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The Importance of the Gastrointestinal Tract in Controlling Food Intake and Regulating Energy Balance

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Abstract
The gastrointestinal (GI) tract, the key interface between ingested nutrients and the body, plays a critical role in regulating energy homeostasis. Gut-derived signals convey information regarding incoming nutrients to the brain, initiating changes in eating behavior and energy expenditure, to maintain energy balance. Here we review hormonal, neural and nutrient signals emanating from the GI tract and evidence for their role in controlling feeding behavior. Mechanistic studies that have utilized pharmacological and/or transgenic approaches targeting an individual hormone/mediator have yielded somewhat disappointing bodyweight changes, often leading to the hormone/mediator in question being dismissed as a potential obesity therapy. However, the recent finding of sustained weight-reduction in response to systemic administration of a long-acting analog of the gut-hormone glucagon-like peptide-1 (GLP-1) highlights the therapeutic potential of gut-derived signals acting via non-physiological mechanisms. Thus, we also review therapeutics strategies being utilized or developed to leverage GI signals in order to treat obesity.

Keywords (3-4)
Enteroendocrine cells, obesity, gastrointestinal peptides

Abbreviations used: Apo A-IV, Apolipoprotein A-IV; BA, bile acids; CB1R, cannabinoid receptor 1 receptor CB1R; CART, CCK, cholecystokinin; CCK-1R, CCK-1 receptor; CNS, central nervous system; DAG, des-acyl ghrelin; DIO, diet-induced obesity; DPP-4, dipeptidyl peptidase 4; EEC, Enteroendocrine cell; ENS, enteric nervous system; FFAR, free fatty acid receptor; FTO, fat mass and obesity-associated; fMRI, functional magnetic resonance imaging; GI, gastrointestinal tract; GIP, glucose-dependent insulinotropic polypeptide, GLP-1, glucagon-like peptide-1; GCGR, glucagon receptor; GOAT, ghrelin-O-acyl transferase; GN, guanylin; GUC2C, guanylate cyclase 2C; GPCR, G protein-coupled receptor; GSR1a, growth hormone secretagogue receptor type 1a; IP, intra-peritoneal; MC4R, melanocortin-4 receptor; NT, neuropeptide; OEA; oleoylethanolamide; OXM, oxyntomodulin; PP, pancreatic polypeptide; PYY, peptide YY; PWS, Prader-Willi syndrome, RYGB, Roux-en-Y gastric bypass; T1R, Taste receptor 1; T2R, Taste receptor 2; T2D, type 2 diabetes; SCFA, short chain fatty acid; UGN, uroguanylin; YR, neuropeptide Y receptor.
1.0 Introduction

Multiple GI-derived signals including peptides/hormones that originate from intestinal epithelial cells, bile acids and molecules produced by the gut microbiota, together with neural signals convey information regarding incoming nutrients\textsuperscript{1-3}. These gut-derived signals act to co-ordinate the various organs involved in digestion and provide the brain with key information regarding the energy content of ingested nutrients (Figure 1). The effectiveness of bariatric surgery in producing sustained weight-reduction and amelioration of obesity-associated co-morbidities, together with the recent positive GLP-1 analogues clinical trial data highlight the opportunity afforded by modulating GI signals to tackle the growing obesity epidemic\textsuperscript{4-6}. This review highlights our current understanding of peptides/hormones and lipid mediators that emanate from the GI tract with a focus on their effects on modulating eating behavior and bodyweight. Attention is also given to the emerging evidence that vagal afferent neurons function as integrators of peripheral energy homeostatic signals\textsuperscript{2}. Neuroendocrine signals from the liver and pancreas also contribute to energy homeostasis regulation. However, this review is limited to signals from the GI tract per se. Several GI-derived peptides are also synthesized within the brain where they act as neuromodulators and/or neurotransmitters. This adds a layer of complexity in terms of utilizing pharmacological and transgenic approaches to delineate the physiological role of GI-derived peptides alone. However, these central receptors can be targeted therapeutically to modulate bodyweight by systemic administration of supra-physiological hormone doses or receptor agonists e.g. GLP-1 receptor agonists\textsuperscript{7}. Moreover, in light of the apparent multiplicity and in-built redundancy in the mechanisms involved in regulating energy homeostasis, therapeutic strategies are now moving away from a single-target approach towards combination therapies and these will also be discussed\textsuperscript{8}.

2.0 Enteroendocrine cells (EECs)

The GI tract represents the largest endocrine organ in the body harboring EECs along its entirety. EECs are the primary sensors of ingested nutrients and synthesize and release an array of peptides and hormones that act as autocrine, paracrine or endocrine regulators of digestive function, glucose homeostasis and energy balance\textsuperscript{9}.
Recent, transgenic advances have enabled the isolation and characterization of the previously enigmatic EECs. These studies have revealed the presence of a complex array of receptors and transporters that enable EECs to sense the luminal and lamina propria environments and position EECs as key integrators of metabolic and inflammatory signaling. Moreover, recent evidence suggests that EECs interact directly with neurons via synapse-like structure named neuropods, further adding to their integrative role. Classically, EECs have been characterized by their location within the GI tract and by the peptides that they produced (Table 1). However, this view has been challenged by the finding that EECs express a broad repertoire of peptide hormone precursors and that the number, density and secretory profile can be modified. Table 2 summarizes the known gut peptides, evidence for their role in controlling food intake, regulating bodyweight and their therapeutic potential.

Below we summarize our current understanding of the regulation of EEC secretion and also review in more detail the gut peptides/mediators cholecystokinin (CCK), GLP-1, oxyntomodulin (OXM), peptide YY (PYY), neurotensin (NT) and apolipoprotein A-IV (Apo A-IV).

### 2.1 Cholecystokinin (CCK)

CCK, the archetypal GI satiation peptide, first shown to acutely inhibit food intake over three decades ago, is produced by I-cells in the duodenal and jejunal mucosa, in the enteric nervous system (ENS) and within the central nervous system (CNS). CCK mediates its biological effects via the CCK-1 receptor (CCK1R), which predominates in the periphery and CCK2R found mainly in the brain. Intestinal CCK secreted in response to luminal nutrients, in particular lipids and proteins, facilitates optimal digestion by stimulating the release of lipolytic and proteolytic enzymes from the gallbladder and pancreas regulating gastric emptying. Although CCK is released into the circulation, chemical and surgical ablation experiments have revealed that CCK’s anorectic effects are exerted via a paracrine mechanism involving CCK1R on vagal afferents that project to the nucleus tractus solitarius and then to the paraventricular nucleus. A physiological role for CCK1R in satiation is supported by the findings that rats-lacking CCK1R show increased meal size and gradually become obese and that CCK1R antagonist administration leads to
increased hunger and increased meal size in humans\textsuperscript{17}. Despite these findings CCK1R-deficient mice have no bodyweight phenotype and chronic administration leaves bodyweight unaltered, due to a compensatory increase in meal frequency\textsuperscript{18, 19}. Furthermore, whilst potent receptor agonists have been developed, these have not reached clinical practice due to limited efficacy and side effects. However, it is now clear that CCK1R activation stimulates multiple interacting signaling cascades and hopefully biased agonists targeting the satiety effects alone will overcome previous problems\textsuperscript{20}. An alternative approach is to use positive allosteric modulators without intrinsic agonist activity to enhance the satiety effect of nutrient-stimulated endogenous CCK\textsuperscript{20}. Furthermore, given that CCK has been shown to interact synergistically with leptin, amylin and glucagon, combination therapies based upon these synergies are likely to be more effective than a single-agent approach.

2.2 Proglucagon gene products:

The proglucagon gene encodes pre-proglucagon which undergoes tissue specific post-translational processing to generate GLP-1, GLP-2, OXM, glucagon and glicentin\textsuperscript{21}. Here we review the data pertaining to the effects of GLP-1 and OXM on energy intake and bodyweight.

2.2.1 GLP-1

GLP-1 is primarily expressed within L-cells of the GI tract and in the brainstem. L-cell derived GLP-1 is synthesized as GLP-1\textsubscript{1-37} and GLP-1\textsubscript{1-36 amide}. These undergo N-terminus cleavage to produce the biologically active fragments GLP-1\textsubscript{7-37} and GLP-1\textsubscript{7-36 amide} that bind to and activate the GLP-1 receptor (GLP-1R). In response to nutrient-ingestion circulating GLP-1 concentrations show a biphasic response\textsuperscript{22}: a rapid at 15-30 minutes then a later peak at 60-90 minutes\textsuperscript{21}. Nutrients entering the proximal GI tract are thought to trigger GLP-1 release from ileal L-cells by a neurohumoral reflex generating the early increase. However, GLP-1 expressing EECs have now been identified within the human proximal intestine\textsuperscript{23}. Thus, direct EEC nutrient-sensing may contribute to the early GLP-1 rise, in addition to mediating the later increase in circulating GLP-1 levels. GLP-1 secretion is also modulated directly and indirectly by chemical, neural and humoral factors\textsuperscript{9}. Once secreted, active GLP-1 is almost
immediately degraded by dipeptidyl peptidase-4 (DPP-4), consequently only approximately 25% reaches the hepatoportal circulation and less than 10% reaches the systemic circulation\textsuperscript{21, 24}.

The physiological role of GLP-1/GLP-1R in regulating glucose homeostasis is well established and therapies targeting the GLP-1/GLP-1R system are widely used to treat people with type 2 diabetes (T2D)\textsuperscript{5}. In contrast, the physiological role of endogenous GLP-1 in regulating feeding behavior and bodyweight is less clear-cut. Whilst peripheral administration of GLP-1 or GLP-1R agonists reduce energy intake and chronic administration reduces bodyweight, GLP-1R deficient mice exhibit normal feeding behavior and bodyweight\textsuperscript{25}. These findings suggest that the GLP-1/GLP-1R system is not necessary for energy homeostasis. The anorectic effects of intra-peritoneal (IP) GLP-1 administration are abolished by vagotomy or capsaicin administration, implicating a paracrine mechanism action\textsuperscript{26}. A paracrine mechanism of action is also in keeping with GLP-1’s short half-life, by the presence of GLP-1R on vagal afferents and the finding that IP GLP-1 administration leads to increased vagal firing\textsuperscript{27}. In contrast, the anorectic effect of intravenous or subcutaneous GLP-1 is unaffected by vagotomy or capsaicin treatment, suggesting a direct effect via CNS GLP-1Rs. Moreover, comparative studies utilizing a long-acting GLP-1R agonist have confirmed a direct modulatory effect upon central appetite-regulating circuits. However, it is unlikely that endogenous GLP-1 circulating concentrations reach the levels required for a direct effect, albeit with the caveat of following bariatric procedures where L-cell stimulation is markedly enhanced\textsuperscript{28}. Importantly, long-acting GLP-1R agonist administration has been shown to lead to sustained weight-reduction with amelioration of obesity-associated comorbidities\textsuperscript{4}. These findings highlight that gut-derived signals working through non-physiological means represent attractive therapeutic strategies for obesity.

\textbf{2.2.2 OXM}

OXM is a 37-amino-acid peptide, containing the 29-amino-acid sequence of glucagon followed by an 8-amino-acid carboxy-terminal extension, activates GLP-1Rs and glucagon receptors (GCGR)\textsuperscript{29}. Within the GI tract OXM is co-distributed, co-expressed
and co-secreted with GLP-1 and is also inactivated by DPP4. Similar to GLP-1, OXM causes glucose-dependent insulin secretion and improves glucose tolerance in mice and humans with T2D despite acting as a GCGR agonist. Peripheral administration of OXM reduces energy intake in rodents, lean and obese human subjects. Repeated subcutaneous administration of OXM to overweight/obese people reduces energy intake, increases energy expenditure and leads to weight loss. Studies on gip-1r null and GCRG-null mice have identified that the anorectic effects of OXM require the GLP-1R and are thought to involve both vagal and direct CNS activation. While stimulatory effects of OXM on energy expenditure are mediated by the GCGR. However, despite mediating its anorectic effects via the GLP-1R, peripheral administered OXM and GLP-1 produce differential c-fos activation patterns within the hypothalamus suggesting differential mechanisms of action and implies the presence of an as yet to be identified OXM receptor. Overall OXM represents a promising weight-loss therapy that appears well tolerated in humans. However, its clinical utility is limited by its short half-life. Thus OXM analogues and modified OXM molecules with altered GLP-1 receptor binding affinity and increased duration of action in vivo have been developed and tested as a proof-of-concept while several others are currently being actively investigated by the pharmaceutical industry.

2.3 Peptide YY
Peptide YY together with neuropeptide Y (NPY) and pancreatic polypeptide (PP) belong to the PP-fold family and mediate their effects via Y-receptors (YR) that belong to G-protein-coupled receptor (GPCR) superfamily. PYY is expressed within GI EECs, the pancreas and brainstem neurons. PYY is a 36-amino-acid peptide (PYY1-36) often described as a distal GI tract hormone due to its predominance within L-cells of the ileum and colon. However, this classical view has been debunked by the finding of PYY expression within the stomach, duodenum and jejunum together with the newfound ability of EECs to adapt their secretory profile. It is also now clear that in addition to being co-secreted with GLP-1, other gut peptides such as CCK, secretin, glucose-dependent insulinotropic polypeptide (GIP) and neurotensin (NT) may be co-secreted with PYY. Circulating PYY levels are low and progressively decrease in the fasted state. In response to nutrient-ingestion, PYY levels rapidly increase to reach a
plateau at 60-90 minutes and then remain elevated for several hours post-ingestion with caloric content and macronutrient composition impacting upon secretion. PYY3-36, produced by N-terminal cleavage of PYY1-36 via DPP4, is the main circulating form. Importantly, PYY1-36 and PYY3-36 exhibit divergent effects on feeding behavior as a consequence of differential YR affinities. PYY1-36 has equivalent affinities for the Y1R and Y2R and increases food intake when administered centrally, whereas PYY3-36 is a high-affinity ligand for the Y2R and reduces energy intake when administered peripherally. Studies y2r-null mice coupled with pharmacological approaches using a selective Y2R antagonist have identified a crucial role for the Y2R in mediating the anorectic effects of PYY3-36. A physiological role for PYY3-36 in bodyweight regulation is suggested by the finding that PYY-null mice are hyperphagic and obese and that PYY3-36 replacement reverses their obese phenotype. Translational studies indicate that PYY3-36 mediates its anorectic effects predominantly by acting upon central appetite-regulating circuits with the hypothalamic arcuate nucleus and brainstem regions. Y2Rs are present on vagal afferents and vagal firing is increased in response to IP PYY3-36 suggesting a paracrine mechanism of action. However, evidence suggests that the main anorectic effect of PYY3-36 is mediated by a direct effect upon central appetite-regulating circuits. Importantly, human brain functional magnetic resonance imaging (fMRI) studies revealed that peripheral PYY3-36 administration altered neural activity within homeostatic, reward and polymodal brain regions. PYY3-36 was the first gut hormone shown to regulate homeostatic and hedonic brain circuits in humans. However, subsequently additional gut hormones and gut-derived signals have also been shown to act in this manner. More recently PYY3-36 has been shown to have beneficial effects upon glucose homeostasis and has been implicated in contributing to the immediate glycemic improvements observed following bariatric surgery. Taken together, PYY3-36/Y2R system represents an attractive therapeutic target for treating obesity and T2D. However, whilst acute PYY3-36 administration to people with overweight and obesity led to a marked reduction in 24-hour energy intake, effective, safe PYY3-36/Y2 agonists have yet to reach the clinical arena. Studies combining PYY3-36 administration with other gut peptides
have revealed synergistic anorectic effects and suggest that Y2R agonist in combination with other gut peptides are likely to offer increased efficacy.

2.4 Neurotensin (NT)

NT is a 13 amino-acid peptide expressed in the CNS and GI tract EECs. NT exerts its pleiotropic biological actions via three NT receptors; NTR1, NTR2, and NTR3, that are widely distributed with the brain and peripherally. Central NT administration reduces energy intake via the NTR1 and is implicated in controlling food reward interacting with the dopaminergic system and leptin. GI NT is released in response to nutrient-ingestion, in particular to fat, and regulates GI motility, pancreatic and biliary secretion, facilitates fat translocation and acts as an incretin. Circulating NT has a very short half-life of around 30 seconds. Peripheral administration of NT causes a transient reduction in energy intake, an effect that is blocked by NT1R antagonist administration. Systemic administration of PEGylated NT, with an increased half-life causes a sustained reduction in food intake coupled with increased hypothalamic POMC expression. Studies examining the impact of vagotomy on the anorectic effect of peripherally administered NT suggest that NT acts via both endocrine and paracrine mechanisms with high circulating NT levels directly accessing CNS appetite-regulatory circuits and paracrine vagal mechanisms mediating the anorectic effect when circulating NT levels are low. Recent studies have revealed that within the GI tract NT, PYY and GLP-1 are not only co-expressed but also co-secreted in response to a wide range of stimuli including bile acids (BA). Moreover, NT has been shown to acts synergistically with GLP-1 and PYY when administered peripherally. In addition, circulating NT concentrations are increased following bariatric procedures that increase EEC exposure to nutrients. Furthermore, administration of an NTR1 antagonist to rats following Roux-en-Y gastric bypass (RYGB) was associated with increased food intake, suggesting that circulating NT post-RYGB contributes to reduced energy intake. Taken together these findings suggest that peripheral administration of long-acting NT or NTR1 agonists might be beneficial for weight-reduction. However, the widespread distribution of NTR1, NTR2 and NTR3 and the pleiotropic effects of NT including cardiovascular effects may limit its therapeutic potential as an obesity therapy.
Moreover, the recent finding that NT-deficient mice exhibit reduced intestinal fat absorption and are protected against diet-induced obesity (DIO) despite having a similar feeding behaviour and energy expenditure compared to their wild-type littermates further complicates the picture\textsuperscript{52}.

### 2.5 Ghrelin

Ghrelin, a 28-amino-acid peptide hormone\textsuperscript{53}, is secreted from P/D1-type cells in humans, X/A like-type cells in rodents, which are a distinct population of endocrine cells located within the gastric oxyntic mucosa\textsuperscript{54}. Ghrelin-producing cells are also present throughout the small intestine with the greatest numbers in the duodenum. Of note, majority of the proximal P/D1-type cells are closed-type and thus not regulated directly by the stomach luminal contents. In contrast, ghrelin-producing cells in the duodenum and jejunum are open-type\textsuperscript{55}. Circulating ghrelin levels increase before spontaneously initiated meals and fall rapidly after nutrient-ingestion in proportion to the energy intake through as yet poorly characterized mechanism\textsuperscript{56, 57}. In vivo and in vitro studies have shown that epinephrine, norepinephrine, endothelin and secretin increase ghrelin release, whereas hyperglycemia, insulin, gastrin releasing peptide, PYY3-36, OXM, GLP-1, CCK, GIP and somatostatin decrease ghrelin release. However, it remains unclear whether these factors act directly on ghrelin cells or indirectly via neighboring cells and for many the physiological relevance to be determined\textsuperscript{55}.

In order to bind to its only known receptor, the growth hormone secretagouge receptor type 1a (GHSR1a), ghrelin requires attachment of a fatty acid side chain to its serine-3 residue. This post-translation acylation is mediated by ghrelin O-acyl-transferase (GOAT)\textsuperscript{58}. Acyl-ghrelin is absent in GOAT null mice indicating that GOAT is the only enzyme capable of activating ghrelin in vivo. In rodents, peripheral acyl-ghrelin administration increases energy intake and repeated administration results in increased adiposity. Similarly in humans, peripheral acyl-ghrelin administration increases hunger and energy intake in lean, obese, healthy and malnourished individuals\textsuperscript{59, 60}. The orexigenic effect of ghrelin is specifically modulated through GHSR1a, as ghrelin fails to promote food intake in GHSR1a-null mice\textsuperscript{61}. Ghrelin acts
directly upon CNS appetite-regulating circuits, in particular the arcuate nucleus of the hypothalamus\textsuperscript{62} and also indirectly via the vagus\textsuperscript{63}. Acyl-ghrelin administration shifts the food preference toward fat-rich diets and leads to increased consumption of palatable saccharin solution\textsuperscript{64}. Ghrelin-null and GOAT-null mice are indistinguishable from wild-type mice when fed normal chow\textsuperscript{55}, however, they are protected from DIO\textsuperscript{55} and display reduced hedonic feeding. Together these data suggest that ghrelin’s main feeding effect is on ‘reward based/hedonic’ feeding. Furthermore, ghrelin administration to human subjects during fMRI brain scanning increases the neural response to food pictures in several brain regions implicated in hedonic feeding\textsuperscript{55}. More recently, attenuated post-meal acyl-ghrelin suppression and altered CNS responsivity to circulating acyl-ghrelin have been implicated as drivers of the increased appetite and hedonic food preferences observed in people homozygous for the obesity-risk A-allele of the fat mass and obesity-associated gene (FTO) linked single nucleotide polymorphism rs9939609\textsuperscript{66}. Moreover, an increased circulating acyl-ghrelin levels in response to dietary restriction seems to play a causative role in weight regain and recidivism after diet-induced weight loss\textsuperscript{67} and in patients with Prader-Willi syndrome (PWS), in whom high ghrelin levels are suggested to cause hyperphagia and weight gain\textsuperscript{68}. In light of the weight-promoting effects of acyl-ghrelin, a novel ghrelin-receptor agonist anamorelin, is being trialled to assess the safety and effectiveness for the treatment of patients with cancer anorexia and cachexia\textsuperscript{69}.

Des-acyl ghrelin (DAG) represent approximately 80% of circulating ghrelin and was initially viewed purely as an acyl-ghrelin degradation product\textsuperscript{70}. However, emerging evidence suggests that DAG has opposing biological actions to acyl-ghrelin and moreover that the ratio of acyl-ghrelin to DAG (acyl-ghrelin/DAG) is important. In support of this hypothesis, in patient patients with PWS, hyperphagia and weight gain has been shown to coincide with an increase in acyl-ghrelin/DAG\textsuperscript{71}. Furthermore, a stabilized DAG peptide analog, AZP-531, has been designed and is being tested for the treatment of PWS and for common obesity. Other approaches include GOAT inhibition, ghrelin receptor antagonists and immune mediated ghrelin inactivation\textsuperscript{72,73}.
2.6 Nesfatin-1
In 2006, centrally produced nesfatin-1 was identified as an anorexigenic peptide and suggested to be a physiological regulator of energy balance on the basis that reduced nesfatin-1 signalling increased feeding\textsuperscript{74}. Nesfatin-1, derived from a precursor peptide NEFA/nucleobindin-2, is widely expressed within the CNS and periphery where it exerts pleiotropic effects including influencing digestive function, glucose homeostasis, sleep and anxiety\textsuperscript{75}. However, the corresponding receptor(s) remain to be identified. Within the periphery the stomach exhibits the highest nesfatin-1 expression levels and here nesfatin-1 is co-expressed with ghrelin within P/D1 cells, though stored within different vesicles\textsuperscript{76}. Circulating nesfatin-1 levels are low in the fasted state and increase with feeding suggesting that peripheral nesfatin-1 might also regulate feeding behaviour acting through autocrine, paracrine and/or endocrine routes. However, the effect of peripheral nesfatin-1 administration upon feeding behaviour has yielded mixed findings. Thus the role of peripheral and in particular gastric nesfatin-1 remains to be determined\textsuperscript{77}. Nevertheless, nesfatin-1 still represents a potential therapeutic target to treat people with obesity in light of its beneficial metabolic effects when administered centrally and the finding that peripheral nesfatin-1 can cross the blood brain barrier.

2.7 Gastric leptin
Leptin is expressed in, and secreted by gastric chief cells and gastric endocrine P-cells, into the gastric lumen\textsuperscript{78,79}. Gastric leptin is implicated in the short-term regulation of food intake and is rapidly secreted in response to food intake and peptide hormones, such as CCK and insulin, a mechanism that is vagally mediated\textsuperscript{80}. Leptin receptors are present on gastric vagal afferents and interestingly studies have shown that the response of vagal gastric afferents to leptin is dynamic and dependent on nutritional status. In the fasted state or following 12-weeks consumption of high-fat diet, gastric leptin inhibits gastric tension sensitive vagal afferents, facilitating increased food intake. In contrast, in the fed state gastric leptin has an excitatory effect on gastric vagal afferents\textsuperscript{81}. Gastric leptin is secreted bound to a soluble leptin receptor that protects it from proteolysis. Upon reaching the duodenum, it attaches to a duodenal enterocyte
transmembrane leptin receptor and is transcytosed to the luminal basal membrane and absorbed into the circulation, impacting upon energy homeostasis through paracrine and endocrine mechanisms\textsuperscript{79}.

2.8 Regulation of EEC secretion and nutrient-sensing

A combination of nutrient, neural and hormonal pathways regulate expression and secretion of gut peptides from EECs\textsuperscript{82}. Moreover, EECs are influenced by diet, microbiota, inflammation and host genetic factors. Of particular note, in light of the prevalence of genetic variation within the melanocortin-4 receptor (MC4R), is the finding that MC4R are present on EECs where they regulate the secretion of PYY and ghrelin\textsuperscript{83}. EECs detect an array of luminal nutrient stimuli via through GPCRs, ion and solute transporters, intra-cellular metabolism, which lead to calcium influx and gut peptide release into the subcellular space. Luminal stimuli include sweet and bitter tastants, monosaccharides, free fatty acids, monacylglycerols, amino acids, di/tripeptides, indole and short-chain fatty acids (SCFA). EECs also sense a number of non-nutrient and luminal paracrine signals including cytokines, BA, gut hormones and enteric neurotransmitters. Endocrine and paracrine signals acting via surface receptors on the basolateral surface of neighboring enterocytes and/or other EECs modulate EEC function. BAs act as non-nutrient stimuli of L-cells via activating TGR5 leading to release of L-cell gut peptides\textsuperscript{84}. In humans, administration of BAs has been associated with increased plasma GLP-1 levels and improved glucose homeostasis.

Targeting secretion of EECs in order to recapitulate the gut peptide milieu following gastric bypass surgery represents an attractive therapeutic strategy for the treatment of obesity. Indeed, evidence suggests that altered EEC secretion as a consequence of altered nutrient stimulation contributes to the weight-loss and metabolic benefits of bariatric surgery\textsuperscript{85}. Similarly, preventing nutrient contact with the duodenal mucosa by placing an endoscopic liner known as Endobarrier\textsuperscript{86}, or thermic ablation of the duodenal mucosa\textsuperscript{87}, results in improved metabolic profiles hypothetically based on their impact upon EEC functioning. Given the rapid recent advancement in our understanding of EEC biology functional foods or designer drugs aimed at modulating EEC secretion will soon reach clinical practice.
2.9 Taste receptors
Taste receptors that respond to sweet, bitter and umami stimuli, together with their associated G-proteins have been identified within the GI mucosa co-localized with several gut peptides including ghrelin GLP-1, GIP, and CCK\textsuperscript{88,89}. Sweet, umami and bitter-tasting stimuli are detected by members of two GPCR families, the taste 1 receptor (T1R) and taste 2-receptor family (T2R). Subtypes of T1R heterodimerize to detect sweet tastants (T1R2 and T1R3) and umami tastants (T1R1 and T1R3), while the T2R receptor family members can detect bitter stimuli.

\textit{In vitro} and \textit{in vivo} studies suggest that taste receptors may play a functional role in detecting luminal nutrients with subsequent modulation of hormonal or neural signaling engendering downstream effects of glucose and energy homeostasis\textsuperscript{90}. For example, gustducin-coupled sweet taste receptors, which sense glucose and artificial sweeteners, have been shown to be co-localized in L-cells and mice-lacking alpha-gustducin exhibit an attenuated GLP-1 and insulin release in response to enteral glucose\textsuperscript{91}. These findings suggest a functional role for the sweet taste receptor in modulating GLP-1 release. However, the finding artificial sweeteners fail to increase GLP-1\textsuperscript{92-94} calls into question the functional role of sweet taste receptors in modulate GLP-1 secretion\textsuperscript{95}. Whilst the precise role of taste receptors within the GI mucosa remains to be determined they offer a potential mechanisms for modulating EEC secretion.

3.0 Additional GI epithelium derived signals
3.1 Apolipoprotein A-IV (Apo A-IV)
Apo A-IV is a glycoprotein produced within enterocytes of the small intestine, the hypothalamus and in rodents, within the liver. Intestinal Apo A-IV is synthesized and secreted in response to the absorption and uptake of long-chain fatty acid into chylomicrons. Neither medium-chain fatty acids nor SCFA impact upon its secretion. In response to lipid ingestion circulating Apo-AIV levels increase within 15 minutes and remain elevated until 30 minutes post-meal\textsuperscript{96}. This finding coupled with the fact that Apo A-IV infusion that reproduces post-meal circulating levels, dose-
dependently inhibits food intake has led to Apo A-IV being proposed as a short-term satiety signal\textsuperscript{97}. A role for CNS sites as mediators of the anorectic effects of Apo A-IV is supported by studies showing a reduction in feeding with central Apo A-IV injection and increased food intake with central administration of Apo A-IV neutralising antibodies. However, these studies are unable to differentiate the relative contribution of GI-derived Apo A-IV compared to centrally released Apo A-IV in mediating the anorectic effects of Apo A-IV. Vagotomy abolishes the anorectic actions of Apo A-IV suggesting a key role for vagal afferents\textsuperscript{98}.

Evidence for cross-talk between CCK and Apo A-IV is suggested by the following observations: CCK and Apo A-IV secretion are both dependent upon chylomicron formation; Apo A-IV increases CCK-elicited vagal firing; Apo A-IV induced satiation requires an intact CCK system including vagal afferent CCK-1R; the anorectic action of CCK is increased in Apo A-IV null mice; CCK and Apo A-IV act synergistically to reduce food intake. Taken together these findings suggest that CCK and Apo A-IV work co-dependently to reduce food intake. In addition to modulating food intake, Apo A-IV has beneficial glycemic effects and has been shown to act as an incretin but with a substantially longer half-life than GLP-1 or GIP\textsuperscript{96}. Thus, Apo A-IV represents a potential therapeutic target either alone or in combination. However, despite the marked advances in our understanding of the biological roles of Apo A-IV the receptor(s) through which it mediates its effects remain to be identified.

3.2 Guanylin and Uroguanylin

Guanylin/guanylate cyclase activator 2A (GN/GUCA2A) and uroguanylin/guanylate cyclase activator 2B (UGN/ GUCA2B) were identified in 1992\textsuperscript{99} and 1993\textsuperscript{100} respectively during a search for endogenous ligands for intestinal guanylate cyclase 2C (GUCY2C), a transmembrane receptor that had been identified as the target of diarrheagenic bacterial heat-stable endotoxins. GN and UGN are secreted as prohormones; proguanylin and proUGN, and undergo proteolytic cleavage to generate c-terminal active peptides GN and UGN. The guanylate peptide family not only regulate fluid and electrolyte balance but also epithelial cell homeostatic programs in the gut with GUCY2C being implicated as a tumor suppressor that can
potentially be targeted for tumor chemoprevention. Within the GI tract, GN increases in the cranio-caudal direction whilst UGN decrease, however the cellular sources are debated and include goblet cells, entero-/colonocytes, EECs and tuft cells\textsuperscript{101}.

In addition to their paracrine actions pro-UGN and pro-GN are secreted into the circulation where they can act in an endocrine manner. Circulating pro-UGN and pro-GN levels are nutritionally regulated being low in fasted state and increasing with feeding. Moreover, UGN levels are regulated in a leptin-dependent manner\textsuperscript{102}. UGN was first suggested to regulate energy balance by Vallentino et al., who reported that pro-UGN is cleaved within the hypothalamus to generate UGN, which activates GUCY2C in hypothalamic neurons and anorexigenic pathways\textsuperscript{103}. They also reported that GUCY2C null mice were hyperphagic with increased bodyweight that was exacerbated by high-fat feeding\textsuperscript{103}. However, another group were unable to elicit an anorectic effect of central UGN or GUCY2C receptor agonist and reported that GUCY2C null mice only exhibited a modest increase in body weight, adiposity and glucose intolerance when exposed to high-fat diet (HFD)\textsuperscript{104}.

Recently, adipose tissue has been identified as a target for the guanylin system with GN and UGN stimulating lipolysis\textsuperscript{105}. Moreover, Folguiera and colleagues have reported that chronic central UGN administration reduced weight gain and adiposity in DIO mice. However, these effects were independent of changes in food intake and were due to increased brown adipose tissue thermogenesis, browning of white adipose tissue together with increased lipolysis due to sympathetic nervous system activation. In addition, increased fecal output was noted due to parasympathetic activation\textsuperscript{102}. Whilst many questions remain to be addressed the guanylate system still holds promise as a potential therapeutic target.

### 3.3 Intestinal lipid-derived signals that sense dietary fat

#### 3.3.1 Lipid-derived orexigenic endocannabinoid messengers

The presence of oral long chain unsaturated fatty acids in the mouth has been reported to lead to increased production of lipid-derived endocannabinoid
messengers such as anadamide that are high affinity agonists for the cannabinoid receptors CB1 and CB2. Activation of the CB1 receptor (CB1R), which expressed centrally and peripherally, stimulates food intake whilst CB1 receptor blockade reduces feeding. The mechanisms by which CB1R activation increases food intake are unclear but they do not require the vagus\(^{106}\). Similarly, the chemical source of fat taste and the receptor(s) mediating fat detection remain to be determined. However, release of non-esterified fatty acids from triacylglycerols is required in order for fat to be detected. Studies in genetically modified mice had implicated CD36, GPR40, GP120 and TRPM5 in fat sensing\(^{107}\). However, more recent data question that role of GPR40 and GPR120 in fat ‘taste’\(^{108}\).

### 3.3.2 Anorexic lipid mediators

As fat enters the small intestine oleic acid liberated from the digestion of triacylglycerols is captured by the epithelial lining and converted into the anorexic lipid mediator oleoylethanolamide (OEA). OEA leads to reduced food intake, increased fatty acid absorption from the small intestine and stimulates lipolysis. OEA is increased in the duodenal and jejunum only not in the circulation suggesting a paracrine mechanism of action. OEA also binds to GPR119 on L-cells stimulating secretion of L-cell products. The mechanisms by which OEA promotes satiety remain unclear but require PPAR alpha-receptor\(^{109}\). OEA-induced satiety is described as being abolished by vagotomy\(^8\) and capsaicin treatment\(^{110}\), but a subsequent paper reported that selective afferent vagotomy did not block feeding inhibition\(^{111}\).

### 3.4 Appetition

Studies report that the presence of dietary sugar or fat in the gut can generate postoral signals that lead to conditioned food preferences and stimulation of appetite and energy intake, a process termed “appetition” by Sclafani and colleagues\(^{112}\). Recent data suggest that SGLT1 functions as an intestinal sugar sensor that promotes the appetite for glucose-rich foods. However, the identity of the appetition signal generated by SGLT1 remains to be identified\(^{113}\). Moreover, studies in mice undergoing bariatric surgery suggest that sugar sensing in the duodenum plays a key role in mediating dorsal striatal dopamine release in response to sugar ingestion.
which is necessary for sweet appetite conditioning\textsuperscript{114}. Studies utilizing transgenic mice suggest that intestinal GPR40 and GPR120 fatty acid sensors have critical roles in post-oral intake stimulation and preference conditioning by dietary fat\textsuperscript{115}. The mechanisms underlying appetite are still being investigated but may involve other non-dopaminergic neurochemical systems and/or presently undiscovered hormonal mediators released from the gut mucosa. A greater understanding of the relative importance of post-oral appetition in humans and the underlying mechanisms is warranted.

4.0 Neural Signals

4.1 Vagal afferent neurons as early integrators of peripheral energy signals

It is now apparent that vagal afferent firing is influenced by leptin, a wide range of gut hormones, gut-derived lipid mediators, shifts in gut microbiota and gut inflammation. Moreover, rodent studies show that vagal afferent neurons exhibit plasticity in terms of their receptor and neuropeptide expression that is regulated by nutrient status. For example, fasting for a few hours reduces expression of Y2R and anorectic cocaine amphetamine related-transcript but increases expression of CB1R and orexigenic melanin concentrating hormone, promoting orexigenic signaling. These changes are normally rapidly reversed to favor anorexigenic signaling upon re-feeding\textsuperscript{3}. A role for CCK in mediating this switch is suggested by the finding that CCK1R antagonism prevents this fast/fed vagal afferent neurochemical phenotype switch. More importantly, this fast/fed switch is impacted by obesity, inflammation and infections. For examples, in DIO animals vagal afferent sensitivity to anorectic and GI distention signals is reduced whilst sensitivity to ghrelin is increased. These changes are accompanied by a loss of this switching of vagal afferent phenotype in response to feeding with vagal afferents locked in a fasted state phenotype\textsuperscript{3}. These findings highlight the role of vagal afferents act as early integrators peripheral energy signals.

4.2 Enteric Nervous System

The enteric nervous system (ENS) is a network of nerve fibers and ganglia that innervates the intestine and interacts with sympathetic and parasympathetic
nervous system and utilizes a wide variety of peptide neurotransmitters in addition to acetylcholine and noradrenaline\textsuperscript{10}. Gut hormone secretion is modulated by signals from other EEC populations and from the ENS. Enteric neurons are also capable of sensing certain absorbed nutrients directly. For example, subsets of enteric neurons express the SCFA receptor FFAR3. In turn, the ENS fine-tunes the function of EECs.

5.0 Microbiota
The role of the gut microbiota is covered by Nieuwdorp and colleagues, in this issue, thus here we provide only a brief overview. The gut microbiota release and interact with an array of metabolites that can impact upon EEC secretion or directly influence energy regulation. For example, SCFA including acetate, butyrate and propionate are derived from microbial fermentation of non-digestable carbohydrate\textsuperscript{82}. SCFA are ligands for two GPCRs, free fatty acid receptor 2 (FFAR2) and FFAR3 expressed in the GI tract. FFAR2 is present on L-cell and P/D1 cells and FFAR2 activation increases GLP-1 secretion and reduces ghrelin secretion. SCFA, given both orally or directly into the intestine reduce food intake and body weight in diabetic and healthy rodents and humans\textsuperscript{1}.

6.0 Inflammation
Obesity is associated with a mild inflammatory state with increased intestinal permeability to bacterial lipopolysaccharide. This binds to toll-like receptor-4 on vagal afferent neurons leading to increased expression of the suppressor of cytokine signaling and attenuates the ability of leptin to activate vagal afferent neurons. This in turn impacts upon CCK signaling\textsuperscript{1}.

7.0 Leveraging GI signals to treat obesity and metabolic disorders
Given the multiplicity, redundancy in, and compensatory capacity of, the mechanisms involved in regulating appetite and energy homeostasis, strategies to treat obesity are now focused upon targeting multiple systems. As discussed in Section 2.7, targeting EECs to release multiple endogenous is an attractive strategy. Alternative approaches that are being trialed include is exogenous administration of combined therapies and monomeric peptides co- and tri-agonists combinations of
endogenous peptides into a single molecule with receptor occupancy patterns that more closely resemble physiological regulation. For example GLP-1/GIP dual agonist GLP-1/GCG dual-agonist and tri-agonist GLP-1/GIP/GCG.

8.0 Conclusions and Future Perspectives
Over the last decade our understanding of the elegant interrelated mechanisms by which GI-derived signals regulate food intake and bodyweight has markedly increased informing the rationale development of anti-obesity strategies. In addition, the long-acting GLP-1R analogue, liraglutide has become accepted as a therapeutic option for managing T2D and obesity, albeit acting through non-physiological mechanisms. The redundancy and compensatory capacity of the mechanisms that govern energy balance are now recognized, leading to a new focus of therapeutic strategies that influence multiple systems. Despite the marked recent advances in our understanding of GI-derived energy signals many questions remain unanswered and additional research is needed in order for their full therapeutic potential to be leveraged. It is also clear that weight-loss response and weight-loss maintenance is highly variable in response to lifestyle interventions, pharmacotherapies and bariatric surgery. Thus in addition to the development of new therapeutic strategies we also need to develop more sophisticated phenotyping protocols for people with overweight and obesity in order to identify specific subpopulations and enable the development of precision medicine based approaches.
**Table 1:** Enteroendocrine cell types (EEC), peptide(s)/hormone(s) secreted, predominant location and proposed physiological role of peptide(s)/hormone(s)

<table>
<thead>
<tr>
<th><strong>Cell type</strong></th>
<th><strong>Hormone(s)</strong></th>
<th><strong>Predominant location</strong></th>
<th><strong>Function</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>X/A* P/D1**</td>
<td>Ghrelin, nesfatin-1</td>
<td>Gastric oxyntic mucosa and proximal small intestine</td>
<td>Appetite control, food intake, glucose homeostasis, growth hormone release, sleep and mood</td>
</tr>
<tr>
<td>Chief Cells</td>
<td>Gastric leptin</td>
<td>Gastric mucosa</td>
<td>Short-term regulation of food intake</td>
</tr>
<tr>
<td>P Cells</td>
<td>P Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Somatostatin</td>
<td>Stomach, small intestine</td>
<td>Gastrointestinal hormone inhibition</td>
</tr>
<tr>
<td>I</td>
<td>CCK</td>
<td>Duodenum, proximal jejunal mucosa, enteric nervous system</td>
<td>Stimulates gallbladder contraction, inhibits stomach emptying and food intake</td>
</tr>
<tr>
<td>K</td>
<td>GIP</td>
<td>Proximal small intestine</td>
<td>Insulin release, gastric acid secretion,</td>
</tr>
<tr>
<td>L</td>
<td>GLP-1, GLP-2, PYY, oxyntomodulin, neurotensin</td>
<td>Along the entire small intestine with ileal and colonic predominance</td>
<td>Nutrient sensing, GI motility, stimulation of insulin release, inhibition of glucagon release, appetite suppression and promotion of energy expenditure.</td>
</tr>
</tbody>
</table>

*Rodents; **Humans
Table 2: Enteroendocrine Cell Nutrient-Sensing Mechanisms

<table>
<thead>
<tr>
<th>Carbohydrate sensing</th>
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<tbody>
<tr>
<td><strong>Nutrient</strong></td>
<td><strong>Receptor activated</strong></td>
<td><strong>Secreted peptide</strong></td>
</tr>
<tr>
<td>Glucose</td>
<td>T1R2/T1R3 sweet taste receptor</td>
<td>GLP-1</td>
</tr>
<tr>
<td>Glucose α-methylglucopyranoside</td>
<td>SGLT1</td>
<td>GLP-1, GIP</td>
</tr>
<tr>
<td>Glucose</td>
<td>kATP</td>
<td>Role unclear, may stimulate GLP-1 release</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein sensing</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutrient</strong></td>
<td><strong>Receptor activated</strong></td>
<td><strong>Secreted peptide</strong></td>
</tr>
<tr>
<td>Peptones (via PEPT1)</td>
<td>GPR93</td>
<td>CCK, GLP-1</td>
</tr>
<tr>
<td>L amino-acids</td>
<td>CaSR</td>
<td>CCK, PYY</td>
</tr>
<tr>
<td>L amino-acids</td>
<td>GPR6a</td>
<td>GLP-1</td>
</tr>
<tr>
<td>L-glutamine L-aspartate</td>
<td>T1R1/T1R3 umami taste receptor</td>
<td>GLP-1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>mGluR4 glutamate receptor</td>
<td>GLP-1</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Lipid sensing</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Nutrient</strong></td>
<td><strong>Receptor activated</strong></td>
<td><strong>Secreted peptide</strong></td>
</tr>
<tr>
<td>Endogenous saturated fatty acids (ethanolamides)</td>
<td>GPR 119</td>
<td>GLP-1, Glucose-dependent insulin secretion</td>
</tr>
<tr>
<td>Short chain fatty acids</td>
<td>FFAR2</td>
<td>PYY, GLP-1</td>
</tr>
<tr>
<td>Short chain fatty acids</td>
<td>FFAR3</td>
<td>PYY</td>
</tr>
<tr>
<td>Medium &amp; long chain fatty acids</td>
<td>FFAR1</td>
<td>GLP-1, CCK, GIP</td>
</tr>
<tr>
<td>Medium &amp; long chain fatty acids</td>
<td>FFAR4</td>
<td>GLP-1, GIP</td>
</tr>
<tr>
<td>Medium &amp; long chain fatty acids</td>
<td>CD36</td>
<td>CCK, Secretin</td>
</tr>
</tbody>
</table>

Summary of macronutrient sensing receptors expressed in enteroendocrine cells and the gut peptide(s) they release \(^1\), \(^3\), \(^9\), \(^10\), \(^82\)
Figure 1:
Several signals arising from the GI tract are able to regulate energy homeostasis and body weight. These comprise GI peptides/hormones, which are secreted by different discrete enteroendocrine cell populations distributed along the entire GI tract from the stomach to the distal colonic mucosa and include the orexigenic hormone ghrelin and anorexigenic hormones (CCK, GLP-1, OXM PYY, nesfatin-1 and leptin); intestinal epithelium derived signals, such as ApoA-IV, guanylin and uroguanylin; anorexigenic and orexigenic lipid-derived molecules (oleoyethanolamide [OEA] and endocannabinoid); and nutrient metabolites produced by gut microbiota (acetate, butyrate and propionate). The enteric nervous system (ENS) interacts with the autonomic nervous system, EEC products and is able to directly sense absorbed nutrients. In turn, the ENS fine-tunes the function of EECs. Vagal afferent firing is influenced by a wide-range of gut peptides, gut-derived lipid-mediators, shifts in gut microbiota, gut inflammation and leptin.
References


Supplementary References


**Duodenum**
- CCK
- GIP
- Ghrelin

**Jejunum**
- GIP
- GLP1
- Apo A-IV
- Guanylin
- Uroguanylin

**Ileum**
- GLP1
- Oxyntomodulin
- PYY
- Neurotensin
- Apo A-IV
- Guanylin
- Uroguanylin

**Stomach**
- Ghrelin
- Nesfatin-1
- Leptin

**Lipid derived molecules**
- Endocannabinoid agonists
- Anorexic lipid OEA

**Colon**
- GLP1
- GLP2
- Oxyntomodulin
- PYY

*Vagus nerve*

*Bile acids*

*ENS*

*Nutrient sensors*

*Microbiome*