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The Role of Gut Peptides in the Regulation of Body Weight

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Introduction and Methodological Considerations

The recent success of therapeutics based on gut hormones, in particular the insulin-stimulating incretin hormones (glucose-dependent insulinotropic polypeptide [GIP] and glucagon-like peptide-1 [GLP-1]), has drawn attention to the gut as an organ involved in the regulation of food intake and body weight. In this review, we will discuss the specific role of gut hormones in the overall regulation of body weight. The fundamental assumption is that body weight is tightly regulated; indeed, overfeeding and starvation experiments, also in humans, have provided ample evidence that weight changes elicited by overfeeding as well as starvation elicit counterregulatory responses that eventually restore body weight [1, 2]. The adjustment of food intake to correspond to the energy expended (due to basic metabolism as well as the individual's physical activity level), thereby ensuring energy balance, is referred to as *homeostatic* regulation of food intake. However, the homeostatic regulation can be overridden by *hedonic* processes that reflect the rewarding, palatable, and pleasurable properties of food. Hedonic feeding has been increasingly invoked to explain how obesity may develop despite the presence of adaptive, homeostatic mechanisms that should protect against overeating. Immediately, one would anticipate that both the hedonic and the homeostatic regulation were matters for the central nervous system, as indicated by numerous studies of the importance of hypothalamic nuclei, including for instance the arcuate nucleus and its interactions with the paraventricular nucleus and other nuclei [3]. However, since the original description by Gibbs et al. that injections of (impure) cholecystokinin (CCK) reduced short-term food intake in a dose-related manner in rats [4], there has been an interest in understanding the role of the gut in the regulation. Gibbs et al. suggested a set of criteria for evaluation of hormones like CCK as physiological regulators: (1) the hormonal signal should be activated by feeding; (2) the hormone, administered exogenously, should significantly decrease

meal size; (3) it should have a rapid onset and a brief duration of action (if it were to be involved in meal termination or meal frequency); (4) its effect should not be due to illness (e.g., evaluated by conditioned taste aversion); and (5) the proposed signal should be effective in physiological doses. The last criterion would be consistent with an endocrine mode of action, i.e., that the signal was transmitted to the brain via the circulation. Subsequent research has shown that this may not be relevant for all gastrointestinal hormones. Furthermore, such criteria, designed for evaluation of the physiological role of the gut hormones, are not necessarily relevant when it comes to the evaluation of the pharmacological potential of the hormones. In early experimental studies, emphasis was often put on effects on meal size versus meal frequency, which again would be physiologically relevant, but may be less important for the therapeutic exploitation of a hormone. The number of gut hormones (which are mainly peptides) evaluated for effect on food intake has been growing steadily over the years; for many years these peptides were thought of as signals released to the bloodstream to act on distant targets (in this case the brain), but many of the newer peptides identified in the gut were eventually localized to nerve cells and fibers of the enteric nervous system, suggestive of nonendocrine mechanisms of action. For many of these *neuropeptides* of the gut, it is currently not possible to estimate their role in food intake because too little is known about their projections, the localization of the receptors, and their mechanisms of activation; for some of them, it seems evident that they have local actions as parts of the enteric nervous system. Many of the peptides are also expressed in the central nervous system where they may serve additional regulatory functions. This makes evaluations very complex. A good example is CCK itself, which probably mainly acts as a classical hormone when it comes to regulation of gall bladder emptying and pancreatic enzyme secretion, but for which other actions depend on interaction with vagal sensory fibers expressing the CCK receptors [5]. Such receptors are also expressed in numerous cortical and noncortical neurons of the brain, and CCK is also produced in numerous CNS neurons where it appears to have a transmitter function, the nature of which and possible role in feeding are still unclear.

CCK will be discussed further below, but otherwise focus will be on the hormones ghrelin, GLP-1, and peptide YY (PYY), while also the potential of glucose-dependent insulinotropic polypeptide (GIP) and oxyntomodulin will be discussed. All these hormones have the actions with which they were originally associated, such as regulation of insulin secretion (GIP and GLP-1), gastrointestinal motility and secretion (GlP-1, ghrelin, and PYY), and stimulation of growth hormone (GH) secretion (ghrelin), but in addition they have all been reported to inhibit food intake (in the case of ghrelin, increase food intake). But what is the evidence that they take part in the physiological regulation of food intake? The fact that they under certain circumstances can inhibit food intake does not necessarily mean that they are important for normal regulation.

Mimicry

Traditionally, in gastrointestinal hormone research, physiological relevance is initially established using the mimicry approach, whereby the assumed actions

of the endogenous hormones are mimicked by administration (infusion) of an exogenous hormone. This requires that the release of the hormone, e.g., during meal intake, is measured (the availability of an accurate assay is therefore essential), followed by infusion of the hormone in question to concentrations similar to those observed during the meal. A positive response (reduction of food intake) supports a physiological function for the peptide, but, of course, only represents a necessary, not a sufficient, condition for this. Indeed, to establish a positive mimicry experiment is often sufficiently difficult to render fulfillment of this criterion problematic – for instance, measurements of the actual hormone concentrations (particularly in rodents) have often been unreliable.

Hormone Antagonists

A much better way of establishing the importance of a new peptide is to acutely block the activities of the peptide with a receptor antagonist. This, of course, requires knowledge about the responsible receptor and, furthermore, requires access to reliable (specific, potent) antagonists, which is clearly a limiting factor. Genetic manipulations may be very useful but also have serious limitations; a knock-in experiment is useless since hyper-expression/-secretion will always be unphysiological. Global knockout, although sometimes informative, very often leads to compensatory changes, which may completely perturb the picture, and acute targeted knockout experiments are difficult to produce and most often involve mice, which are difficult to study and not always relevant for human physiology. A good example is the hormone GLP-1 and its analogs, some of which are among the most powerful appetite and food intake suppressors we know, but where a global knockout of the receptor does not lead to changes in body weight or food intake [6]. Does this mean that endogenous GLP-1 does not participate in the regulation of food intake? Probably not, but a global knockout experiment is not the way to demonstrate this.

Activation and Inactivation

Is there a reliable way to demonstrate unequivocally that the gut hormones contribute to the regulation of food intake under physiological conditions? As already discussed, the most reliable tool we have is blockade with hormone receptor antagonists, since it is a fundamental dogma that these peptides act on (a) specific receptor(s); therefore, acute blockade of these should remove the effect they are responsible for. The use of receptor antagonists has one more very important advantage: if we are talking *small molecule* antagonists (and most are at least as small as the hormones themselves), they are likely to access the same tissues and compartments as the hormones. This means that it will also be possible for the antagonists to interfere with paracrine interactions and activations of neural mechanisms that might be involved, provided the effects depend on interaction with the receptors. In line with this, there are other approaches that may yield similarly specific and relevant results. Thus, if the hormones can somehow be deactivated or their activation prevented, this may have a similar effect as antagonism.

An illustrative example is the use of dipeptidyl peptidase 4 (DPP-4) inhibitors to prevent the conversion of the orexigenic primary secretion product of PYY, PYY 1–36, to PYY 3–36, which is generated by cleavage of the peptide by the enzyme DPP-4 [7]. Conversely, the DPP-4 inhibitors prevent the DPP-4 mediated inactivation of GLP-1 and GIP (and several other substrates), and the plasma concentration of the active forms of these hormones will therefore increase, leading, for instance, to increased insulin secretion [8]. For ghrelin, its biological activity depends on the octanoylation of the serine residue in position no. 2, and inhibitors of the enzyme, ghrelin O-acyl transferase (GOAT) that catalyzes this modification, will inactivate the hormone [9].

Somatostatin as a Tool

There is another approach, which might be described as a physiological sledgehammer: the use of somatostatin. Somatostatin acts via its five different receptors [10], and virtually all gut endocrine cells express one or more of these receptors, which typically inhibit adenylate cyclase via a Gi protein subunit. Administration of somatostatin therefore inhibits the secretion of most (all?) gut hormones [11]. Some of the more long-acting somatostatin analogs developed for clinical use differentially activate the various receptor subtypes (e.g., lanreotide has greater affinity for peripheral rather than central receptors). Somatostatin, however, has actions on many tissues and cells and is an important regulator of pituitary secretion, for instance of growth hormone. In addition, it regulates gastrointestinal function via presynaptic inhibition of neurons of the enteric nervous system. Even more problematic, somatostatin also inhibits the secretion of pancreatic islet hormones [12], which have all been suspected of influencing appetite and food intake. Thus, an effect observed after somatostatin infusion (or injection) is not necessarily due to specific inhibition of (a) gut hormone(s), but might, of course, be compatible with such an effect. Indeed, in experiments with octreotide, a long-acting somatostatin agonist, an increase in food intake was *not* observed in healthy controls, whereas in patients after esophagectomy and with elevated secretion of gut hormones, a positive response, i.e., an increase in food intake, was observed [13].

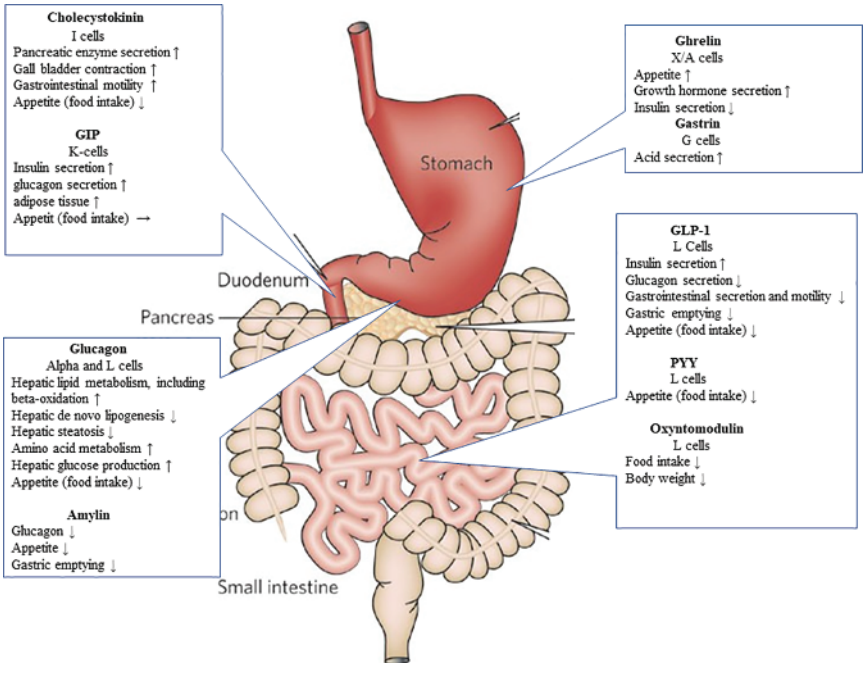
An even more dramatic experimental approach is to study patients with short bowel syndrome. These individuals have a natural drive to eat since insufficient energy intake in their situation would be deleterious. Fortunately, the patients show a ferocious appetite and hyperphagia [14], but, of course, have a clear homeostatic problem to solve. Complicating matters further, a discordance between secretion of gut-derived appetite hormones and energy intake in humans was recently reported [15]. In these studies, volunteers ingested a low-fat (LF) or a low-carbohydrate (LC) diet in a crossover design. The LC meal increased plasma concentrations of GLP-1, GIP, and PYY but lowered ghrelin levels compared to the isocaloric LF meal, while energy intake was 550 kcal greater on the LC meal. Thus, the effects of gut-derived appetite hormones on ad libitum energy intake can be overridden by other diet-related factors, at least in the short-term [15].

In conclusion, in spite of 50 years of research, there is no clear answer to the question of the role of the gut hormones in the regulation of appetite and food intake

under physiological conditions. It appears that the closest we can get is to evaluate, for each hormone, the evidence derived from mimicry and other experiments with exogenous hormone administration and studies with whatever antagonists have been available. In the following, we will discuss some of the evidence for the contribution of the most important gut hormone candidates for regulation of food intake.

Gut Hormones with a Possible Role in Food Intake

An overview of the gut hormones and their main actions discussed in this chapter are presented next.



Cholecystokinin

CCK was discovered in 1929 by Ivy and Oldberg as a physiological principle (endocrine regulation of gall bladder emptying), but it took another 40 years before the chemical nature of the hormone was revealed: a peptide of 33 amino acids [16]. During the purification of CCK, it became clear that the specific bioactivity of the compound in terms of gall bladder motility and stimulation of pancreatic enzyme secretion increased in parallel; eventually, the pure (and subsequently synthetic) peptide exhibited similar dual bioactivity and it was concluded that CCK and pancreozymin were one and the same peptide [16]. The peptide is produced in

the endocrine I-cells of the upper small intestinal mucosa and, upon development of radioimmunoassays for CCK, it could be demonstrated to be released in response to protein and lipid ingestion [17]. Like other peptides, CCK is produced as a larger precursor molecule (95 amino acids), which undergoes posttranslational cleavage and modification including sulfation and amidation of the C-terminus, both of which are essential for its bioactivity, but it has been difficult to reach an agreement regarding the chain length of "cholecystokinin." As mentioned, the structure was originally determined to comprise 33 amino acids, but incomplete cleavage of the precursor and metabolism of the various cleavage fragments results in the formation of several molecular forms, among which the 33-amino acid peptide is only one. A C-terminal (amidated and sulfated) fragment of 8 amino acids (CCK-8) has apparently full biological activity, and several other components, including a 22- and a 58-amino acid component, have been described [18]. The various components have different potencies and different half-lives in the circulation, and these factors complicate interpretation and determination of the physiological functions of "CCK." Furthermore, CCK has two receptors, the CCK 1 and 2 (or A and B) receptors, for which CCK-8 has about the same affinity and potency, whereas the related stomach hormone, gastrin (their C-terminal structures are very similar), also has affinity for the B-form (which is therefore commonly referred to as the gastrin receptor although it has affinity for both), and there is a differential expression of the two receptors [19]. Complicating matters even further, CCK and the CCK receptors are also expressed in several regions of the central nervous system [20] where CCK seems to exert a transmitter function (typically exerted by CCK-8). Obviously, experiments consisting of simple infusions of "CCK" are difficult to interpret.

However, with the advent of specific receptor antagonists (loxiglumid, proglumide), it became possible to analyze at least some aspects of CCK physiology. Smith et al. were again the first to show that the inhibition of feeding with CCK depended on intact afferent fibers of the vagus [21]. Subsequently, Smith and coworkers could demonstrate that a specific CCK1 receptor antagonist resulted in significant increases in meal size, strongly supporting a role for CCK in the regulation of food intake at least in rats [22]. Furthermore, a rat strain with inactive CCK-A receptors (Olefsky rats) showed disordered food intake and obesity [23]. It should be noted that CCK also importantly influences gastric emptying, apparently involving a vago-vagal reflex activity, and it has been suspected that this effect could explain some of the anorexic effects. The CCK receptors are found in numerous locations in the brain [24], but they hardly serve as targets for peripheral CCK, but rather for CCK released from local CCK-containing neurons. Importantly, CCK receptors can also be demonstrated to be expressed on vagal afferent neurons with cell bodies in the nodose ganglion, and there is substantial evidence for the importance of this pathway for the actions of CCK on food intake [25]. Given the solid status of CCK in feeding regulation in rodents, one would expect CCK/CCK receptors to be an attractive target for therapy of humans. However, the role of the CCK system in humans is not nearly as clear as it is in rodents, and long-acting CCK-A receptor agonists did not result in weight loss in overweight or obese individuals [25, 26]. In addition,

CCK-1 receptor agonists may induce pathological findings, including pancreatitis and pancreatic neoplasia, and a long-acting agonist was demonstrated to cause increased pancreas weight and neoplasia at the expected therapeutic exposure levels in monkeys [27]. Indeed, the agonists would also be expected to increase the risk of bile system complications including gallstones and gallstone-related pancreatitis [27]. As discussed earlier, investigations in patients subjected to bariatric surgery, in particular gastric bypass surgery, generally reveal elevations of gut hormones, secondary to accelerated intestinal exposure of nutrients, and these elevations are thought to contribute importantly to the weight loss observed. CCK concentrations are mildly elevated after gastric bypass surgery [28, 29], which may be surprising since the operation bypasses the site of most dense localization of I-cells, but apparently more distally located I-cells are sufficient to explain the elevation. The other operation, sleeve gastrectomy (SG), is also associated with increased intestinal nutrient exposure and shows exaggerated release of several gut hormones, but here the increase in CCK secretion is nevertheless minor [29]. In conclusion, while the evidence for an important role in the regulation of food intake in rodents is extensive, the situation in humans is less clear, and utilization of CCK agonism for therapeutic purposes seems far away.

GLP-1

GLP-1 is a 30-amino acid peptide product of intestinal expression of the gene encoding proglucagon (PG), the precursor of glucagon [30]. When produced in the gut, the 160-amino acid prohormone is cleaved to produce glicentin (corresponding to amino acids no. 1–69 of PG, a fraction of which [about a third] may be cleaved to oxyntomodulin [PG 33–69]), GLP-1 (PG 78–107), and GLP-2 (PG 126–158). In the pancreas, the major products are glucagon (PG 33–61) and the Major Proglucagon Fragment (PG 72–158) [31]. Because GLP-1 was originally thought to correspond to PG 72–107 (which is formed in small amounts in the pancreas and is inactive), the naturally occurring form is often designated GLP-1 7–37 or, since it is often amidated C-terminally (a process that involves the C-terminal Gly residue), GLP-1 (7–36) amide. The cellular origin is the endocrine L-cell [32], one of the most abundant gut endocrine cells, a cell type that also gives rise to the gut hormone PYY (see below). The L-cells are found in increasing numbers along the intestine with a majority in the colon, even in the distal colon and the rectum [33]. The proglucagon products are secreted in response to ingestion of all the major macronutrients, because the L-cells are equipped with both apical and basolateral proteins [34] that either sense G-protein coupled receptors (GPCRs) or transport digested nutrients (like SGLT-1). For instance, for lipids, the cells express the GPCR, GPR 119, reacting with 2-monoacyl glycerols, a major digestive product of nutritional triglycerides [35]. And for protein breakdown products, several apical and basolateral membrane-bound proteins (GPCRs and transporters) are known to react to amino acids and stimulate secretion [36]. Immediately after its release, and already in the capillaries draining the mucosa of the gut, GLP-1 is broken down by the enzyme DPP-4, which is expressed by the endothelial cells of

the capillaries [37]. DPP-4 cleaves off the two N-terminal amino acids, and the metabolite GLP-1 9–39 is inactive. The half-life in the circulation is one to two minutes. Thus, very little GLP-1 reaches the liver in the intact form and even less reaches the peripheral circulation [38]. This creates a problem regarding relevant measurements. It is possible to measure the intact hormone with sandwich ELISA targeting the terminals of the peptide. But the concentrations are very low and do not reflect the secretion of the peptide. Instead, it is possible in humans to measure the metabolite GLP-1 9–36 amide, the concentration of which will reflect secretion [31]. However, it is now believed that GLP-1 mainly signals by activating afferent sensory pathways, a process that may take place in the lamina propria before the entry of the hormone into the mucosal capillaries and degradation by DPP-4 [39, 40]. Measurement of the metabolite may therefore also reflect the actions of the hormone. In mice, measurements are even more difficult because of the actions of the enzyme neprilysin, which destroys the metabolite [41, 42].

Role of Endogenous GLP-1 in Food Intake Regulation

Although discovered on the basis of its insulin releasing properties, GLP-1 is also considered one of the ileal brake hormones, i.e., an enterogastrone hormone, released from the ileal mucosa in response to remaining nutrients, with inhibitory effect on upper gastrointestinal secretion and motility ([43, 44]). This hormonal axis also involves inhibition of appetite and food intake. GLP-1 was shown in 1996 to inhibit food intake, when administered intra-cerebro-ventricularly (ICV) [45], but it is doubtful whether this mode of administration is relevant for peripherally secreted GLP-1 or for subcutaneously injected GLP-1 receptor agonists. Rather, the numerous cerebral receptors are targets for GLP-1 produced by a small group of neurons in the nucleus of the solitary tract of the brain stem [46]. These neurons appear to receive excitatory input from peripheral stimuli, e.g., gastric distension [47], but they are not activated by peripherally administered GLP-1. Appetite suppression and food intake inhibition by peripheral GLP-1 were first demonstrated in 1998 by Flint et al. [48], and soon confirmed by many other groups, and in 2001 it was determined in a meta-analysis that the effect was dose dependent, i.e., the more GLP-1 infused the more food intake would be inhibited [49]. In spite of this, mice with deletion of the GLP-1 receptor do not become obese. Similarly, the antagonist of the GLP-1 receptor, exendin 9–39, only variably and weakly influences food intake and appetite sensations [50] in humans although it apparently did block CNS responses to palatable food consumption as determined by functional Magnetic Resonance Imaging (fMRI) [51]. The antagonist is probably doing its job, since it blocks the food intake inhibiting actions of exogenous GLP-1 receptor agonists. Because the lack of effect of the antagonist contrasts to the powerful inhibition of food intake with GLP-1 receptor agonists, one may ask whether exendin 9–39 elicits other effects that may counteract the effects of the GLP-1 blockade on appetite and food intake. Thus, in humans, Ex9 invariably increases plasma concentrations of glucagon and glucose, which may both dampen appetite. It may be that effects of GLP-1 are mainly seen during conditions of strong stimulation of secretion as in conditions with accelerated gastric emptying [31]. Attempting to identify the roles

of the exaggerated secretion of both GLP-1 and PYY (see below) for the weight loss after gastric bypass surgery, Svane et al. [7] studied ad libitum food intake after blockade of either or both of these hormones. For GLP-1 receptor blockade, they used the GLP-1 receptor antagonist exendin 9–39. For PYY, there is no antagonist for human use, but the conversion of PYY 1–36 to PYY 3–36, which is required for activation (see below), can be prevented by inhibitors of the responsible enzyme, DPP-4. Svane et al. also studied the secretion of the two hormones in response to a test meal. Importantly, blockade of the GLP-1 receptor led to greatly augmented PYY 3–36 responses (caused by a negative feedback mechanism regulating L-cell activity), and DPP-4 inhibition greatly increased intact GLP-1 responses; thus, as the effects of one hormone were prevented, secretion of the other hormones increased, presumably negating the effect of the inhibition and vice versa. Consistent with this, combined blockade resulted in a 20% increase in ad libitum intake, providing strong support for the importance of these two hormones for weight loss after gastric bypass surgery.

Mechanism of Action of Exogenous GLP-1 Receptor Agonists

As mentioned earlier, the injected agonists of the GLP-1 receptor powerfully inhibit food intake, and it has been demonstrated that their activity depends on interaction with cerebral receptors [52]. Fluorescent or radioactive GLP-1 agonists can be shown to access several of the circumventricular organs, where the blood-brain barrier is leaky, including the median eminence, the area postrema, and the subfornical organ [53, 54]. Notably, no binding is observed in animals with a deletion of the GLP-1 receptor gene, suggesting that GLP-1 and its analogs do need to enter the brain to exert activity. A subsequent activation (as determined by c-Fos staining) can be demonstrated in the nucleus of the solitary tract, in several nuclei of the hypothalamus, in the amygdala, and in the parabrachial nucleus [53]. These, therefore, should be considered the initially engaged pathways.

Development of GLP-1 Receptor Agonists for Obesity Therapy

In the earliest studies, GLP-1 was infused to mimic high physiological increases in plasma concentration and did result in significant inhibition of food intake and appetite sensations, consistent with a physiological effect on food intake [48], and, as already mentioned, the response could also be demonstrated to be dose-dependent. However, side effects consisting of nausea, vomiting, and stomach pain, observed during sudden increases in plasma levels of GLP-1, prevented investigations of higher doses [55]. With the advent of GLP-1 receptor agonists, developed for diabetes therapy, similar problems were observed [56], but further studies showed that a slow up-titration of dosing to some extent could mitigate the side effects [57], and in a study where liraglutide was given in a fixed combination with the long-acting insulin analog, insulin degludec, and where the up-titration was done according to the insulin component (fasting blood glucose) and thus took place over a 14-week period, there were virtually no gastrointestinal side effects [58]. Subsequent careful dose-response studies with increasing doses of liraglutide

revealed that higher doses of this once-daily agonist resulted in weight losses up to 7–8 kg over a 20-week period [59]. This led to the SCALE trials, phase 3 trials [60] of 3 mg liraglutide daily for obesity therapy. The large SCALE 1 trial, which was extended over three years, showed persistent weight loss and an 80% reduction in the risk of developing diabetes during the trial [61]. The SCALE trials led to approval of liraglutide 3 mg for obesity therapy, under the name of Saxenda. This marked the opening of the therapy of obesity with the GLP-1 agonists. A second generation agonist, semaglutide, developed for improved actions on both food intake and insulin secretion and for a longer half-life (weekly) [62] was also tested in a dose-response study (with daily doses to avoid side effects of the larger weekly doses) [63]. The trial showed that 0.4 mg daily over a year would result in a weight loss of 14%. A series of phase 3 trials, the STEP trials [64], with semaglutide at 2.4 mg per week given to people with obesity but no diabetes were then initiated. In these studies, weight losses of up to 18% were observed, and the compound was approved for therapy of obesity under the trade name of Wegovy. In March 2026, a higher dose of semaglutide was approved for chronic weight management.

It has been discussed whether the side effects of high doses of GlP-1 receptor agonists are inseparably associated with the appetite reducing effect and involve the same cerebral pathways [65]. However, the fact that it is possible to develop some insensitivity to the effect, while the effect on food intake is preserved, suggests that separate mechanisms are involved. This raises the possibility that agonists without these side effects could be developed. Recently, it has been proposed that co-administration of GIP with GLP-1 reduces the side effects of GLP-1 [66], and this might explain why the GLP-1-GIP coagonist tirzepatide can be administered in higher doses and therefore may have an even greater effect on body weight (up to 25%). Proof for this hypothesis is still lacking, however.

Oxyntomodulin and Glucagon-GLP-1 Coagonists

Oxyntomodulin is a 37-amino acid product of proglucagon, corresponding to residues no. 33–69 of proglucagon [67]. The peptide was known to exist as a glucagon-related peptide in gut extracts by size exclusion chromatography of "gut glucagon" (enteroglucagon) [68], and its definitive structure was determined in 1982 [69, 70]. Dominique Bataille felt that it had special effects on oxyntic gland secretion (gastric glands) in experimental models and therefore suggested the catchy name oxyntomodulin. In extracts of human intestinal mucosa, it constitutes about a third of the glucagon-containing products of proglucagon (with glicentin [PG 1–69] responsible for the remaining 2/3), and it is released from the L-cells in about the same proportion [68]. This also means that its concentration is rather low under most circumstances and that its secretion follows the secretion patterns of glicentin and GLP-1. Subsequent studies revealed that it binds to the glucagon receptor and the GLP-1 receptor but with about 1/100 of the affinity of the cognate ligands [71]. Thus, it is difficult to see that it plays a major physiological role under normal circumstances. However, under conditions with severely increased secretion of the proglucagon-derived intestinal products, the levels may rise sufficiently

to influence these receptors [72]. Such conditions are almost all associated with accelerated gastric emptying, and the secretion is particularly pronounced after gastric bypass operations [72], where secretion and, therefore, the levels of glicentin and oxyntomodulin may increase up to 30-fold.

The current interest in oxyntomodulin relates to the discovery that it is a natural glucagon-GLP-1 coagonist. Two groups showed in 2009, based on studies with oxyntomodulin, that stabilized and long-acting analogs might have a particularly pronounced effect on food intake and body weight, as expected because of the anorexic properties of both GLP-1 and glucagon. It was also found that the combination with GLP-1 ensured that the hyperglycemic effects of their glucagon agonism did not predominate [73, 74]. Thus, glucagon-GLP-1 coagonists appeared to have treatment potential. Since then, several companies have engaged in the production of glucagon-GLP-1 coagonists. This development is surprising in view of the efforts previously made to produce glucagon *antagonists* for diabetes therapy in recognition of the importance of glucagon for diabetic hyperglycemia [75]. Although the glucose-lowering effects of such antagonists were confirmed with several compounds, they also had unwanted effects on lipid metabolism, with aggravation of hepatic steatosis and hyperlipidemia [76]. Glucagon *agonism*, on the other hand, importantly affects hepatic lipid metabolism, including increased beta-oxidation, lipolysis, and inhibition of de novo lipogenesis [77]. GLP-1 was of course already effective in antidiabetic treatment; thus, by combining glucagon and GLP-1 action, one would further augment the anorexic effect and improve the metabolism-associated steatosis of most patients with obesity and type 2 diabetes. This assumption seems to be correct. Several published studies with various GLP-1/glucagon coagonists have shown important reductions in hepatic steatosis and weight loss in patients both with and without type 2 diabetes [78]. So far, only a single compound (Mazdutide) has reached the market (in China), and there may be outstanding issues with adverse effects – both GLP-1 and glucagon may cause nausea and vomiting and both increase heart rate. In addition, the powerful effects of glucagon on amino acid metabolism may be associated with unwanted increases in protein turnover.

PYY

PYY is a peptide of 36 amino acids with tyrosine residues (one letter code: Y) at both terminals (hence the name) and a member of a family of peptides that also comprises the very similar 36-amino acid peptides, pancreatic polypeptide (PP) and neuropeptide Y (NPY). It was discovered by Kazuhiko Tatemoto who, working with Victor Mutt in Stockholm, isolated the peptide from pig intestinal extracts using an original assay for amidated peptides [79]. Thus, it was isolated without any notions about biological activity [79]. Early studies suggested that it would inhibit pancreatic and gastric secretion. It is produced in the endocrine L-cells of the intestinal mucosa, most of which also produce proglucagon and the proglucagon-derived peptides (GLP-1 and oxyntomodulin); indeed, PYY is even localized to the same granules as these

hormones [79, 80]. However, not all L-cells contain both hormones; most of the proximal L-cells of the small intestine do not contain PYY [81], whereas some of the distal cells do not secrete proglucagon-derived peptides [82]. The density of L-cells increases distally and is highest in the colon [83]. This family of peptides is also called the PP-fold peptides, because of a definite secondary structure: the peptides make a turn around the middle so that the two termini come close together in a hairpin-like shape. The proximity of the termini is essential for the receptor interaction [84]. The PP-fold family of peptides binds to the so-called Y or NPY receptors [85], comprising five subtypes; thus, the type and proximity of the terminals of the peptides determine the interaction with the extracellular compartment of the Y receptors. In the gut, PYY is produced as PYY 1–36, which has a high affinity for the Y1 receptor, which transmits increased appetite, and initially PYY was also found to stimulate food intake, undoubtedly because of its Y1 receptor activation in the models investigated, for instance intracerebroventricular injection. Even in 2002, PYY was designated "a key mediator of orexigenic behavior" [86]. However, a truncated form, produced by cleavage mediated by the enzyme dipeptidyl peptidase-4 (DPP-4), was also identified [87]. The product, PYY 3–36, no longer binds to Y1 but instead to the Y2 receptor [88]. Batterham et al. demonstrated inhibition of food intake by exogenous PYY 3–36 in both rats and humans acting via the Y2 receptor [89] and showed that PYY 3–36 was effective also in obese individuals (unlike leptin) [90]. These reports gave rise to considerable controversies [91, 92]. However, Boey et al. studied mice with knockout of PYY and found that they were prone to the development of obesity and glucose intolerance [93], and today there seems to be agreement regarding the inhibitory effects of PYY on food intake [94]. Batterham et al. also reported lower levels of PYY in plasma, suggesting that PYY might be involved in the pathogenesis of obesity. The effect is thought to be exerted by interaction of the peptide with Y2 receptor containing neurons in the arcuate nucleus, suggesting that circulating PYY 3–36 reaches these regions of the brain, possibly via the median eminence (one of the circumventricular organs with a leaky blood-brain barrier). This hypothesis is supported by the finding that peripheral PYY can cross the blood-brain barrier by a nonsaturable mechanism [95]. However, other data point toward a vagal route of PYY signaling since the food intake-reducing effect of PYY following intraperitoneal injection was found to be blocked by vagotomy in rats. Moreover, vagotomy also greatly decreased the PYY-induced expression of Fos in the arcuate nucleus (ARC) [96, 97]. Therefore, peripheral PYY is likely to signal to the brain via the vagus nerve and via direct interaction with hypothalamic nuclei after crossing the blood-brain barrier.

Measurements of PYY in plasma are by no means simple. First, the close structural relationship between the members of this family of peptides (also including the neuropeptide NPY and the islet peptide, pancreatic polypeptide) poses great demands on the specificity of the antibodies employed for immunoassays; second, to be interpretable in terms of metabolic consequences of secretion, it is necessary to distinguish between the 1–36 and the 3–36 forms. In addition, it turns out that the peptide is also degraded rapidly from the C-terminus, where plasma enzymes are responsible for sequential cleavage of the 36 and 35 residues, rendering PYY

3–34 a significant metabolite [98]. Importantly, both PYY 3–34 and the intermediate, PYY 3–35, are biologically inactive. Thus, there are no immunoassays currently available that can accurately measure active PYY 3–36, and predictions on the biological impact of changes in its plasma concentrations cannot be made. However, perhaps with recent mass spectrometry methods, this might now be possible [99].

Side effects (nausea and vomiting) during infusion into humans were also a concern, and in a series of studies, Sloth et al. attempted to identify conditions that would result in robust inhibition of food intake with both PYY 1–36 and 3–36 in humans, while minimizing the side effects [100, 101]. However, the effects of both hormones were disappointing after subcutaneous injections, and inhibition of appetite was invariably associated with adverse effects and signs of sympathoadrenal activation during intravenous infusions of PYY 3–36, whereas PYY 1–36 was without effect. Support for a role for PYY in appetite regulation in humans came from studies of gastric bypass surgery, where the bypass was associated with greatly elevated levels of PYY and GLP-1 [102, 103] (see above).

In further studies, Neary et al. [104] found additive effects of infusions of PYY and GLP-1 on appetite and food intake, and Schmidt et al., who studied very low doses of the two hormones, were able to identify a clearly potentiating interaction between the two, meaning that the inhibition of food intake after combined infusion was greater than the sum of the inhibitory effects of the separate infusions [105]. Attempting to identify the roles of the exaggerated secretion of both PYY and GLP-1 in the weight loss after gastric bypass surgery, Svane et al. studied ad libitum food intake after blockade of either or both hormones using the GLP-1 receptor antagonist exendin 9–39 and inhibiting conversion of PYY 1–36 to PYY 3–36 with an inhibitor of the enzyme DPP-4, as described earlier [7]. Combined blockade resulted in a 20% increase in ad libitum intake, supporting an important interaction between the two hormones and providing strong support for the importance of these two hormones for the weight loss after gastric bypass surgery.

So far there are no reports of a suitable PYY agonist for human use in the treatment of obesity, but a dual agonist with efficacy for both GLP-1 and Y2 receptors has been described [106]. Furthermore, coadministration of PYY, GLP-1, and oxyntomodulin, mimicking the pattern observed after gastric bypass operations in humans, by subcutaneous infusions for four weeks, has been effective in providing weight loss in healthy humans [107] as well as individuals with prediabetes and obesity.

GIP

The 42-amino acid hormone, glucose-dependent insulinotropic polypeptide, GIP, was discovered by John Brown, working with Victor Mutt in Stockholm, on the basis of its inhibitory effects on acid secretion in denervated gastric pouches in dogs – hence its original name: gastric inhibitory polypeptide[108]. After the discovery that it also stimulated insulin secretion in humans [109] (whereas its role in gastric inhibition in humans was less clear), it got its new name, retaining the

acronym, and a series of mimicry experiments indicated that the new hormone was likely to be one of the long-sought incretin hormones [110], hormones from the gut that would enhance insulin secretion during oral intake of glucose (and perhaps other nutrients). In 2017, a GIP receptor antagonist suitable for human use was discovered [111], and in experiments with antagonists against the GIP and the GLP-1 receptor, it could be demonstrated that GIP was an important incretin hormone in humans, responsible for almost half of the incretin effect [112].

GIP is secreted from the endocrine K-cells of the duodenal and the proximal jejunal epithelium in response to carbohydrate and fat ingestions [113]. Like GLP-1 and PYY, it is degraded by the enzyme DPP-4, and the metabolite of GIP is inactive [114, 115]. The half-life of GIP is about 7 minutes in humans, and that of the metabolite about 30 minutes, compatible with renal elimination by glomerular filtration [116]. GIP is processed from a 92-amino acid precursor molecule, which is not known to produce alternative molecular forms of GIP or additional bioactive molecules [117]. A shorter form, GIP 1–30 amide, which is fully biologically active on the GIP receptor, may be formed in very small amounts, but does not seem to play a specific role [118, 119]. As for the other substrates of DPP-4, the hormone circulates in an intact form (GIP 1–42), representing about 50%, and as the truncated metabolite, GIP 3–42, but the ratio between the two molecular forms does not seem to change much. Regarding its circulating concentrations, measurements of the intact form would of course be preferable, but in qualitative terms more or less similar results are obtained with total GIP assays [120]. Assays employed in the older literature showed cross reactions with poorly characterized substances, among these is the so-called GIP 8000, with little or no relationship to GIP secretion [121]. Current assays seem to be more specific. The GIP receptor is expressed in the beta cells of the pancreatic islets in agreement with the activity on insulin secretion (but also in the other islet cell types) [122]. There is also a somewhat diffuse expression in numerous other tissues, including the adipose tissues, where the receptor may be expressed on both adipocytes and nonadipocyte cell types [123, 124]. The glucose-dependent insulinotropic polypeptide receptor (GIPR) is apparently also expressed in the brain [125], but currently the more precise localization is uncertain [126], and in particular expression in the human brain is unclear. Interestingly, the effect of GIP on insulin secretion in people with type 2 diabetes is lost or nearly absent [127]. The reason for this is unknown, but it may be related to defective glucose-induced insulin secretion.

The current interest in GIP and its effects in food intake derives from the remarkable effects of the GIP-GLP-1 coagonist, tirzepatide [128]. In the phase 3 trials for the obesity indication, treatment with this compound resulted in up to 25% weight loss in the course of 72–88 weeks [129], seemingly greater than those obtained with semaglutide, which is a monoagonist of the GLP-1 receptor. Also, the effect on glucose control in people with T2DM seems to be greater than that of semaglutide, with more than 50% of treated individuals reaching hemoglobin A1c values below 5.7% [130].

These findings are surprising indeed, since GIP has been thought to *promote* obesity based on numerous experimental observations. The early literature was summarized by Vincent Marks in 1988 in his paper "GIP – The Obesity Hormone"

[131], and a detailed follow-up was provided by Yip and Wolfe in 2000 [132]. The notion of a role of GIP in promoting obesity was supported by several findings including the following: it appears to stimulate fatty acid synthesis in the adipose tissues; it enhances insulin-stimulated incorporation of fatty acids into triglycerides and may increase insulin receptor affinity and insulin-stimulated glucose transport; and it was also reported to enhance postprandial lipid clearance from the circulation. A lipolytic activity has also been demonstrated, and it has been proposed that insulin may act as a switch between lipolysis and lipogenesis. Importantly, mice with knockout of GIP or the GIP receptor are resistant to diet-induced obesity [133]. Furthermore, the GIP receptor antagonist was demonstrated to block the effects of GIP on adipose tissue blood flow and lipid uptake, and the antagonist also impairs GIP-induced increases in splanchnic blood flow and nutrient uptake [134]. In genetic studies, inactivating variants of the GIP receptor are associated with leanness [135]. The lack of activity of GIP on glucose control in patients with type 2 diabetes has been confirmed in many studies, but its effects on food intake are more important for the current discussion. The development of tirzepatide was preceded by rodent studies carried out in Munich, where a GIP-GLP-1 coagonist was demonstrated to have good effects in experimental animals in 2013 [136]. In 2019, in a paper summarizing the effects of GIP on food intake and body weight in rodents, it was shown that while GIP mono-agonism did not have impressive effects on body weight, combinations with GLP-1 were remarkably effective [137]. Paradoxically, in other laboratories, GIP antagonists, developed on the basis of assumptions of its role to promote obesity, showed similar results [124], and indeed currently a GIP antagonist (a monoclonal antibody directed against the GIP receptor to which is attached a GLP-1 moiety) has shown remarkable weight-reducing and glycemia-improving effects [138] and is currently in phase 3 clinical trials.

Isolated experiments, designed to demonstrate effects of GIP on food intake in humans, have so far not been able to reveal any effects [139]. Addition of GIP to GLP-1 administrations has had only negative effects on plasma glucose and lipids, on liver fat, and has shown no effect on food intake [140]. Development of a long-acting GIP mono-agonist produced by NovoNordisk was terminated in 2023 due to lack of additional activity when combined with semaglutide (press release April 2023). A long-acting GIP agonist was presented by Eli Lilly at diabetes conferences in 2023 but showed very moderate and barely significant effects on blood glucose levels and body weight, and similar findings have been presented by NovoNordisk.

Two interesting hypotheses have been presented to explain the apparent superiority of tirzepatide. Tirzepatide has been demonstrated to be a "wild-type" agonist for the GIP receptor but a biased agonist on the GLP-1 receptor with preference for the cAMP pathway compared to the beta-arrestin pathway, which is responsible for receptor internalization and termination of activity [141]. Presumably a biased agonist may therefore have prolonged activity. The other proposal is that coadministration of GIP decreases the gastrointestinal side effect of GLP-1 administration [66]. With a GIP-GLP-1 coagonist, it may, therefore, be possible to use higher doses of the coagonist without running into the limiting side effects of GLP-1 agonism.

In conclusion, the effects of GIP on the control of food intake, particularly with regard to humans, are uncertain. However, the GIP/GLP-1 coagonist, tirzepatide, no doubt represents an remarkable advancement in the treatment of both diabetes and obesity.

Ghrelin

Ghrelin was discovered in 1999 as a ligand for the secretagogue receptor of the growth hormone-producing somatotrophs in the pituitary (the GHSR-1A receptor) [142]. Surprisingly, its natural ligand, ghrelin, turned out to be produced and secreted from endocrine cells of the gastric mucosa, until then designated A-like or X-cells. Expression of the gene gives rise to a preproghrelin of 117 amino acids [143], which is homologous to promotilin. Ghrelin is a 28-amino acid peptide carrying an unusual posttranslational modification, octanoylation of the serine residue in position 3, catalyzed by the enzyme GOAT (ghrelin O-acyl-transferase) [9]. The octanoylation (other fatty acids may also be used) may be lost after release in the circulation, and the deacylated molecule loses some of its actions. In agreement with its receptor localization, ghrelin stimulates GH secretion, but measurements of its secretion revealed an unusual pattern, with increasing concentrations in the interprandial periods, followed after initiation of food intake by decreases proportional to the size of the meal [144, 145]. In agreement with this pattern, ghrelin is thought to increase appetite and contribute to meal initiation. The most important receptors for this activity appear to be localized on the food intake-promoting NPY and agouti-related protein (AGRP) cells of the arcuate nucleus of the hypothalamus [146]. It is not clear how peripheral ghrelin gets there, and a hypothalamic source of ghrelin has been proposed, but remains controversial. Alternatively, ghrelin seems to engage afferent vagal fibers [5], and the growth hormone secretagogue receptor (GHSR) also appears to be expressed in gastrointestinal sensory neurons emanating from spinal dorsal root ganglia; thus, sensory neuron GHSR deletion attenuated diet-induced obesity [147]. Furthermore, viral tracing suggested crosstalk between gut non-vagal sensory afferents and adipose sympathetic outflow, pointing to alternative pathways for regulation of energy homeostasis [147]. Interestingly, ghrelin and GLP-1 may have opposing effects on behavioral patterns related to obtaining and consuming palatable foods. While meal anticipation is largely driven by endogenous ghrelin, GLP-1 has opposing effects. Preference for high-fat/sugar foods is also heightened by ghrelin and reduced by GLP-1 signaling [148].

Peripheral administration of ghrelin has been reported to lead to increased food intake in both rats and humans [149, 150], but endogenous ghrelin levels are lower in obesity, suggesting that the hormone is not directly responsible for the increased food intake [144, 151]. On the other hand, ghrelin levels increase in response to weight loss and may contribute to the difficulties of maintaining weight loss. Ghrelin appears to be involved also in glucose regulation, perhaps in particular the ghrelin produced locally in the epsilon cells of the pancreatic

islets. Here, ghrelin increases somatostatin secretion and inhibits insulin secretion with resulting impairments of glucose tolerance [152]. Interestingly, ghrelin levels have been found to be elevated in individuals with the Prader-Willi syndrome, a syndrome of obesity associated with chromosomal deletions and associated with "ferocious appetite," which may be partly due to the high ghrelin levels [153]. The biology of ghrelin has been extensively studied in rodents and the number of publications is overwhelming (see Muller et al. [154] for comprehensive review [154]). Stimulation of food intake in humans was found already in 2001 [149] and has been confirmed in several subsequent studies, also including obese subjects [155]. The studies have generally included few individuals, and in some studies, for instance, in vagotomized individuals [156], there was no effect of the infusion. Also in gastrectomized subjects, there were no effects [157]. These results would be compatible with an essential role of the stomach and/or the vagus for the effects of ghrelin. The studies generally used high doses (5 pmol/kg × minutes) that give rise to supraphysiological plasma concentrations, and one group concluded that circulating ghrelin was unlikely to function as a physiological regulator of food intake since more physiological doses had no effects on food intake [158]. Such experiments obviously depend on the accuracy of the assays employed, but with a peptide the size of ghrelin, infusion rates of 5 pmol/kg × minutes would be expected to result in very high levels compared to those usually reported for peptides in human studies.

Knockout studies have not been entirely conclusive; ghrelin knockout mice (ghrelin−/−) have a normal body size, body composition, bone density, growth rate, gastric emptying, food intake, reproduction, gross behavior, and tissue pathology [159, 160]; and *Ghsr*-null mice are not dwarfs; their appetite and body composition are comparable to that of wild-type littermates [161]. Other investigators using another knockout model concluded that ghrelin signaling is required for the development of the full phenotype of diet-induced obesity, but also in those studies, the spontaneous effects of the knockout were inconspicuous [162] and a clear role in the development of obesity could not be identified [163].

Antagonist studies have so far not led to compounds with a clear therapeutic potential in obesity, and small molecule agonists and inverse agonists have not yet reached clinical development [164].

An interesting pharmacological approach is the application of inhibitors of the GOAT enzyme, preventing the octanoylation and thereby inactivating the circulating ghrelin, but this approach has so far not been clinically successful [9].

Measurements of ghrelin after bariatric surgery are generally consistent with a contribution of ghrelin to the weight loss observed since plasma levels fall considerably after surgery as might have been suspected [165]. In a study where the patients were especially selected to be comparable regarding both preoperative weight and weight loss, it was possible to evaluate the importance for the weight loss of differences regarding hormonal changes [7]. In this study (the results of which differ markedly from those obtained in consecutive patient groups without weight matching ([166])), postoperative levels of PYY and GLP-1 were much less elevated after gastric sleeve compared to gastric bypass, and ghrelin levels were

much lower (in agreement with the removal of a large part of the stomach). This pointed to a particularly important role for ghrelin changes after SG. A follow-up study was therefore performed to evaluate the importance of the ghrelin changes by substitution. Twelve participants scheduled for SG were included. Before and three months after surgery, a mixed-meal test followed by an ad libitum meal test were performed with concomitant infusions of acyl-ghrelin (1 pmol/kg/minutes) or placebo. Infusions began 60 minutes prior to meal intake to reach a steady state before the mixed-meal and were continued throughout the study day. Two additional experimental days with 0.25 pmol/kg × minutes and 10 pmol/kg × minutes and acyl-ghrelin infusions were conducted three months after surgery. Both before and after SG, postprandial glucose concentrations increased dose-dependently during ghrelin infusions compared with placebo infusions (notably, complete substitution to preoperative levels was obtained with the 0.25 pmol/kg × minutes dose). Ghrelin infusion inhibited basal and postprandial insulin secretion rates, resulting in lowered measures of β-cell function. Ad libitum meal intake did not differ during ghrelin administration compared with placebo. It was concluded that the permanently lower concentration of ghrelin following SG surgery may contribute to the improved glycemic control after SG, but apparently had no influence on food intake [167].

LEAP2 is a liver-produced antimicrobial peptide that was demonstrated to act as a specific antagonist of the ghrelin receptor [168, 169]. Infusions of LEAP2 were recently found to decrease appetite and food intake [169]. The release of LEAP2 from the liver (and intestine) is not yet well defined, but the findings so far suggest that ghrelin levels should be compared with circulating levels of LEAP2 to estimate the impact of the ghrelin system on, e.g., food intake.

Conclusion

It is clear from the aforementioned presentation that the gut harbors several peptides with powerful effects on food intake. The results of bariatric surgery, where in particular Roux-en-Y gastric bypass is associated with grossly elevated plasma levels of several of these gut hormones, indicate that this endogenous appetite-regulating system is sufficiently powerful to cause weight losses that correspond to the aspirations of many obese individuals. It is also sufficient to cause great improvements in the health status of the patients with reductions in the risk of development of the complications of obesity and extension of life expectancies, by five years in patients with normal glucose tolerance and nine years in those with diabetes. Fortunately, it seems that several of the hormones, when applied in forms with extended half-lives in the circulation, are capable of producing weight losses approaching those of bariatric surgery, with for instance tirzepatide, the GIP-GLP-1 coagonist, causing weight losses of 25% after 88 weeks of treatment. If one assumes – and admittedly, it remains an assumption, although well supported by experimental and observational data – that the results of the surgery are mainly due to these hormonal changes, then it follows that similar results may be obtained after long-term treatment with the exogenous agonists. If true, this is greatly

encouraging and raises hopes of much improved future therapy for many more people living with obesity and elevated risk of complications. Conversely, the results also support the notions about the importance of the gut hormones for appetite and food intake regulation in healthy individuals, although the evidence for their role is weaker. Thus, the normal regulation of food intake undoubtedly involves many more factors than merely the release of gut hormones.

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