a meal empties into the intestine before meal termination (20). Therefore, pregastric, gastric, and intestinal satiation signals commence almost simultaneously, and they function in unison, augmenting each other's satiating effects (21). Gastric satiation signals arise primarily from mechanical distention, whereas those from the intestine derive largely from the chemical effects of food (22). Although the stomach can sense nutrients (for example, to regulate gastrin release) (23), this does not seem to contribute to satiation. The stomach wall is endowed with discrete neural sensors of tension (24), stretch (25), and volume (21). Output from these mechanoreceptors is relayed to the brain by vagal and spinal sensory nerves (21, 26), using a complex array of neurotransmitters and neuromodulators, including glutamate, acetylcholine, nitric oxide, calcitonin-gene-related peptide, substance P, galanin, and cocaine-andamphetamine-related transcript (21).

Intestinal Satiation. The generally accepted assertion that "gastric satiation is volumetric, intestinal satiation is nutritive" (22) reflects the importance of nutrients in mediating intestinal satiation, with a limited role for distention. Intestinal nutrient infusions reduce food intake in many species, including humans (21) — an effect that commences within seconds of nutrient infusion, indicating that at least some of the associated satiation signals emanate from the gut, rather than from postabsorptive sources (27). These, and other, findings demonstrate that the intestines play a dominant role in satiation. Many intestinal satiation signals inhibit gastric emptying, and this probably helps limit ingestion by enhancing gastric mechanoreceptor stimulation. However, sham feeding experiments show that a delay of gastric emptying is not required for intestinal signals to elicit satiation. Mediators of intestinal satiation include a cadre of gut peptides that are secreted from enteroendocrine cells in response to ingested food. In conjunction with gastric distention, satiation peptides educe the perception of GI fullness, promoting meal termination (21).

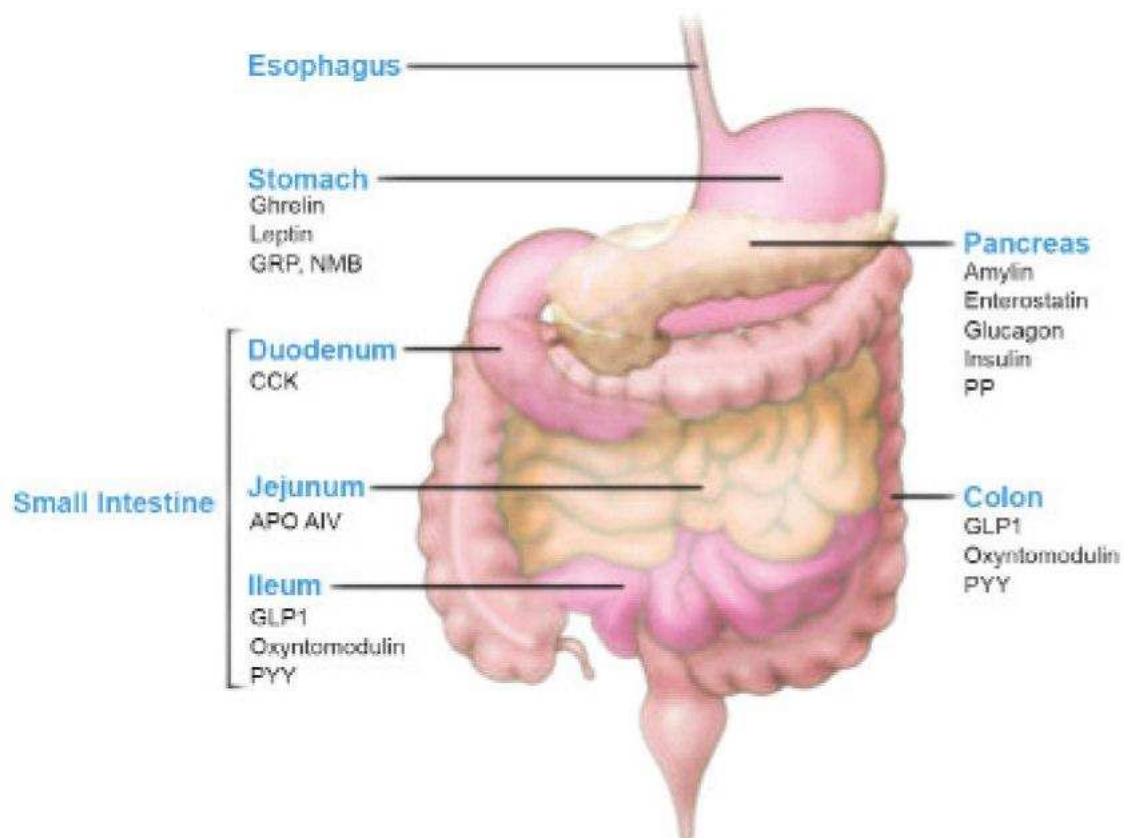


Figure 1. Principal sites of synthesis of GI peptides implicated in the regulation of food intake (Adapted with permission from Cummings DE *et al.*).

Fat – specific satiation peptides: enterostatin and apolipoprotein A-IV. Some GI peptides are specifically stimulated by fat ingestion and subsequently regulate intake and/or metabolism of lipids. Enterostatin is a pentapeptide cleaved from procolipase, which is secreted from the exocrine pancreas in response to ingested fats to facilitate their digestion. Procolipase is also produced in the gut and several brain areas pertinent to energy homeostasis (28). Both peripheral and central enterostatin administration decreases dietary fat intake in animals, and enterostatin-receptor antagonists do the opposite (29). The mechanisms underlying these effects seem complex but involve the F1-ATPase β subunit as the putative enterostatin receptor (30), with downstream mediators including melanocortins and the 5-hydroxytryptamine (serotonin) receptor 1B (31). Unfortunately, enterostatin administration to humans has thus far shown no effects on food intake, appetite, energy expenditure, or body weight (32).

Apolipoprotein A-IV (APO AIV) is a glycoprotein secreted from the intestine in response to fat absorption and chylomicron formation (33). It is used to package digested lipids for transit through lymphatics to blood. It is also produced in the hypothalamic arcuate nucleus. Exogenous administration of APO AIV decreases meal size, food intake, and weight gain in rats, whereas APO AIV-specific antibodies do the opposite (34). APO AIV is

hypothesized to represent a link between short- and long-term regulation of lipid-related energy balance (33).

Pancreatic satiation peptides: PP and amylin. PP is produced in specialized islet cells under vagal control, and its secretion is stimulated postprandially in proportion to caloric load (34). Acting primarily on peripheral and central Y4R and Y5R, it influences biliary and exocrine pancreatic function, gastric acid secretion, and GI motility. Whether PP has an important role in energy homeostasis is controversial, in part because peripheral administration decreases feeding, whereas central administration increases it. Reminiscent of PYY, this disparity might result from differential access to Y receptors — circulating PP decreasing food intake through Y4R in the AP and central PP increasing it through Y5R deeper in the brain. Peripheral PP injections reduce food intake and weight gain in wild-type and genetically obese *ob/ob* mice (36), and administration to humans decreases appetite and food intake, independently of gastric emptying (37).

Amylin, a peptide cosecreted with insulin postprandially by pancreatic β cells, inhibits gastric emptying, gastric acid, and glucagon secretion. It can also decrease meal size and food intake after peripheral or central administration (38,39).

Interactions of Long-Term Adiposity Signals & Short-Term Satiation Signals

There is no doubt that food intake in humans is influenced by emotional factors, social cues, and learned behavior. These influences overlay highly conserved systems within the brain that sense and integrate signals reflecting overall energy stores, recent energy intake, and presence of specific classes of nutrients. The hypothalamus, especially the arcuate nucleus, is relatively accessible to circulating factors and also receives inputs from other areas of the brain. Here, signals are received that relate to total energy stores in fat and to immediate changes in energy availability, including nutrients within the gut. These two categories of signals are not exclusive, because signals relating to long term energy stores, including insulin and leptin, can modulate responses to short-term nutritional inputs. The hypothalamus integrates these peripheral and central signals and exerts homeostatic control over food intake, levels of physical activity, basal energy expenditure, and endocrine systems, including those that determine reproductive competence (4).

Although there are many peripheral signals that can contribute to feeding behavior and body weight regulation, it is important to recognize that short-term and long-term food intake and energy balance are regulated through distinct, but interacting, mechanisms. In this context, some signals (e.g., nutrients and gastrointestinal [GI] hormones) act primarily as determinants of satiety to limit the size of individual meals. These short-term signals have a markedly different function than the long-term regulators of energy homeostasis that are activated in proportion to both body adipose stores and to the amount of energy consumed over a more prolonged period of time.

Insulin and leptin are two such long-term signals. These hormones regulate food intake and energy expenditure to ensure that energy homeostasis is maintained and that body weight and adiposity remain relatively constant. In contrast, short-term signals are not primary determinants of body adiposity since they can be overridden by long-term regulatory signals. Nonetheless, the short-term and long-

term mechanisms need to function in concert to integrate energy intake and energy expenditure to ensure that energy balance is maintained (40).

Insulin, produced by pancreatic β -cells, is vital for regulating the storage of absorbed nutrients and also acts as an adiposity signal to the brain for the regulation of energy balance (41). Mechanisms exist for transporting insulin across the blood-brain barrier and insulin receptors are expressed in appetite-controlling areas of the brain. Administration of insulin to the brain not only reduces appetite in rodents and subhuman primates but also potentiates satiety factors such as cholecystokinin (CCK).

Leptin is an adipocyte-derived factor, or adipokine, that is the dominant long-term signal informing the brain of adipose energy reserves (42). Similar to insulin, leptin is transported across the blood-brain barrier, where it binds to specific receptors on appetite-modulating neurons, most notably but not exclusively in the arcuate nucleus (43). The long form of the leptin receptor activates Janus kinase–signal transducer and activator of transcription (JAKSTAT) signaling among several other signal transduction pathways. Exogenous leptin reverses obesity caused by its absence in mice and humans (44, 45); however, absolute leptin deficiency is an uncommon cause of human obesity. Instead, leptin resistance results from defects in transport across the blood-brain barrier or impaired intracellular signaling. Suppressor of cytokine signaling 3 (SOCS3) is an intracellular protein that acts to limit leptin signaling and is an important mediator of leptin resistance.

Because SOCS3 also limits insulin signaling, its expression provides a potentially important point of interaction between insulin and leptin pathways that may be important in the pathogenesis of the metabolic syndrome (46). Protein tyrosine phosphatase-1b is another factor implicated through both gain- and loss-of-function experiments as a key regulator of insulin and leptin signaling (47).

Long-acting adiposity hormones that regulate body weight, such as leptin and insulin, must ultimately influence eating behavior at individual meals. Accordingly, leptin and insulin acting in the brain, especially the hypothalamus, enhance central sensitivity to input from short-acting peripheral satiation signals, such as CCK (18,19). Emerging evidence suggests that analogous synergism between long- and short-acting signals occurs in the gut. For example, leptin and insulin receptors are expressed on L cells, and activation of these receptors augments GLP1 secretion (48). Conversely, and similarly to what occurs in the hypothalamus, L cells display diet-induced leptin and insulin resistance, with diminished GLP1 release. These findings suggest that long- and short-acting anorectic

signals cooperate at the level of gut-peptide secretion.

Similar interactions occur at the level of vagal sensitivity to gut peptides. A functional signaling isoform of the leptin receptor is coexpressed with CCK1R by vagal-afferent nerve terminals in the stomach and duodenum (49). CCK activation of cultured vagal sensory neurons from these regions is enhanced by leptin (50), and the two peptides function synergistically to increase discharge of vagal-afferent fibers (51), just as they potentiate the anorectic actions of each other (18,19). Some authors speculate that these findings establish a neuroanatomical substrate for complementary interactions between gastric leptin and intestinal CCK in short-term satiation. It is probably true that the gastric leptin secreted from chief cells into the stomach lumen during meals travels to the duodenum and stimulates CCK release (52). It is not clear, however, whether gastric leptin secreted from P cells into the circulation reaches duodenal vagal fibers before passing through the liver and being diluted in the general circulation, where leptin levels fluctuate only very minimally with meals. Therefore, the enhancement of CCK-induced duodenal vagal-afferent signaling by leptin might reflect a long-acting adiposity hormone (adipocyte leptin) increasing peripheral neural sensitivity to a short-acting GI satiation factor (8).

Just as the hypothalamus and hindbrain integrate input from catabolic and anabolic peripheral signals reflecting energy status, the vagus nerve seems to perform an analogous assimilative role in the gut. GI vagal-afferent fibers display extensive colocalization of receptors for gut peptides that are anorexigenic, such as CCK and leptin, as well as orexigenic, such as ghrelin, melanin-concentrating hormone (MCH), and orexins (53). At least some of these receptors are regulated adaptively by alterations in nutritional state, and they interact with one another in a coordinated, logical manner. For example, receptors for ghrelin and CCK are coexpressed on vagal-afferent neurons (53), and these two ligands exert antagonistic effects on vagal-afferent discharges (54). Similarly, catabolic gut peptides tend to suppress secretion of anabolic gut peptides and vice versa, whereas ghrelin increases expression of orexigenic cannabinoid-1 and MCH-1 receptors on vagal afferents (53). These observations suggest that GI peptides act in a coordinated manner, belying their diffuse anatomical distribution. Moreover, alterations in nutritional state influence gut-brain satiation signaling by recalibrating vagal sensitivity to GI signals (8).

In addition to leptin, a number of other factors secreted by adipocytes or that regulate adipocyte metabolism have been implicated in the regulation of energy balance. For example, acylation stimulating protein (ASP), which

is produced by adipocytes as a result of interaction of complement factor C3, factor B, and adipsin, has a role in increasing the efficiency of triacylglycerol synthesis (55,56). Mice that lack the ability to synthesize ASP have delayed postprandial lipid clearance (57). However, ASP deficiency also has a major impact on energy balance and insulin action. Despite increased energy intake, C3/ASP knockout mice have significantly reduced adipose tissue depots compared with wild-type animals fed either chow or high fat diet, indicating that these animals have elevated energy expenditure (58).

Enhancement of adipocyte lipolysis can have a similar effect on energy balance. This is indicated by the finding that mice with a knockout of the gene for perilipin and hence an elevated activity of hormone-sensitive lipase are hyperphagic and lean with smaller adipocytes and that the absence of perilipin reverses obesity even in leptin receptor-deficient db/db mice (59,60). Another adipocyte protein that is of considerable interest with regard to the regulation of energy balance and insulin action is adiponectin (61,62),

which is also known as gACRP30, a cleavage product of adipocyte complement-related protein 30 (63), and as adipoQ (64). Circulating adiponectin levels are reduced in obese humans (62) and rhesus monkeys (65). In humans, adiponectin levels are negatively correlated with fasting insulin concentrations and positively correlated with insulin sensitivity (66).

Short-term signals are primarily from the GI tract (e.g., CCK and GI stretch receptors) and are involved in promoting sensations of satiety that lead to meal termination. These short-term signals by themselves are not sufficient to regulate energy balance and body adiposity. The long-term signals insulin and leptin are produced and circulate in proportion to recent energy intake and body adiposity. Together, the short- and long-term signals interact to regulate energy balance in that insulin and leptin appear to determine the sensitivity of the brain to the satiety-producing effects of the short-term signals from the GI tract (40).

Gut Hormones

Numerous circulating peptides and steroids produced in the body influence appetite through their actions on the hypothalamus, the brain stem and the autonomic nervous system. These hormones come from three major sites – fat cells, the gastrointestinal tract and the endocrine pancreas (67). Today more than 30 peptide hormone genes are known to be expressed throughout the digestive tract, which makes the gut the largest endocrine organ in the body (68).

GHRELIN

Ghrelin is a peptide hormone released into circulation from the stomach that was first discovered as an endogenous ligand for the growth-hormone-secretagogue receptor (GHS-R). Ghrelin is composed of 28 amino acids and is uniquely modified by the addition of an octanoyl group to the serine residue at position three. This acylation is necessary for ghrelin to bind to the GHS-R and to cross the blood-brain barrier (69).

Ghrelin is the only known factor to increase appetite through the circulation. The pattern of ghrelin release suggests that it governs feelings of hunger. Circulating ghrelin levels are increased by fasting, and fall after a meal. Central or peripheral administration of acylated ghrelin to rats acutely stimulates food intake and growth hormone release, and chronic administration causes weight

gain. Intravenous infusion or subcutaneous injection of ghrelin to humans increases both feelings of hunger and food intake. Ghrelin is often referred to as the 'hunger hormone'. It has been reported that peripheral ghrelin administration reduces fat use (70) and that chronic central ghrelin infusion increases the expression of enzymes that promote fat storage in adipose tissue (71). The metabolic effects of ghrelin might not, therefore, be entirely dependent on increased food intake.

Contrary to satiation peptides, ghrelin increases GI motility and decreases insulin secretion. Also in contrast to satiation peptides, circulating levels surge shortly before meals and are suppressed by ingested nutrients (with carbohydrates being more effective than proteins, which are more effective than lipids). Postprandial suppression does not require luminal nutrient exposure in either the stomach or the duodenum, where 80%–90% of ghrelin production occurs, but results instead from neurally transmitted (nonvagal) intestinal signals, augmented by insulin (72). Ghrelin is implicated in mealtime hunger and meal initiation because of its marked pre-meal surges (73). Moreover, ghrelin enhances food intake by increasing the number of meals initiated, without altering their size, and it elicits numerous appetitive feeding behaviors. Preprandial ghrelin secretion seems to be a cephalic response, possibly stimulated by the sympathetic nervous system (74). Pre-

meal ghrelin surges can be entrained to regularly scheduled meals, and they might participate in the anticipatory processes that enable animals to prepare for food intake and nutrient disposition (75).

In addition to being produced in the stomach, ghrelin may also be produced within the brain. A new set of ghrelin-positive neurons within the hypothalamus, lying between the dorsal, ventral, paraventricular and arcuate nuclei were identified by immunohistochemistry (76). The functional relevance of brain-derived ghrelin remains to be determined. However, there is now evidence that the central actions of ghrelin are of physiological relevance in the control of adipocyte metabolism (77).

Thus, central ghrelin appears to partition nutrients toward fat storage by favouring an increase in glucose and triglyceride uptake, increasing lipogenesis and inhibiting lipid oxidation in white adipocytes. This fits in well with the phenotype of ghrelin-deficient mice described earlier, where under conditions of abundant dietary fat, the impaired adipocyte metabolism becomes functionally relevant and leads to a decreased susceptibility to diet-induced obesity. Further, this may also suggest an alternative role for the well-known pre-meal surge in ghrelin. Rather than being a signal of meal initiation, this change in circulating ghrelin may trigger processes in the central nervous system that prepare the body to receive and appropriately process incoming nutrients (67).

Beyond its proposed role in short-term feeding control, ghrelin also fulfills established criteria for a hormone contributing to long-term body-weight regulation (72). First, circulating levels respond in a compensatory manner to bidirectional body-weight changes achieved by diverse means, increasing with weight loss and vice versa. Second, ghrelin influences neuronal activity through its receptor in several areas of the brain governing long-term energy homeostasis, including the hypothalamus (specifically arcuate NPY/AGRP neurons), caudal brainstem, and mesolimbic reward centers. The ghrelin receptor is also expressed by vagal-afferent nerves, which are inhibited by ghrelin (opposite to satiation factors) (54), although the importance of this for ghrelin-stimulated feeding is controversial. Third, chronic ghrelin administration increases body weight through numerous anabolic effects on food intake, energy expenditure, and fuel utilization. Finally, pharmacologic ghrelin blockade in adult animals decreases food intake and body weight, and mice lacking ghrelin signaling resist diet-induced obesity (77,78).

Chronic central ghrelin infusion resulted in increases in the glucose utilization rate of white and brown adipose tissue without affecting skeletal muscle. In white adipocytes, mRNA expression of various fat storage-promoting

enzymes such as lipoprotein lipase, acetyl-CoA carboxylase a, fatty acid synthase, and stearoyl-CoA desaturase-1 was markedly increased, while that of the rate-limiting step in fat oxidation, carnitine palmitoyl transferase-1 α , was decreased. In brown adipocytes, central ghrelin infusion resulted in lowered expression of the thermogenesis-related mitochondrial uncoupling proteins 1 and 3. These ghrelin effects were dose dependent, occurred independently from ghrelin-induced hyperphagia, and seemed to be mediated by the sympathetic nervous system. Additionally, the expression of some fat storage enzymes was decreased in ghrelin-deficient mice, which led us to conclude that central ghrelin is of physiological relevance in the control of cell metabolism in adipose tissue (77).

Environmental factors may modulate ghrelin's activity and have a role in obesity. Furthermore, constitutive activation of the growth hormone secretagogue receptor, polymorphisms in the gene for this receptor, and dysregulation of postprandial ghrelin suppression may contribute to the obese phenotype (80).

OBESTATIN

Recently, it was demonstrated that proghrelin also encodes another secreted peptide, termed obestatin due to its reported anorexigenic and BW-reducing effects (81). Obestatin is a 23-amino acid peptide that has been reported to require amidation to be biologically active. It was also reported to be the endogenous ligand of the G protein-coupled receptor, GPR-39, which belongs to the GH secretagogue receptor family (81). This previous orphan receptor is localized in multiple regions of the brain as well as in peripheral tissues (82). Obestatin has been reported to have actions opposite to ghrelin, such as decreasing food intake, BW, and delaying gastric emptying (81), and to antagonize the actions of ghrelin when both peptides are coadministered. However, obestatin did not alter GH secretion. More surprisingly, obestatin did not modify leptin serum levels, and circulating obestatin levels were not increased after fasting (81). Moreover, it has been suggested that obestatin readily crosses the blood-brain barrier but is rapidly degraded (83). Until now, all available data have suggested that obestatin is a new and relevant player in energy balance regulation, which could open up the possibility of targeting the GRP-39 receptor for the development of antiobesity drugs (84).

CHOLECYSTOKININ

CCK is the archetypal intestinal satiation peptide, first described as such three decades ago (12). It is produced by I cells in the duodenal and jejunal mucosa, as well as in the brain and enteric nervous system. Intestinal CCK is

secreted in response to luminal nutrients, especially lipids and proteins. The CCK prepropeptide is processed by endoproteolytic cleavage into at least six peptides, ranging from 8 to 83 amino acids in length (85). The multiple bioactive forms pertinent to feeding share a common carboxy-terminal octapeptide with an O-sulfated tyrosine. The major circulating moieties are CCK8, CCK22, CCK33, and CCK58, although recent evidence suggests that CCK58 might be the only relevant endocrine form in some species (86). CCK peptides interact with two receptors expressed in the gut and brain. CCK receptor 1 (CCK1R, formerly known as CCK-A, for "alimentary") predominates in the GI system, whereas CCK2R (formerly known as CCK-B, for "brain") predominates in the brain. Through endocrine and/or neural mechanisms, CCK regulates many GI functions, including satiation.

As is mentioned above, CCK-induced satiation could result in part from inhibition of gastric emptying, thereby augmenting gastric mechanoreceptor stimulation. Some vagal-afferent fibers respond synergistically to gastric distention and CCK (87), and subthreshold doses of CCK reduce food intake in monkeys if combined with gastric saline preloads (88). Similarly, gastric distention augments the anorectic effects of CCK8 in humans (89). These and other observations indicate that CCK causes satiation through mechanisms additional to enhancing gastric distention signals.

The obesity is fairly mild, however, and is not present in CCK1R-deficient mice (90); this is consistent with the proposed function of CCK as a short-acting satiation signal. CCK1R antagonists also increase meal size and food intake in experimental animals (91,92), and they increase hunger, meal size, and caloric intake in humans (93). Despite the role of CCK in terminating individual meals, its importance in long-term body-weight regulation and its potential as an antiobesity target are questionable. Chronic CCK administration in animals, with up to 20 peripheral injections per day, reduces meal size, but this is offset by increased meal frequency, leaving body weight unaffected (94). CCK administration decreases food intake acutely in humans by shortening meals (95), but anorectic effects dissipate after only 24 hours of continuous infusion. Not surprisingly, trials of CCK1R agonists as antiobesity therapeutics have been unsuccessful to date. The most important role for CCK in body-weight regulation might be its synergistic interaction with long-term adiposity signals, such as leptin (18,19).

The demonstration that supramaximal CCK58 did not induce pancreatitis in rats was the most recent example of a marked biological difference between CCK58 and shorter forms of CCK. Other unique roles for CCK58

compared with shorter forms (CCK8 or CCK33) have previously been demonstrated in the modulation of central catecholaminergic mechanisms and neuroendocrine functions (96), the pattern of gallbladder muscle contraction (97,98), and in intestinal afferent nerve discharge (99). More recently, it was shown that CCK58 inhibited food intake much more strongly than CCK8 by profoundly increasing the intermeal interval (100). These new and previously reported distinctive qualitative differences in biological actions between CCK58 and shorter forms of CCK indicate that future investigations of CCK biological functions should include use of the major endogenous form of the peptide, CCK58 (101).

Although most work implies that CCK1R mediates the control of food intake, a role for CCK2R in the inhibitory action of CCK on food intake cannot be totally excluded to date. Indeed, CCK2R is expressed in the vagus nerve-brain stem complex. Therefore, it is conceivable that it contributes to the relay of the satiety effect of peripheral CCK toward the hypothalamus (102,103). Moreover, the CCK2R is the predominant CCK receptor form found in the central nervous system, particularly in hypothalamic areas controlling body energy balance, and therefore, is an ideally positioned candidate for the mediation of the action of CCK (104,105,106).

CCK2 receptor inactivation in mice alters the regulation of body weight and food intake and leads to obesity with insulin and leptin resistance that are known to predispose to diabetic state.

An important issue of study by Clerc et al showed that genetic variants of CCK2 receptor could contribute to obesity susceptibility and pathogenesis of this complex disease. Their data give also strong support to a contribution of CCK2 receptors to the antiobesity effect of CCK. Therefore, they are important to take into account when designing synthetic antiobesity satiating drugs activating the CCK system. Indeed, they raise the hypothesis that activation of both CCK1 and CCK2 receptors, rather than specific targeting of CCK1 receptor may be necessary to achieve a full satiety effect (107).

PEPTIDE YY

The PP-fold peptide family includes neuropeptide Y (NPY), peptide YY (PYY), and pancreatic polypeptide (PP). All are produced as 36-amino acid, tyrosine-containing peptides and are recognized by a family of receptors. NPY, produced in the arcuate nucleus, is the most potent short-term stimulus for appetite. PYY is produced in enteroendocrine cells in the ileum and colon and is secreted after a meal, acting as an "ileal brake" to delay gastric emptying (4).

Polypeptide YY (PYY) is co-secreted with GLP-1 from L cells in the distal ileum. Previous investigation suggested that PYY acts through Y2 receptors to inhibit NPY/AgRP neurons and stimulate POMC neurons in the arcuate nucleus; however, a recent study demonstrates inhibition of both POMC and NPY neurons in the hypothalamus, calling into question the conventional understanding of hypothalamic integration of gut signaling (108). Like GLP-1, PYY is degraded by DPP-4, but the cleaved product, PYY3-36, also expresses biologic activity. PYY levels appear to be decreased following meals in obesity, and fasting levels are negatively correlated with BMI. These findings have not been consistent across studies, however, perhaps due to difference in assays for intact compared with total PYY (109,110). Conversely, PYY levels are increased in cachectic states and in preterm infants (111). Following Roux-en-Y gastric bypass, increased levels of postprandial PYY are thought to mediate continued weight loss and anorexia after surgery (112). Intravenous infusion of PYY reduces calorie intake in both lean and obese humans (113,114). Anorexigenic effects have been reproducible in human studies, but variable in animal models. PYY's role in glucose homeostasis is still unclear, though preliminary reports indicate that PYY1-36 acts to inhibit glucose-stimulated insulin secretion, whereas PYY3-36 improves insulin sensitivity (115). Pregnancy is one clinical situation when weight gain is expected, but extreme gains of weight pose increased health risks to both mother and infant. Interestingly, PYY levels were decreased at baseline and after meal ingestion in morbidly pregnant women who gained significantly more weight (>1.5 kg/week) during the second trimester of pregnancy compared with healthy-weight pregnant controls (116).

Fasting and postprandial endogenous plasma PYY levels were attenuated in obese humans and rodents. The PYY3-36 infusion study showed that the degree of plasma PYY reduction in obese subjects were likely associated with decreased satiety and relatively increased food intake. Thus, obese subjects have a PYY deficiency that would reduce satiety and could thus reinforce their obesity (109).

These findings lend some support for the association between PYY and obesity that could lead to possible new therapeutic options in obesity. PYY exerts anorexigenic effects; it is possible that surgical weight-loss procedures work synergistically with PYY to promote weight loss. Further investigation is needed to clarify whether PYY actually causes reduced calorie intake or whether the rate of food delivery to the ileo-colonic segment influences PYY levels, thus affecting satiety (117).

Peptide YY plays a role in the integrative regulation of metabolism. The emerging hedonic effects of peptide YY

together with the weight-reducing effects observed in obese rodents suggest that targeting the peptide YY system may offer a therapeutic strategy for obesity (118, 119, 120).

GLUCAGON – LIKE PEPTIDE – 1

Glucagon-like peptide-1 (GLP-1) is cleaved from proglucagon by prohormone convertase-1 and is released from the L cells of the intestine in response to carbohydrates, lipids, and neurohormonal stimulation from the proximal small intestine. GLP-1 possesses two biologically active forms: GLP-17-37 and GLP-17-36; the latter is the major form in humans (121). The half-life is relatively short, lasting only 2 min, due to rapid degradation by dipeptidyl peptidase 4 (DPP4) (122). GLP-1 effects on satiety include appetite suppression, increased energy expenditure, and decreased gastric and intestinal motility via an 'ileal brake' (8). GLP-1 is also an incretin, with effects on postprandial glucose homeostasis. Incretins are hormones that enhance insulin release in response to oral compared with intravenous glucose (123). Contributions to glucose homeostasis occur through postprandial inhibition of alpha cells, and increased expression of sulfonylurea receptors and inwardly rectifying K⁺ channels on beta cells. Insulin gene transcription and mRNA stability are promoted, and beta-cell neogenesis occurs from ductal precursor cells while beta-cell apoptosis is inhibited (124). Patients with T2DM have lower baseline levels of GLP-1 and demonstrate blunted secretion of GLP-1 90-150 min postprandially (125). GLP-1 receptor knockout mouse models and GLP-1R antagonist (exendrin9-39) administration result in impaired glucose tolerance and decreased insulin secretion in response to a glucose load (126,127). A randomized cross-over study showed exercise induced increases in GLP-1 and pancreatic polypeptide and clinical anorexia that persisted after exercise, indicating that exercise-mediated effects on weight loss may be mediated by gut-hormone regulation in addition to calorie expenditure (128). Fasting GLP-1 levels are decreased in obesity, but increase after weight reduction (129). Clinically, pharmacotherapy utilizing GLP-1-related glucohomeostatic effects has offered novel therapies for type 2 diabetes mellitus and obesity. GLP-1 analogues include injectable exendin-4 and a number of orally administered DPP4 inhibitors. Patients treated with exendin demonstrate improved glucose homeostasis, as well as decrease in body weight (124).

The GLP-1 (7-36) amide has a dual function. First, it binds to the known GLP-1R on the β -cell and stimulates insulin release. Increased insulin levels result in wellknown metabolic effects, including reduction of NEFAs, suppression of Ra and decreases in glucagon levels. GLP-1 (7-36) amide is then cleaved by DPP-4, rendering GLP-1

(9-36) amide. This peptide then binds to a second receptor (GLP-1mR) and initiates a series of events that result in further reductions in Ra, independent of plasma glucose levels and the actions of insulin. In the postabsorptive state glucose oxidation is reduced and NEFA oxidation is activated, even in lean subjects. Because the effects of GLP-1m were indeed more pronounced in the obese state we can speculate that GLP-1m is acting in some manner to suppress fatty acid oxidation and thereby inhibiting production of glucose by the liver, through effects on the glucose–fatty acid oxidation cycle (130).

An analogy for different actions of GLP-1 and GLP-1m with respect to action and affinity to receptor subtype is the well described physiological functions of peptide YY(1–36) (PYY (1–36)) and the second biologically active form of PYY, PYY(3–36), which is formed by the removal of amino-terminal dipeptide Tyr-Pro by DPP-4 (131). PYY (3–36) has different biological action than intact PYY (1–36), mediated by different receptors (132).

Exendin (Ex)-4 is an agonist of the glucagon-like peptide (GLP)-1 receptor (GLP-1R) that has anorexigenic and fat-reducing properties. Ex-4 reduces the levels of ghrelin by up to 74% in fasted rats. These effects are dose dependent and long lasting (up to 8 h), and they can be detected after both central and peripheral administration of Ex-4. Suppression of ghrelin was neither mimicked by GLP-1(7–36)-NH₂ nor blocked by the GLP-1R antagonist Ex-(9–39). Moreover, it was independent of the levels of leptin and insulin. The decrease in ghrelin levels induced by Ex-4 may explain the reduced food intake in fasted rats, justifying the more potent anorexigenic effects of Ex-4 when compared with GLP-1. As well as the potential benefits of Ex-4 in type 2 diabetes (133).

GLP-1 is emerging as a regulatory factor with a broad range of actions related to substrate and energy metabolism. With the recent development of medications based on GLP-1R signaling for diabetes treatment, these new findings suggest the promise of further application of this system for the treatment of other conditions such as obesity and cardiovascular disease (134).

GLUCOSE – DEPENDENT INSULINOTROPIC POLYPEPTIDE

Glucose-dependent insulintropic polypeptide (GIP) is a 42 amino-acid peptide released from K cells in the upper small intestine in response to nutrients, especially fats. GIP effects are mediated by the GIP receptor GIPR, which is a G-protein-coupled receptor of the glucagon superfamily and widely expressed in many tissues (pancreas, stomach, small intestine, adipose, adrenal cortex, lung, pituitary,

heart, testes, vascular endothelium, bone and brain). While GIP does not appear to play a major role in satiation, it is a key player in glucose homeostasis. GIP acts as an incretin, but its action is decreased in T2DM and levels are increased in obesity. GIP receptor knockout mice are protected from high-fat diet-induced obesity. In ob/ob mice, GIP receptor antagonists improve insulin sensitivity and correct islet-cell hypertrophy (124,135).

GIP has not been reported to have an acute influence on food intake. However, GIP-receptor-knockout mice are resistant to obesity when fed a high-fat diet. The reason for this resistance is unclear and there has been speculation that it might reflect a direct effect on adipocytes rather than on central appetite-regulating circuits (136,137).

Recent studies support the hypothesis that, in addition to its principal role as an incretin, GIP/GIPR signaling may represent an important etiologic factor in the pathogenesis of obesity. Overnutrition composed of high-fat and high glucose appears to lead to enhanced synthesis and secretion of GIP from the small intestine, which would lead not only to increased insulin secretion, but also to increases in adipocyte nutrient uptake by both insulin and GIP, resulting in obesity. Eventually, insulin resistance would occur and subsequently lead to hyperinsulinemia and subsequently to the development of T2DM, a vicious cycle that would continue until broken. A thorough understanding of the pathways regulated by GIP will enable us to break this vicious cycle and potentially enable the development of methods for preventing and treating obesity (138).

OXYNTOMODULIN

Oxyntomodulin (OXM) is a proglucagon-derived hormone of 37 amino acids released from enteroendocrine L cells in response to nutrients, especially fats. A definitive receptor for oxyntomodulin has not yet been identified; however, evidence suggests that oxyntomodulin acts at the GLP-1 receptor. GLP-1 knockout mice and GLP-1 receptor antagonists cause diminished response to oxyntomodulin administration (3, 139) When administered to obese subjects, human oxyntomodulin suppresses appetite and energy intake immediately after administration and up to 12 h later (140). In obese subjects, administration of human oxyntomodulin contributes to increased energy expenditure measured by combined heart rate and movement monitoring in home environments (141).

Study of Wynne et al suggests that energy expenditure and energy intake may be coupled in humans and demonstrates a possible mechanism of weight-loss during oxyntomodulin administration (141).

Recent studies have shown that chronic peripheral administration of oxyntomodulin to rats reduces both

food intake and weight gain. Studies in human subjects have demonstrated that acute administration reduces food intake by 19%. When given preprandially by subcutaneous injection three times a day, oxyntomodulin results in a reduction in food intake and mean weight loss of 2.8 kg over 4 weeks. Oxyntomodulin thus represents a potential therapy for obesity (142).

Although the therapeutic potential of oxyntomodulin requires further investigation, this data supports the role of oxyntomodulin as a potential anti-obesity therapy (141).

BOMBESIN / BOMBESIN – RELATED PEPTIDE

GRP, a peptide produced by endocrine cells in the gastric mucosa, is the mammalian homologue of a peptide (bombesin) first isolated from glands in the skin of amphibians (143). Mammalian bombesin-related peptides, gastrin-releasing peptide and neuromedin B actions are mediated by two receptors (BB1-receptor, BB2-receptor), which are closely related to the orphan receptor BRS-3 (BB3-receptor) (148). GRP not only regulates secretion of gastrin, but its peripheral administration (and that of bombesin) inhibits food intake in animals (144), and its intravenous infusion reduces appetite and food intake in humans (145). Because GRP/bombesin also potentially delays gastric emptying (146), the extent to which these effects on GI motility contribute to the reduction of food intake is uncertain. However, intracerebroventricular injection of bombesin at a dose that has no peripheral effects decreases the size of meals in free-feeding baboons, suggesting that GRP-related peptides have a role in the central regulation of food intake (147).

ENTEROSTATIN

Enterostatin is a pentapeptide cleaved from procolipase released by the exocrine pancreas. Peripheral and central administration reduce food intake in rats and alter food preference away from fatty foodstuffs, but in humans, recent metabolic studies have failed to show an effect of oral enterostatin on food intake, appetite, energy expenditure, or body weight (32).

APOLIPOPROTEIN AIV

This is a glycoprotein synthesized mainly in the small intestine, and its release is stimulated by fat intake. ICV and intravenous infusions of chylous lymph containing apolipoprotein A-IV (apo A-IV) in rodents have been shown to reduce food intake (149,150). It may have a role in longterm energy balance as both insulin and leptin alter the expression of apo A-IV (151).

OLEOYLETHANOLAMIDE

Oleoylethanolamide (OEA) is a naturally occurring lipid mediator that inhibits food intake and body weight gain and therefore has been a molecule of recent intense scientific interest in the search for therapeutic strategies for the treatment of human obesity (152,153).

OEA is synthesized in brain, adipocytes, and the small intestine and is structurally similar to the endogenous cannabinoid anandamide (arachidonylethanolamide), but it does not bind to or activate cannabinoid receptors. Rather, pharmacological and molecular biological experiments have demonstrated that OEA induces satiety (154) and reduces body weight gain in mice and rats (153) through activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)- α (152). This ligand-activated transcription factor is abundantly expressed in liver and intestine and is known to play a pivotal role in the regulation of lipid homeostasis (155,156). Intestinal OEA has been shown to rise after a meal, suggesting that there is a fairly rapid feedback signal from the gut to the brain, promoting inhibition of further feeding after a meal is consumed. This OEA-mediated event is believed to involve the activation of intestinal PPAR- α and signaling via vagal sensory fibers to the brain stem (157). OEA also has been reported to alter serum triglyceride levels, suggesting a possible participation of OEA in the control of energy expenditure and accumulation. However, the effect of OEA on the small intestinal function is not well described (153).

The fact that OEA reduces food intake and body weight while it increases enterocyte fatty acid uptake suggests that OEA-mediated enterocyte fatty acid uptake may be due to decreased food intake and body weight. The increases of the fatty acid uptake by OEA would enhance the utilization of nutrients in the small intestine. These results suggest that OEA may contribute to the peripheral regulation of feeding and that FAT/CD36 plays an important role in OEA-mediated fatty acid uptake. Yang et al findings support evidence that FAT/CD36 may play an important role in OEA-mediated reduction of food intake and body weight control (158).

INSULIN

Insulin action is peripherally adipogenic but centrally, insulin exerts appetite-suppressive effects. Insulin receptors are found in multiple parts of the brain and are particularly concentrated in the hypothalamus, hippocampus and cortex (159). Insulin crosses the blood-brain barrier and, like leptin, acts via phosphatidylinositol-3 kinase pathways (160,161). Direct administration of insulin into the brain suppresses eating and inhibits hepatic gluconeogenesis

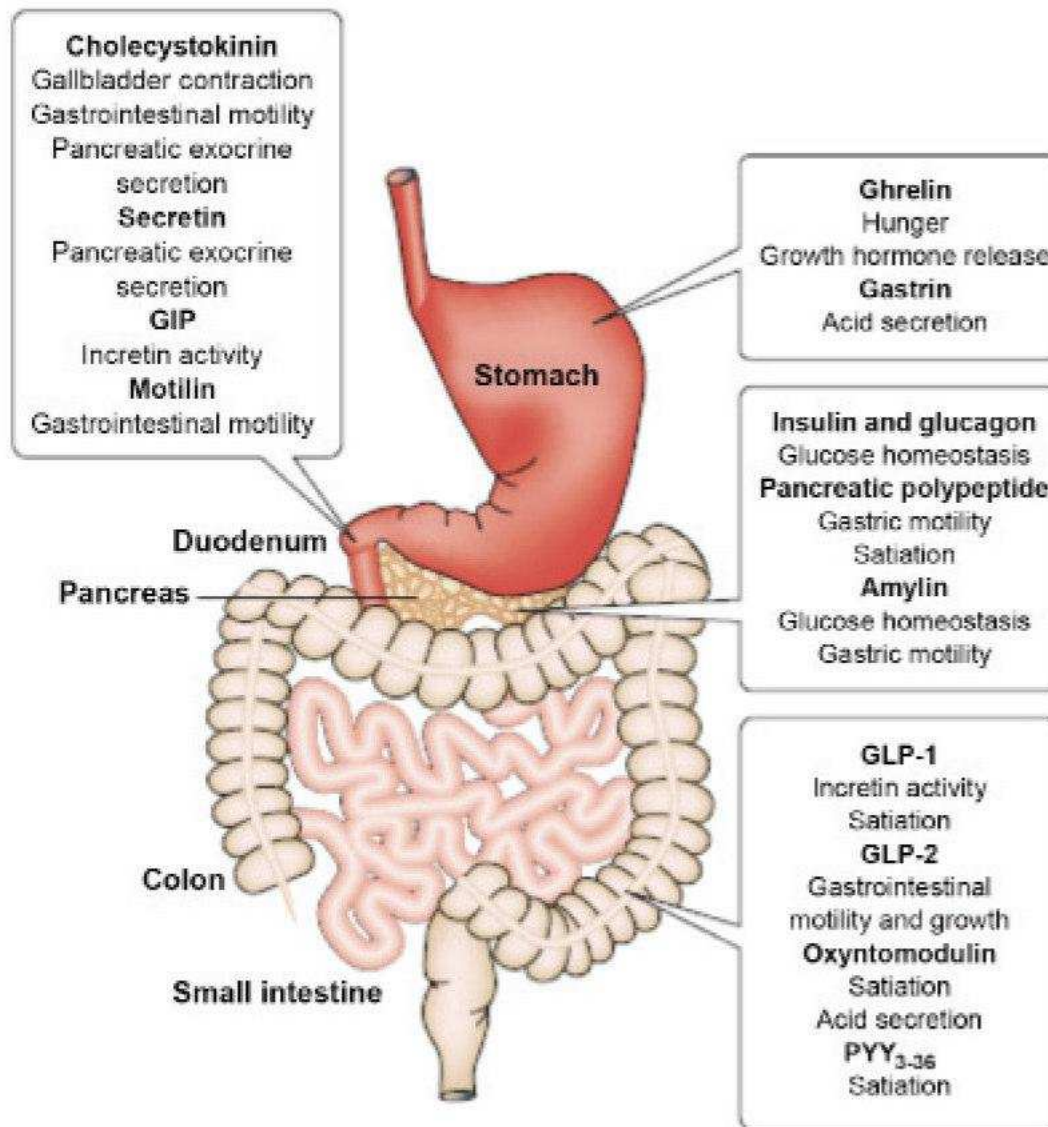


Fig.2. A schematic diagram of the gastrointestinal tract illustrating where particular gut hormones are concentrated and their major putative functions (Adapted with permission from Murphy KG, et al).

in rodents (18,162). In female mice only, the selective deletion of central nervous system insulin receptors results in hyperphagia, obesity and peripheral insulin resistance (163).

PANCREATIC POLYPEPTIDE

Pancreatic polypeptide is a member of the PP fold peptide family that is synthesized and released from the endocrine pancreas. Pancreatic polypeptide shows preferential binding to the Y4 and Y5 receptors. Similarly to PYY₃₋₃₆, it is released after a meal and reduces appetite. Acute peripheral administration to mice and humans reduces

food intake (35,37), and chronic administration reduces body weight in ob/ob obese mice (58). No nausea or gastrointestinal distress was reported in the intravenous study investigating feeding effects in humans. The anorectic effects of pancreatic polypeptide might occur partly as a result of delayed gastric emptying (37,164). The food intake and fat mass of transgenic mice overexpressing pancreatic polypeptide are reduced, but such mice also show reduced gastric emptying (35). However, at present the physiological role of pancreatic polypeptide in energy homeostasis is unknown.

AMYLIN

Amylin, which is also known as islet amyloid polypeptide, is a 37-residue member of the calcitonin peptide family that is released together with insulin from pancreatic β -cells in response to food ingestion. Although its main function is thought to be in glucose homeostasis, given peripherally at supraphysiological levels amylin can reduce food intake. Administration of the amylin agonist pramlintide reduces body weight in type 1 and 2 diabetics by between 0.5 and 1.4 kg for up to 1 year (136,165).

LEPTIN

Leptin is released from adipocytes in direct proportion to total fat mass (166) and is actively transported into the central nervous system. There leptin exerts anorexigenic effects and increases sympathetic tone and energy expenditure (167). Studies of both peripheral and central administration of leptin demonstrate reduction of the volume of food ingested at meals but not the number of meals consumed (168). To date, six alternatively spliced isoforms of the leptin receptor (OB-Ra-f) have been described. Ob-Rb is the principal signal transducer and is highly expressed in hypothalamic and immune cells. It acts via JAK-STAT pathways (169). Leptin is both a 'long-term' signal of energy stores and a short-term modulator of meal termination. It directly depolarizes (stimulates) arcuate anorexigenic POMC neurons, reduces

GABAergic inhibition of POMC, and stimulates cocaine and amphetamine-related transcript in the lateral arcuate nucleus. Concomitantly, leptin hyperpolarizes (inhibits) orexigenic NPY/AgRP neurons. Steroidogenic factor-1 (SF-1)-containing neurons in the ventral medial hypothalamus are necessary for leptin induction of anorexigenic tone (170).

Leptin's influence on food intake and its role in synaptic remodeling are mTOR dependent (171). AMP-activated protein kinase (AMPK), like mTOR, is regulated by the ratio of intracellular AMP to ATP. Its concentrations increase during energy deficit and are inhibited by leptin and various nutrients; AMPK exerts negative feedback on mTOR function (171). During fasting, declining leptin concentrations mediate declines of thyroid and reproductive hormones, sympathetic tone, and thermogenesis; appetite rises and increased energy reserves become available for immune defense. Concentrations of circulating leptin and leptin-secretion rates are proportionate to adiposity, but fasting and feeding alter serum leptin concentrations over a period of hours independent of body fat (166).

Physiologic concentrations of leptin have been shown to stimulate expression of CRP in human hepatocytes. As CRP is associated with increased adiposity and leptin, this inflammatory cytokine may contribute significantly to 'leptin resistance' in obese individuals (172).

Nutritional Influences on Gut Hormone Release

Previous work on cholecystokinin suggested that there is a gut nutrient sensor that signals to appetite centres in the brain to reduce appetite after meals. Recent work has identified the anorexigenic gut hormones PYY, oxyntomodulin and pancreatic polypeptide, and the orexigenic peptide ghrelin, which are all potential targets in weight loss management. Short-term studies infusing these peptides have shown their efficacy in altering appetite and food intake (173).

Understanding how food, nutrients and eating habits influence the pattern of gut hormone release, and whether this in turn is associated with changes in appetite and food intake may help to improve the efficacy of dietary interventions for weight loss (173).

Carbohydrate-rich meals suppress ghrelin, which returns to basal concentrations in the late postprandial period (174). A simple carbohydrate meal caused a larger reduction (41%) in ghrelin compared with a complex carbohydrate meal (33%), supporting glucose as the most

potent ghrelin suppressant (175). Also, a high-carbohydrate meal was more effective in suppressing ghrelin over a 3-h period than a high-fat meal (176). Weight loss achieved with a high-carbohydrate, low-fat diet prevented the increase in ghrelin that usually occurs after energy restriction (177). The mechanisms behind these effects are unknown.

Lipids or high-fat diets are less effective than glucose in suppressing ghrelin (176), but nevertheless cause a significant late-phase postprandial reduction (174,178). In long-term feeding studies, it is difficult to separate the effect of fat intake per se from that of weight gain on ghrelin levels.

Oral glucose produces a greater GLP-1 and insulin response than fructose; fructose administration one hour after glucose elucidates no additional GLP-1 release. The increased GLP-1 release after glucose does not translate to a difference in appetite or food intake (179).

MUFA-rich meals stimulate greater GLP-1 release than SFAs; MUFAs were associated with lower triacylglycerol concentrations and higher high-density lipoproteins than SFAs. This suggests a relationship between fatty acid composition, incretin responses and triacylglycerol metabolism. Oral fat feeding of MUFAs produced a greater GLP-1 release and higher non-esterified fatty acid concentrations than either PUFAs or SFAs (180).

Studies administering lipase inhibitors with oral (181,182) or intraduodenal (183) fat show a reduction or abolishment of GLP-1 release. Oral administration was associated with faster gastric emptying and higher glucose and insulin concentrations.

Several studies on monogastric mammals have demonstrated that the postprandial release of PYY is dependent on the caloric density and meal composition (184). It has long been accepted that PYY increases postprandially with increasing energy intake, reaching a plateau within 1–2 h (185). Water does not stimulate PYY release compared with liquid meals of carbohydrate, fat or protein (184), suggesting that gastric distension does not influence the plasma level of PYY and that nutrient intake is essential to release PYY. Fat was the most potent stimulus of plasma PYY in animals (186) and man (185).

Plasma cholecystokinin rises promptly after a mixed meal and can remain elevated for several hours postprandially (187). Frolehlich et al. (188) found that a meal pure in fat caused a higher cholecystokinin release than a fat-free or mixed meal. It is free fatty acids rather than triglycerides per se that are believed to trigger cholecystokinin secretion, because lipase inhibition prevents the usual cholecystokinin response after fat ingestion (189,190,191). Interestingly, cholecystokinin release appears to be sensitive to the fatty acid chain length.

In contrast to fat and protein, carbohydrate does not greatly influence cholecystokinin secretion. Glucose caused a small and transient elevation of plasma cholecystokinin in humans (187), whereas starch had no significant effect (192).

Study of Feile-Bisset et al demonstrates that, in healthy adults, 1) proximal small intestinal exposure to nutrients, in this case long-chain triglycerides, is sufficient to suppress ghrelin secretion; 2) fat digestion is required for the suppression of ghrelin and stimulation of PYY and PP secretion; and 3) the effects of small intestinal fat on

ghrelin and PYY secretion occur progressively, indicative of modulation by mechanisms that depend on the nutrient load and length of intestine exposed to nutrient (193).

The presence of fat in the small intestine slows gastric emptying, stimulates the release of many gastrointestinal hormones, and suppresses appetite and energy intake as a result of the digestion of fats into free fatty acids; the effects of free fatty acids are, in turn, dependent on their chain length. Given these effects of fat, it is paradoxical that high dietary fat intakes have been linked to increased energy intake and body weight and are considered to play a significant role in the pathogenesis of obesity. However, increasing evidence indicates that a chronic increase in dietary fat is associated with an attenuation of the feedback signals arising from the small intestine induced by fat, with a consequent relative acceleration of gastric emptying, modulation of gastrointestinal hormone secretion, and attenuation of the suppression of energy intake (194).

Obesity can, in the broadest sense, be considered to be the result of an energy intake that exceeds energy expenditure. Signals arising from the gastrointestinal tract play a fundamental role in the regulation of appetite and energy intake, and evidence indicates that the gastrointestinal and hormonal mechanisms involved in the suppression of appetite and energy intake are compromised in obesity (195,196). Hence, obesity may, at least in part, reflect a decreased sensitivity to the gastrointestinal effects of nutrients, particularly in the face of excessive calorie intake. Studies in animals and, to a much more limited extent in humans, indicate that consumption of a high-fat diet has the capacity to modulate the gastrointestinal responses to ingested fat and, thereby, leads to impairments in appetite regulation that favor the development of obesity. A number of factors potentially involved in the regulation of energy intake, including gastric emptying (197,198), intestinal transit (197,199), gastropyloroduodenal motility (200), and the secretion and/or action of gastrointestinal peptides, including ghrelin, CCK, GLP-1, and PYY (201,202,203,204) are altered by a high-fat diet. It is likely that the signals arising from the distal small intestine, as evidenced by the impairment of PYY secretion, and the efficacy of Roux-en-Y gastric bypass, play an important role in the regulation of appetite and energy intake and are of pathogenic relevance to the development of obesity (194).

Taste Receptors in Gastrointestinal Tract

Chemosensing of potentially beneficial or harmful substances in the lumen by the gastrointestinal mucosa triggers various gastrointestinal functions including secretion, motility and regulation of blood flow that are essential for digestion and absorption of nutrients, but that are also important for the initiation of protective responses, including vomiting and food aversive behaviour, through the activation of hormonal and neuronal pathways (205).

The cellular and neural pathways that mediate the biological responses to luminal molecules are still poorly understood. Recent evidence supports a role for members of the Gprotein-coupled receptor (GPCR) superfamily as sensors of luminal contents which, upon stimulation, initiate functional responses through the activation of G protein signaling cascades. The background for this hypothesis resides in the identification of the molecular transduction mechanisms operating in the mouth. Nutrients

and nonnutrients are initially detected in the mouth by interacting with different transduction elements, including ion channels, ligand-gated channels, transporters and GPCRs that are expressed in the apical membranes of specialized epithelial cells, known as taste receptor cells. These cells, localized in taste buds of the oral epithelium, transmit taste information to the brain as changes in neural activity through afferent gustatory fibers (206). This detection process might influence ingestive behavior by stimulating secretions that facilitate digestion and absorption or by inducing a behavior that protects from potentially harmful substances. The sensors detecting sweet and bitter sensations and amino acids have now been identified and characterized as unrelated GPCR families—the T1R and T2R taste receptors families (207,208,209). The T1R family comprises three distinct members that heterodimerize to sense sweetness (T1R2 and T1R3) and

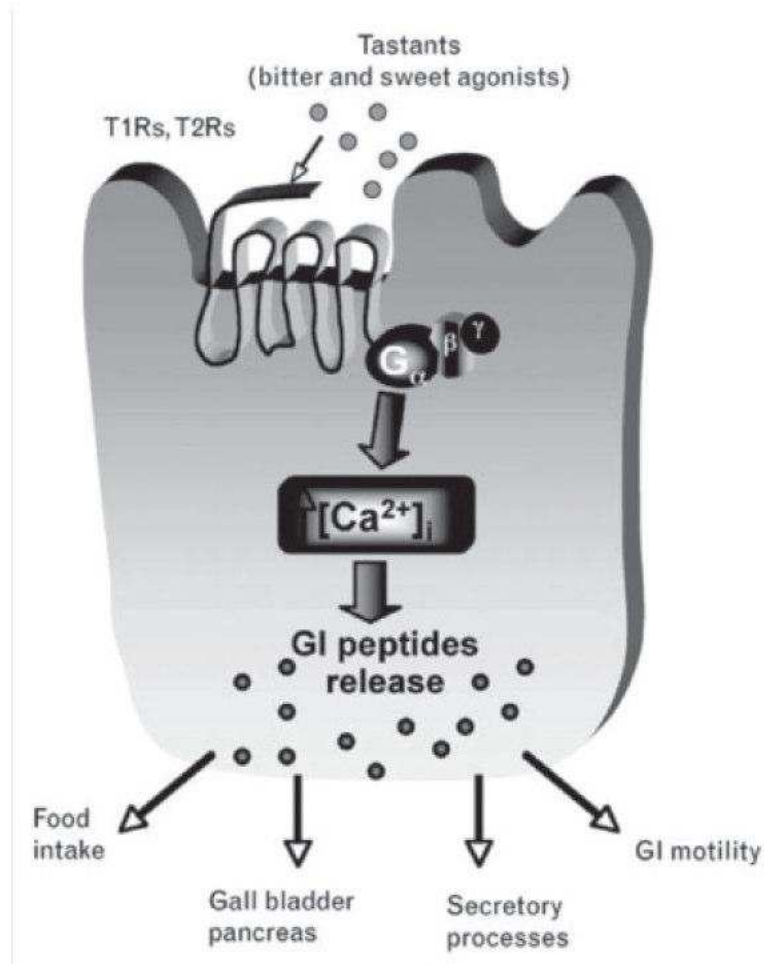


Fig. 3. Postulated mechanism involving sweet (T1R) and bitter (T2R) taste receptors on enteroendocrine cells (Adapted with permission from Sternini C *et al.*)

amino acids (T1R1 and T1R3), whereas the T2R family includes numerous divergent GPCRs that act as broadly tuned bitter sensors (207,210). Taste receptors interact with specific G α subunits, including α -gustducin, a taste-specific signaling protein with a prominent role in bitter taste (211,212).

Other transducers associated with taste signaling include phospholipase C β 2 and transient receptor potential channel type 5, a calcium-activated cation channel (210). Several of these proteins, including α -gustducin, are also involved in sweet-taste transduction (206,210).

Some of the key molecules implicated in taste signaling are expressed in the gastrointestinal mucosa and enteroendocrine cell lines, providing strong support to the hypothesis that transduction mechanisms identified in the oral epithelium also operate in the gut. Multiple T2R transcripts have been detected in the mucosa of the mouse and rat gastrointestinal tract and human colon, some of which have known ligands, like mT2R138 and hT2R38, the phenylthiocarbamide receptor, and mT2R108 and hT2R47, the denatonium benzoate receptor (213,214,215). The sweet taste receptors, T1R2 and T1R3, have also been described

in rodent and human intestine (216,217,218). In addition, transcripts for α -gustducin have been reported in rat and mouse gastrointestinal mucosa (215,216,217,218,219,220). Immunoreactivity for α -gustducin or α -transducin 2 has been localized to the mucosa of mouse stomach and duodenum (215), and α -gustducin cells have been observed in the rat intestinal mucosa and pancreatic duct (221,222), and in human gastric and intestinal mucosa (213). Finally, co-expression of α -gustducin with T1R2 and T1R3 has been described in the mouse duodenum, further supporting the role of this G protein in taste sensation (218).

The recognition that several GPCRs and G proteins that respond to bitter and sweet compounds and amino acids are expressed by gastrointestinal epithelial cells has provided strong support for the concept that the gastrointestinal mucosa lining is equipped with a chemosensory machinery that resembles the one operating in the lingual epithelium. GPCRs are envisaged to function as sensors, which couple to G proteins upon activation by distinct luminal contents, and through second messengers and ion channels lead to intracellular Ca $^{2+}$ increase, the key signal that triggers transmitter release (Fig. 3) (205).

Gut Hormones: The Future of Obesity Therapy

Obesity is a multifactorial disorder that has reached epidemic proportions and represents a major public health burden: Pharmaceutical intervention is one method to attempt to combat increasing incidence of the disease. However, relatively few antiobesity medications are available internationally with only four approved: (i) phentermine, an anorectic catecholamine stimulant; (ii) sibutramine, a serotonin and noradrenaline reuptake inhibitor; (iii) orlistat, a lipase inhibitor; and (iv) rimonabant, a recently approved CB-1 receptor antagonist. Three agents target central hypothalamic systems (i.e. phentermine, sibutramine and rimonabant) and the third targets non-systemic pancreatic and gastric lipases (i.e. orlistat) (223).

Clinical effects of these drugs confer modest improvements, and side effects negatively impact long-term treatment course. This paper suggests single target pharmacological interventions are possibly hampered by the myriad of alternate orexigenic peptidic signals that drive hyperphagia, hence a multiple target model or combination treatment approach is proposed to offer greater therapeutic potential in modulating appetite and managing weight.

Appetite and satiety are mediated by complex neuroendocrine signalling pathways involving over

30 hormones, neuropeptides, enzymes, other chemical messengers and their receptors. Research efforts continue to expand understanding of the role of signalling molecules between central hypothalamic nuclei and peripheral enteroendocrine cells; and discoveries of novel networks and messengers provide new biological insights on how to manipulate appetite-satiety pathways.

Anti-obesity agents are challenged by limitations of efficacy and serious side effects that have previously halted drug development and market availability. Current drugs lead to modest weight loss, and our lack of understanding why anti-obesity drugs are not optimized in vivo provokes scientific enquiry to determine the factors that contribute to or cause this phenomenon, whether they are biological, social, or environmental in origin.

Anti-obesity drug mechanisms targeting central hypothalamic neurotransmitter systems and peripheral metabolic peptides are conceivably countered or attenuated by the numerous orexigenic peptidic signalling cascades and activities of sympathetic neural pathways that drive hyperphagia. In this scenario, attempts to alter the body's native central or peripheral orexigenic signalling biochemistry is hypothesized to result in compensatory

orexigenic pathways relative to the needs for energy. Hence, anti-obesity drugs may lead to a nominal drug effect because alternate orexigenic signalling mechanisms and/or adaptive responses in the brain-gut axis continue along normalized or up-regulated pathways, which override attempts to pharmacologically influence the appetitive control centres. This effect can be partially explained by CCK-PYY activation, which reduces food intake even when melanocortin activities are disrupted (113,224,225), suggesting other mechanisms or systems are operating simultaneously during attempts to modulate one discrete pathway (albeit anorexigenic in this case). Consequential effects of centrally or peripherally acting obesity agents thus result in suboptimal clinical improvements, which are evident in clinical trials (226,227).

Recent developments in unraveling the intricate physiology of appetite and energy expenditure have opened up the possibilities of new pharmacological treatments. Factors that control meal initiation, frequency and termination influence overall energy intake. An important group of these factors are gut hormones released from the gastrointestinal tract in response to changes in the nutritional state. These hormones influence central mechanisms involved in the regulation of energy balance, through a range of bloodborne and neural pathways (139).

The gut hormones are attractive targets for obesity therapy, because unlike indiscriminate chemical agents, they target only the relevant appetite control systems in the brain. In order to maintain permanent weight loss, patients may require life-long treatment and side-effects may be less likely to occur during prolonged treatment with gut hormones, which are endogenously produced daily in normal individuals, compared with non-specific neurotransmitter mimetics or antagonists. Furthermore, inappropriate gut hormone release or signalling may itself contribute to the pathophysiology of obesity. If this is the case, then correcting it by the administration of exogenous gut hormones or their antagonists would be appropriate (6).

The combination of a reduction in food intake and enhanced insulin release makes GLP-1 an attractive target for the treatment of type 2 diabetes and obesity. The therapeutic potential of GLP-1 is limited by its rapid breakdown in the circulation. The GLP-1 receptor agonist exendin-4/exenatide is resistant to enzymatic cleavage, and has recently been approved for the treatment of type 2 diabetes. The long-acting GLP-1 derivative liraglutide is, however, also showing promise by improving glycaemic control and reducing bodyweight in clinical trials (6).

Oxyntomodulin is not as potent an incretin as GLP-1, and therefore may be a better target for the treatment of obesity in non-diabetic individuals. It has recently been shown that the subcutaneous administration of oxyntomodulin three times a day for 4 weeks led to a fourfold increase in weight loss compared with control (7).

The predominant circulating form, PYY3-36, is also a candidate for an antiobesity agent. PYY3-36 dose-dependently reduces food intake in rodents and humans (113). Importantly, obese humans are not resistant to the anorexigenic effects of PYY3-36 (114). Natch recently announced further phase I clinical trials of a PYY3-36 nasal spray. Results from previous phase I trials have shown a significant and sustained caloric reduction after PYY3-36 administration.

There is currently great interest in the possibility of using ghrelin antagonists as anti-obesity agents. Other gut hormone systems exist that might be clinically exploited to tackle obesity. Cholecystokinin was the first gut hormone shown to inhibit feeding after exogenous administration in rodents (228). Its usefulness as a therapeutic agent is, however, limited by a short half-life, and its ineffectiveness in reducing food intake when given more than 30 min before a meal (6).

The gut satiety response to nutrient ingestion is mediated by the coordinated response of multiple hormones. This raises the possibility that the administration of more than one gut hormone may result in an increased anorectic response. A recent exciting advance was the demonstration that the co-administration of PYY3-36 and the GLP-1 receptor agonist, exendin-4, to mice resulted in a greater reduction in food intake than either peptide alone at the same dose (229). Furthermore, the co-administration of submaximal doses of PYY3-36 and GLP-17-36 additively inhibited food intake in healthy human volunteers (230). The co-administration of lower doses of gut hormones may result in a reduction in the side-effect profile, making it an attractive strategy for the treatment of obesity (6).

Strategies to optimize existing drug therapy may involve combination drug and therapy approaches, but clinical trials are needed to determine the safety and efficacy of new combinations, with long-term anti-obesity treatment goals in mind. Novel drugs with novel mechanisms may provide new solutions to the problem of obesity, but lengthy drug development processes will be challenging and it may be several years until new drugs are approved by national medicines agencies (223).

Conclusions

A new understanding of the role of the gut in obesity and energy balance has recently developed. The list of peptide hormones emanating from the gastrointestinal system and influencing energy balance continues to grow, and it seems likely that additional gut hormones will be identified (4). By furthering our knowledge it is hoped it will open up novel therapeutic avenues for the treatment of obesity (6).

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