Part One

# The Physiology of Metabolic Tissues Under Normal and Disease States

# Chapter 1

# Gut as an Endocrine Organ: the Role of Nutrient Sensing in Energy Metabolism

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## INTRODUCTION

Energy homeostasis is balanced by food intake and energy expenditure. Both events are controlled by complex sets of neuronal and hormonal actions. Food intake is driven by a central feeding drive, namely, the appetite, which is induced under the fasting state after energy consumption through physical activities. Following food digestion, the passage of nutrients through the gastrointestinal (GI) tract generates signals that produce sensations of fullness and satiation. In particular, nutrients interact with receptors in the small intestine and stimulate the release of peptide hormones, the actions of which mediate physiological adaptations in response to energy intake. The commonly known GI peptides include the incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide or gastric inhibitory peptide (GIP), as well as peptide tyrosine tyrosine (PYY), cholecystokinin (CCK), and oxyntomodulin. These peptide hormones are secreted from different regions of the small intestine. GLP-1, oxyntomodulin, and PYY are secreted from endocrine L cells that are mainly distributed in the distal small intestine (1, 2), whereas GIP is secreted from endocrine K cells primarily localized in the duodenum (3, 4). CCK is secreted from I cells in the duodenum (5). Nutrients released through the digestive tract induce secretion of GI peptide hormones, which subsequently bind to their respective receptors and trigger a cascade of physiological events. These receptors are expressed in tissues such as the central nervous system (CNS), the GI tract, and pancreas, and upon activation lead to suppression of appetite,

*Metabolic Syndrome: Underlying Mechanisms and Drug Therapies* Edited by Minghan Wang

reduced gastric emptying, and assimilation of nutrients. Nutrients can also suppress the secretion of GI peptides. For example, ghrelin, a peptide hormone released by the stomach under the fasting state that stimulates food intake (6), is suppressed after food ingestion (6).

GI peptides mediate two principal physiological events: (i) the feedback response on the CNS and the stomach to reduce food intake and slow gastric emptying, and (ii) the feedforward response, mediated particularly by the incretins, to prepare tissues for nutrient integration. In this regard, the small intestine is not only an organ for nutrient absorption but also a major site for providing hormonal regulation of energy intake and storage. GLP-1 and GIP are called incretins because they act on the pancreatic β-cells to increase insulin secretion at normal or elevated glucose levels. They also regulate glucagon secretion by pancreatic α-cells. These actions represent a critical step in preparing the body to switch from the fasting state to postprandial activities. By suppressing glucagon secretion, GLP-1 shut down hepatic gluconeogenesis and adipose lipolysis, two key biological pathways in maintaining energy homeostasis under the fasting state. In addition, GLP-1 can act directly on liver and muscle to regulate glucose metabolism independent of its incretin action (7). In the meantime, induction of insulin secretion by the incretins facilitates glucose uptake by the peripheral tissues. GLP-1 is also involved in the feedback response by acting on the CNS to suppress food intake. PYY and CCK exhibit a similar effect in the CNS underscoring the complexity of appetite regulation.

The magnitude and potency of the feedback and feedforward responses depend on both the nutrient content and the length of small intestine exposed. Although both glucose and free fatty acids (FFAs) modulate the secretion of GI peptides, their actions are mediated by distinct mechanisms because they have different residence times in the small intestine and interact with different nutrient-sensing receptors. In fact, even the activity of FFAs varies with their chain length. Moreover, the intestinal length exposed to nutrients and the nutrient contact sites are important determinants in GI peptide secretion.

## FOOD INTAKE AND NUTRIENT-SENSING SYSTEMS IN THE GI TRACT

After ingestion, food chime is mixed with digestive juices in the stomach and propelled into the small intestine. The three segments of the small intestine, the duodenum, the jejunum, and the ileum, perform different digestive functions (Figure 1.1). Nutrients are generated from the digestion of carbohydrates, fat, protein, and other food components. The passage of nutrients through the small intestine not only facilitates absorption but also plays a role in regulating gastric emptying and satiety. The interaction of nutrients with the small intestine segments generates signals that regulate the rate of gastric emptying and food intake. The nutrient-sensing system consists of receptors, channels, and transporters in the open-type cells on the small intestine luminal surface. It responds to macronutrients and activates signaling pathways leading to the release of GI peptides, which subsequently act on the stomach and the CNS to

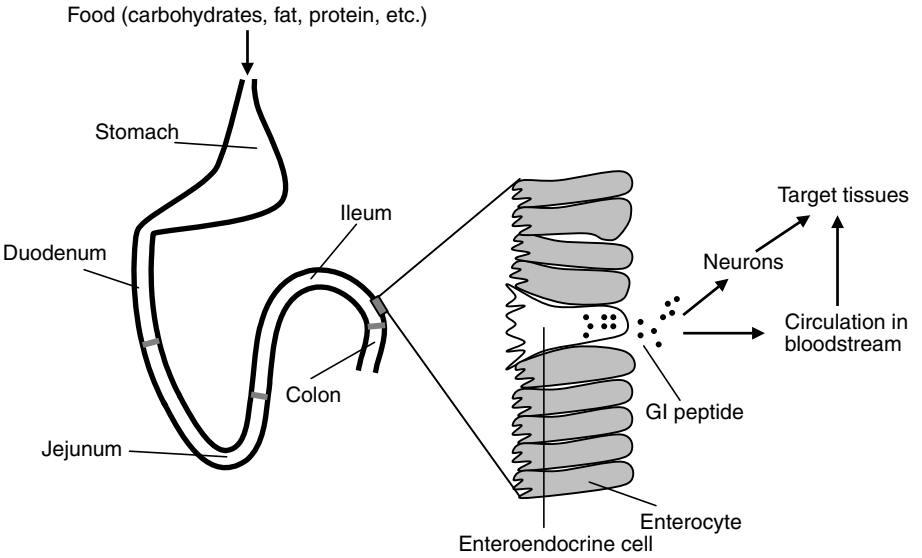


**Figure 1.1** Localization of enteroendocrine cells in the GI tract. Enteroendocrine cells (exemplified by an L cell) are on the surface of the GI tract where their luminal sides detect nutrients passing in the lumen, leading to intracellular signals that stimulate the secretion of GI peptides. GI peptides exert biological effects by acting on their receptors in nearby neurons that transduce signals to target tissues. The peptides are carried to target tissues through the circulation and act locally on sites such as the CNS, the pancreas, and the stomach.

slow gastric emptying and suppress appetite, respectively (Figure 1.1). In addition, some peptides such as the incretins stimulate insulin secretion and regulate glucagon secretion to help integrate nutrients into tissues post absorption (Figure 1.1).

Studies in pigs demonstrated that rapid injection of glucose into the duodenum during or immediately prior to feeding suppressed food intake (8, 9). The reduction in food intake far exceeded the energy content of the infused glucose (8, 9), suggesting that the effect of glucose on food intake is likely to be mediated by signaling events. In the meantime, hepatic portal or jugular infusion of glucose in pigs did not alter short-term food intake (10). These data suggest that the regulatory effect of glucose on food intake is a preabsorptive event and the sites of regulation are in the GI tract. To further understand the mechanisms by which dietary carbohydrates regulate energy intake, glucose was infused into the stomach or different segments of the small intestine in pigs. The infusion started 30 min prior to the meal and continued until the pigs stopped eating (11). It was found that infusion of glucose into the stomach, duodenum, jejunum, or ileum each suppressed food intake (11). But comparatively, jejunal infusion caused more reduction in food intake than elsewhere (11). These data suggest that glucose may interact with receptors or other sensing components expressed in various parts of the small intestine to control short-term energy intake. In addition to glucose, FFAs released from fat digestion also play important albeit more complex roles in controlling energy intake. Healthy human volunteers receiving ileal infusion

of lipids consumed a smaller amount of food and energy and had delayed gastric emptying (12). Ileal lipid infusion also accelerated the sensation of fullness during a meal (12). However, intravenous (i.v.) infusion of lipids did not affect food intake (12), suggesting that lipids may interact with ileal receptors to induce satiety and reduce food consumption. Further studies suggest that digestion is a prerequisite for the inhibitory effect of fat on gastric emptying and energy intake. For example, administration of a lipase inhibitor increased food intake in healthy subjects or type 2 diabetic patients receiving a high-fat meal (13, 14), suggesting that FFAs, the breakdown products of fat after ingestion, rather than triglycerides, are the active nutrients that exert the regulatory effects. Likewise, sugars from carbohydrate digestion, rather than carbohydrates themselves, are the active nutrients that induce intestinal signals. Although both glucose and FFAs can stimulate a set of GI peptides that regulate appetite, gastric emptying, and insulin and glucagon release, they have differential effects. For example, glucose stimulates robust secretion of both GLP-1 and GIP, whereas FFAs from a fat meal elicit only modest GLP-1 secretion despite equally robust GIP secretion (15). Further, not all FFAs are equally active since the stimulatory effect depends on their chain length. Although FFAs with a chain length of greater than $C_{12}$ stimulate CCK release, further increase in chain length has no additional effect, and $C_{11}$ or shorter FFAs are not active (16, 17).

Like carbohydrate and fat meals, protein meals also activate the nutrient-sensing system but in different ways. In healthy human subjects, plasma GIP levels were elevated after both carbohydrate and fat meals but not a protein meal (15). However, intraduodenal amino acid perfusion in human subjects stimulated both GIP and insulin secretion (18, 19). Oral ingestion of mixed amino acids by healthy volunteers also increased plasma GLP-1 levels (20). These findings suggest that amino acids can function as nutrient-sensing agents, and a protein meal is likely to contribute to nutrient sensing in the GI tract. However, since mixed amino acids are not equivalent to a digested protein meal, GLP-1 secretion was studied in humans following a protein meal (15). A transient peak was observed at 30 min followed by a steady-state rise throughout the rest of the 3 h study period (15). The nutrients from the protein meal that stimulated GLP-1 secretion were a mixture of protein hydrolysates but not amino acids *per se*. It is important to carry out studies with protein hydrolysates that mimic the digested products in the GI tract. A protein hydrolysate (peptone) containing 31% free amino acids and 69% peptides induced the secretion of PYY and GLP-1 in the portal effluent of isolated vascularly perfused rat ileum after luminal administration (21). Peptones also induced CCK secretion and transcription in STC-1 cells, an established L cell line (22, 23). Peptones made from both albumin egg hydrolysate and meat hydrolysate stimulated the transcriptional expression of the proglucagon gene encoding GLP-1 in two L cell lines but not pancreatic glucagon-producing cell lines (24), suggesting that the signaling pathways mediating this effect are L cell/small intestine specific. In STC-1 cells, the proglucagon promoter contains elements responsive to peptones (25). In contrast, the mixture of free amino acids is at best a weak stimulant (21, 24). These data suggest that free amino acids may have a limited role in protein meal-stimulated GLP-1 or PYY secretion. However, amino acids are indeed involved in nutrient sensing in the GI tract. Aromatic amino acids may play a

role in gastrin secretion because they activate the calcium-sensing receptor (CaR) on gastrin-secreting antral cells (26, 27). In addition, amino acids also stimulate CCK release (28, 29) and gastric acid secretion (30).

In addition to glucose, FFAs, amino acids, and digested peptides from proteins, other nutrients are also involved in the regulation of GI peptide secretion (21). At physiological concentrations, bile acids stimulate the secretion of PYY, GLP-1, and neurotensin (NT) (21). Interestingly, the threshold concentration of taurocholate for PYY and GLP-1 stimulation is about twofold that required for stimulating NT release (21), suggesting that there is a slight difference in the sensitivity of L cells and N cells to bile acids (21). In addition to the small intestine, the stomach plays an important role in terminating a meal. When rats were implanted with an extra stomach to which a liquid diet was infused, food intake was reduced regardless of whether food was allowed to empty into the small intestine or retained in the stomach (31). This effect is not likely to be mediated by neuronal mechanisms because the implanted stomach was completely denervated (31). This result suggests that the implanted stomach may have generated hormonal signals that affect food intake, and these hormonal signals may mediate the ability of the stomach to sense nutrient quality and quantity to alter the rate of gastric emptying and amount of food ingested (32, 33).

## MOLECULAR MECHANISMS OF NUTRIENT SENSING

It has been recognized that it is the monomeric nutrients that interact with luminal small intestinal receptors or other nutrient-sensing components and regulate the feedback and feedforward responses to food intake. What do we know about these receptors and their downstream pathways? The analogy between the intestinal nutrient sensing and taste reception by the tongue can shed new light on this question. Glucose sensing in taste buds is mediated by taste receptors expressed in the lingual epithelium (34). These receptors are G protein-coupled receptors (GPCRs) in the apical membranes of taste receptor cells (34). All the three members of the taste receptor family 1 (T1R) class of GPCRs are involved in this function by acting in combination to sense different tastes. The T1R2/T1R3 heterodimer senses sweet taste whereas the T1R1/T1R3 heterodimer senses amino acids and umami taste (35). These receptors activate a phospholipase C (PLC) β2-dependent pathway to increase intracellular $Ca^{2+}$ concentrations by coupling to the G proteins gustducin and/or transducin (34). The activated taste receptors may also stimulate the cAMP-dependent pathway (34). In an *in vitro* assay where T1R2/T1R3 were coupled to Gα15, a promiscuous G protein linked to PLC, T1R2/T1R3 responded to sweet taste stimuli, including glucose, fructose, lactose, and galactose, as well as synthetic sweeteners (35). The activity was inhibited by the sweet taste inhibitor lactisole (35). These data indicate that the T1R2/T1R3 complex mediates sweet sensation along with other components such as G proteins and PLC.

Interestingly, the key components of the sweet taste transduction pathways are also expressed in the gut enteroendocrine cells (36), with the signaling events leading to GI peptide secretion by these cells (Figure 1.2). For example, the three members of

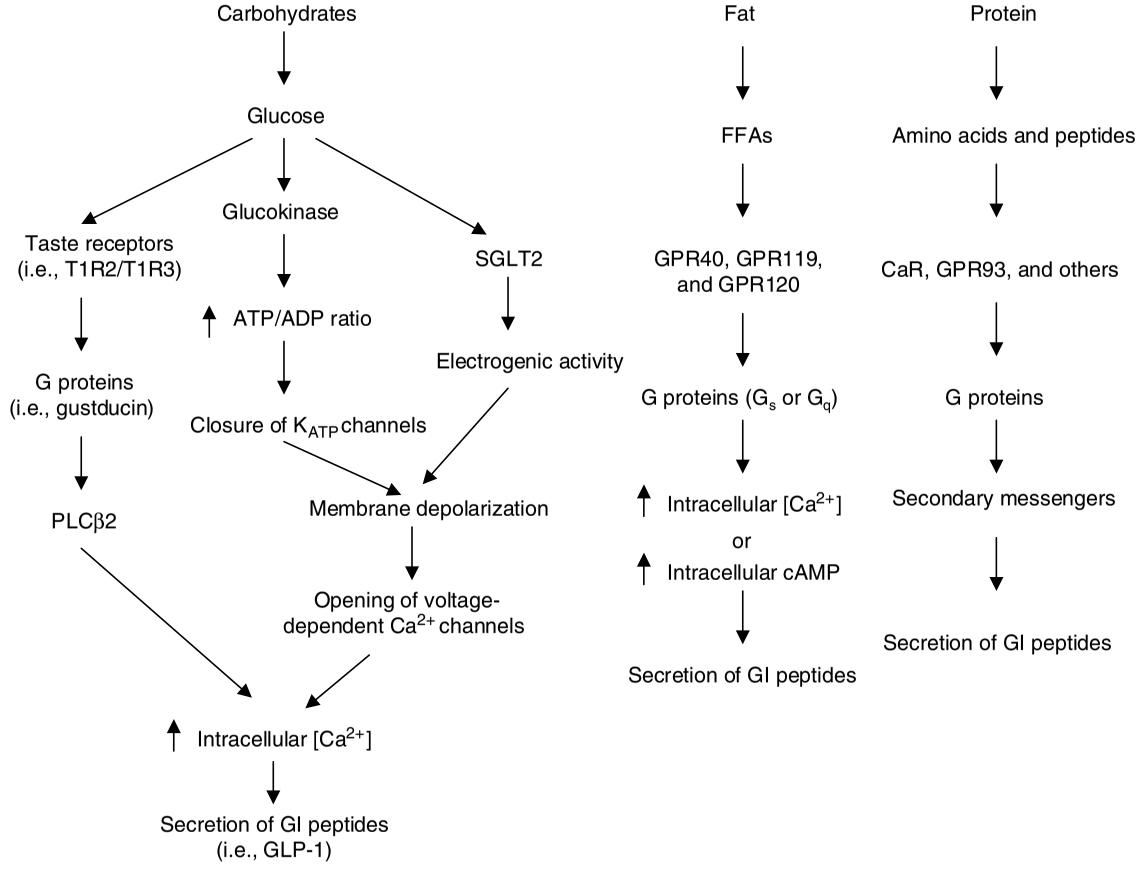


**Figure 1.2** Potential signaling cascades that mediate GI nutrient sensing in response to main nutrients. Macronutrients, including sugars, FFAs, and amino acids/peptides, are derived through digestion from carbohydrates, fat, and protein. There are three potential pathways that can sense glucose: taste receptors, $K_{ATP}$ channels, and SGLT1. FFAs and amino acids/peptides can activate GPCRs expressed in enteroendocrine cells. Activation of downstream signaling by these mechanisms triggers secretion of GI peptides.

the T1R class of GPCRs are detected in brush cells, one form of solitary chemosensory cells (SCCs), in the apical membranes of rat jejunum (37). Also found in these cells are α-gustducin, transducin, and PLCβ2 (37). In addition, α-gustducin is also expressed in brush cells of the stomach, the duodenum, and pancreatic ducts in rats (38, 39). Brush cells have a structure similar to lingual taste cells (39), suggesting that they may use similar nutrient-sensing pathways. Consistent with the findings in rats, T1R2, T1R3, and α-gustducin are expressed in mouse small intestine (40). Taste signaling elements, including the three subunits of gustducin (α-gustducin, Gβ3, and Gγ13), PLCβ2, and taste receptors, were also found in human L cells (41). Taken together, these data suggest that the taste receptors and associated signaling components are present in gut cells and may be involved in nutrient sensing in a fashion similar to that by the lingual epithelium of the tongue. There are two functional consequences upon the activation of the taste receptor systems in the gut. The first is the release of GI peptides such as GLP-1, which mediates both feedback and feedforward responses to food intake as described above. Glucose induces GLP-1 secretion from enteroendocrine L cells by stimulating the taste receptors, the signal of which is mediated by the taste G protein gustducin. The role of gustducin in sugar sensing and glucose homeostasis was exemplified in α-gustducin null mice (41). In wild-type mice, ingestion of glucose induced a marked increase of GLP-1 secretion (41); in contrast, α-gustducin null mice exhibited defective GLP-1 secretion in response to glucose ingestion (41), suggesting that L cells of the gut sense glucose through similar mechanisms used by taste cells of the tongue. Thus, the gut cells can "taste" sugars and release mediators, such as the incretins, that in turn regulate food intake and nutrient assimilation. The second consequence of the taste receptor activation in the GI tract is elevated glucose transporter 2 (GLUT2) insertion on the apical membrane of the gut lumen to increase glucose absorption (37). The basal level of glucose absorption in the gut is mediated by sodium–glucose cotransporter 1 (SGLT1) and GLUT2 when glucose level is around 20 mM (37). At higher local glucose concentrations (30–100 mM), increased insertion of GLUT2 in the apical membrane occurs to facilitate additional glucose absorption (37). GLUT2 provides three to five times more capacity for glucose absorption than the SGLT1 pathway (37).

Despite the above evidence that supports the role of the taste receptor system in mediating nutrient-sensing effects in the GI tract, several research groups have reported findings that dispute this notion. Although the artificial sweetener sucralose was shown to stimulate GLP-1 secretion from human L cells *in vitro* (41), it did not stimulate GLP-1 secretion in primary L cells (42). In addition, it did not stimulate GLP-1 or GIP release in healthy humans when delivered by intragastric infusion (43). This is in agreement with an earlier study in type 2 diabetic patients where the sweetener stevioside had no effect on GLP-1 or GIP release (44). Further, several sweeteners, including sucralose, were tested in Zucker diabetic fatty rats for their nutrient-sensing activity (45). Consistent with the previous reports, none of these sweeteners increased incretin secretion (45). Taken together, these data indicate that the role of the taste receptor system in GI nutrient sensing remains to be further clarified.

Two additional signaling pathways in the GI tract have been proposed that could mediate GLP-1 secretion in response to glucose exposure. The first one is the classical glucose-sensing machinery employed by pancreatic β-cells for eliciting glucose-dependent insulin secretion (46). This machinery includes components such as ATP-sensitive potassium ($K_{ATP}$) channels and glucokinase (Figure 1.2). In this pathway, glucokinase serves as the rate-limiting step in glucose metabolism and therefore is also termed "glucose sensor." Glucose metabolism increases the ATP/ADP ratio, which causes the closure of $K_{ATP}$ channels and depolarization of the β-cell membrane. Next, membrane depolarization leads to opening of voltage-dependent $Ca^{2+}$ channels and accumulation of intracellular $Ca^{2+}$, which triggers insulin release. Both the $K_{ATP}$ channel subunits Kir6.2 and SUR1 and glucokinase were detected in GLUTag cells, an L cell line (46). In these cells, glucose concentrations between 0 and 20 mM decreased membrane conductance, caused membrane depolarization, and triggered action potentials (46). Tolbutamide also triggered action potentials in GLUTag cells (46), presumably by blocking the $K_{ATP}$ channels. These data suggest that the classical glucose-sensing machinery involving glucokinase and $K_{ATP}$ channels mediates glucose-induced GLP-1 release from L cells. However, if this notion is true, GLP-1 and GIP secretion following an oral glucose challenge should be lower in individuals with heterozygous glucokinase mutations that confer reduced activity. Unfortunately, when heterozygous glucokinase mutation carriers were subjected to oral glucose tolerance test (OGTT), they did not have altered GLP-1 or GIP secretion post oral glucose challenge compared to normal controls (47). This observation suggests that the glucokinase and $K_{ATP}$ channel pathway does not mediate incretin secretion in the gut, or it is involved but there are other redundant pathways that can compensate for it. SGLT1 represents another novel glucose-sensing mechanism that triggers GLP-1 secretion (Figure 1.2). Both SGLT1 and SGLT3 are expressed in GLUTag cells (48), and GLP-1 secretion in response to glucose is inhibited by phlorizin, a SGLT inhibitor compound (48). Moreover, the $EC_{50}$ value of glucose for glucose-induced GLP-1 secretion matches the $K_m$ of SGLT1 (49). These data suggest that SGLT1 could directly mediate glucose-induced GLP-1 release. This effect could be attributed to the electrogenic activity of SGLT1 because low glucose concentrations were shown to trigger small inward currents as they enter cells (48). This current could cause membrane depolarization, which could induce GLP-1 release (Figure 1.2).

Like sugars, amino acids and FFAs also regulate endocrine response to food intake through activation of their respective GPCRs in enteroendocrine cells (Figure 1.2). L-Amino acids activate the T1R1/T1R3 heterodimer, which mediates umami taste in taste buds (35). These GPCRs are also expressed in the apical membranes of the gut (37) and couple to the G protein transducin to activate PLCβ2 and stimulate $Ca^{2+}$ mobilization. Through this signaling system, amino acids may mediate GI peptide release and regulate food intake. In addition, the extracellular CaR may also act to sense amino acids released from protein digestion. CaR is abundantly expressed in epithelial cells and neurons of the stomach, the small intestine, and the large intestine (50). In the stomach, CaR is expressed on gastrin-releasing G cells and its activation stimulates intracellular $Ca^{2+}$ mobilization via the activation of PLC (51).

CaR can be activated by aromatic amino acids (52), suggesting that it may act as a nutrient-sensing receptor in response to a protein diet. However, in the absence of $Ca^{2+}$, aromatic amino acids had no effect on CaR-mediated signaling (52), suggesting that aromatic amino acids are not CaR agonists; rather, they may act as allosteric modulators to enhance the sensitivity of CaR to its agonist $Ca^{2+}$. The proposed role of CaR in amino acid sensing has physiological support. Analysis of human jejunal content before and 3 h after ingestion of a protein-rich meal revealed that aromatic amino acids were more preferentially released than acidic, polar, and aliphatic amino acids (53). For example, the phenylalanine concentration in jejunum could reach ~2 mM (53), a level similar to the $EC_{50}$ value of phenylalanine in a $Ca^{2+}$ mobilization assay (52). In addition, L-phenylalanine can activate CCK secretion, presumably through CaR (54, 55). Further, protein hydrolysates directly activate GPR93 in enterocytes, suggesting that multiple GPCRs are involved in sensing of protein nutrients (23). The G protein species to which CaR and GPR93 are coupled are diverse; they depend on specific conditions in different cell types (56) and ligand species (23). As a result, these receptors stimulate the accumulation of a number of secondary messengers. Like glucose and amino acids, longer FFAs appear to interact directly with GPCRs in enteroendocrine cells. The FFA receptor GPR40 is a GPCR highly expressed in pancreatic β-cells mediating the FFA-stimulated glucose-dependent insulin secretion (57). GPR40 is activated by medium- and long-chain FFAs (57, 58). Interestingly, it is also expressed in endocrine L and K cells of the GI tract and mediates GLP-1 and GIP secretion (59). GPR120 is another GPCR expressed in the intestine especially in GLP-1 positive cells and acts as a receptor for unsaturated long-chain FFAs (60). Activation of GPR120 both *in vitro* and *in vivo* led to increased GLP-1 secretion (60), suggesting that GPR120 is a major intestinal FFA sensing receptor that mediates incretin release. Further, a recent study indicates that GPR120 also mediates the stimulation of CCK release by FFAs (61). GPR119, a receptor for endogenous ligands oleoyl-lysophosphatidylcholine (OLPC) and oleoylethanolamide (OEA) (62, 63), is expressed in pancreatic β-cells and upon activation enhances glucose-dependent insulin secretion (63). GPR119 is also localized in L cells and oral administration of a GPR119 agonist increased the release of both GLP-1 and GIP in normal but not GPR119 knockout mice (64), suggesting that GPR119 mediates long-chain FFA-induced incretin release. The three GPCRs trigger different intracellular signaling pathways. GPR40 is coupled to the $G_q$-PLC pathway and upon activation increases the intracellular $Ca^{2+}$ accumulation (65), which leads to incretin secretion. Similarly, GPR120 also induces incretin release by triggering the accumulation of intracellular $Ca^{2+}$ (60). GPR119 is coupled to $G_s$ and stimulates intracellular cAMP accumulation (66).

In addition to enteroendocrine cells, the intestinal mucosa has two other types of sensory systems, neurons and immune cells (67). The sensory neurons are involved in the control of GI motility and signaling to the CNS that controls feeding behavior (67). The immune cells protect against harmful substances that may enter the GI tract. All the three sensing systems work in concert through direct contact with the intestinal contents.

## REGULATION OF INCRETIN SECRETION

In 1902, Bayliss and Starling discovered that acid extracts of intestinal mucosa contained a hormone that could be carried to distal tissues via blood circulation and stimulate the exocrine secretion of the pancreas, and named this factor secretin (68). To test if this factor could be used to treat diabetes, Moore et al. administered duodenal mucosa extracts orally to several type 1 diabetics but did not see clear effects (69). The term "incretin" was first proposed by La Barre in 1932 to describe a hormone extracted from the upper gut mucosa with hypoglycemic effect (70). However, the existence of incretin was not proven until 1964, when two independent research groups discovered that an oral glucose load is associated with a significantly greater insulin response than intravenous administration of the same amount of glucose in human subjects (71, 72). The incretin activity was further evaluated by conducting i.v. glucose infusion isoglycemic to the profile generated from an oral glucose challenge. Despite the identical plasma glucose profiles generated by both the oral and the i.v. routes, the oral glucose challenge stimulated greater levels of insulin and C-peptide (73, 74), suggesting that intestinal factors may be released and involved in the stimulation of insulin secretion after oral glucose ingestion. This so-called "incretin effect" describes the important communication through enteroendocrine factors from the GI tract to pancreas in response to food ingestion. This response is a key part of the feedforward mechanism that increases insulin secretion in anticipation of rising blood glucose after food ingestion.

There are two incretins, GLP-1 and GIP, both of which are rapidly released to the bloodstream after meal ingestion and stimulate glucose-dependent insulin secretion (GSIS) by pancreatic β-cells. In addition, GLP-1 also suppresses glucagon release by pancreatic α-cells, food intake, and gastric emptying, and is cardioprotective. In contrast, GIP does not exhibit these effects. GLP-1 is secreted from intestinal L cells, which are predominantly found in the distal jejunum, ileum, colon, and rectum (1). However, the distribution of L cells throughout the GI tract is somewhat species specific. The overall L cell density in rat or pig GI tract is greater than that in human gut (1), and higher levels are located in the distal jejunum, ileum, and rectum relative to other intestinal regions in humans (1). In dogs, L cells are predominantly concentrated in the jejunum and less so in the ileum (4). Recently, GLP-1 immunoreactive cells were detected in human duodenum (75), and GLP-1 and GIP were colocalized in a subset of endocrine cells in the small intestine (76). GIP is secreted from K cells located primarily in the duodenum (3), but they can be found in other parts of the small intestine (76). For instance, in dogs, GIP-secreting K cells are equally distributed in the duodenum and the jejunum (4). Both L and K cells are open-type endocrine cells that are in immediate contact with nutrients in the intestinal lumen, allowing nutrient-dependent regulation of incretin secretion.

GIP is a 42-amino acid secreted peptide initially isolated from intestinal mucosa. It was named gastric inhibitory peptide but later renamed glucose-dependent insulinotropic peptide for its ability to stimulate insulin secretion (77). The secreted GIP from intestinal K cells is the active form GIP(1–42). GIP is rapidly cleaved at the

N-terminus by dipeptidyl peptidase-4 (DPP-4) (also known as DPP IV, DP 4, CD26, and adenosine deaminase binding protein), an amino peptidase found in almost all organs and tissues (78), producing the inactive form GIP(3–42) (79). DPP-4 also processes other peptides such as GLP-1 (79), chemokines (80–82), and neuropeptides (83). GLP-1 is part of the proglucagon polypeptide that is expressed in both intestinal L cells and pancreatic α-cells. The proglucagon polypeptide is processed posttranslationally by prohormone convertases (PC) 1/3 and 2. PC1/3 is expressed in L cells whereas PC2 is expressed in α-cells. The tissue-specific expression of the convertase isoforms dictates which mature peptides are generated from the proglucagon polypeptides. In the small intestine, the posttranslational processing by PC1/3 produces GLP-1, GLP-2, glicentin, and oxyntomodulin (84, 85). In contrast, in pancreatic α-cells, PC2-mediated posttranslational processing generates glucagon, glicentin-related pancreatic peptide (GRPP), and the major proglucagon fragment (MPGF) that contains the GLP-1 and GLP-2 segments within its sequence (85). There are two equipotent active forms of GLP-1, GLP-1(7–36)amide and GLP-1(7–37). Both forms are prone to proteolytic cleavage by DPP-4 generating inactive GLP-1(9–36)amide and GLP-1(9–37), respectively (79).

Carbohydrate, fat, and protein meals all stimulate GLP-1 secretion in human subjects with glucose being the strongest stimulant (15, 20). Unlike carbohydrates and fat that are also strong stimulants of GIP secretion, protein meals have no effect (15). The plasma concentrations of both hormones increase rapidly within 5–15 min after food ingestion (15, 20) but their actions are short lasting due to rapid proteolytic degradation by DPP-4 and other proteases. The plasma half-lives for intact GLP-1 and GIP are 1–2 and 7 min, respectively (86–88). DPP-4 is the main enzyme for incretin clearance as targeted disruption of the DDP-4 gene in mice led to improved stability of endogenous GLP-1 (89). The tissue distribution of DPP-4 plays an important role in GLP-1 degradation. There is a high level of DPP-4 in the endothelium of the capillaries surrounding L cells, and over 50% of newly secreted intact GLP-1 loses the N-terminal dipeptide and as a result is inactivated before entering the systemic circulation (90). The rapid rise of GLP-1 and GIP in the circulation ensures elevated GSIS in response to a meal, which is essential for the normalization of postprandial glucose. The disappearance of the incretins is in sync with the normalization of postprandial glucose. The first contributor of such a precise regulation is proteolytic degradation. In addition to DPP-4, the neutral endopeptidase 24.11 (NEP-24.11) is also involved in incretin degradation (91, 92). But DPP-4 is the main incretin degradation protease. Like DPP-4, NEP-24.11 is not selective against the incretins; it also processes other hormonal peptides (91). Its catalytic rates on vasoactive intestinal peptides (VIP) and glucagon are much faster than those on the incretins (91). The other factor that contributes to the rapid decline of plasma GLP-1 and GIP levels is a negative feedback mechanism, under which both hormones limit their own secretion by stimulating the somatostatin-mediated paracrine regulation. Somatostatin-positive D cells are located throughout the small intestine in close proximity to both L and K cells (4). *In vitro*, somatostatin inhibits GLP-1 secretion by L cells (93). In perfused porcine intestine, blocking somatostatin activity with a neutralizing monoclonal antibody increased GLP-1 secretion by 8–9-fold (94). Further,

intravascular infusion of somatostain-28 strongly inhibited GLP-1 release in pigs (94). This finding is consistent with other somatostatin infusion studies in rats (95), sheep (96), and human subjects (97), where somatostatin inhibited GLP-1 or GIP secretion *in vivo*. These data suggest that GLP-1 secretion is tonically inhibited by the local release of somatostatin-28 from epithelial paracrine D cells. Compared to somatostatin-28, the enteric neuron-derived somatostatin-14 is much weaker in influencing GLP-1 secretion (94). The suppressive effect of somatostatin on GLP-1 secretion is mediated by somatostatin receptor subtype 5 expressed in L cells (98). Since both GLP-1 and GIP stimulate somatostatin release (99, 100), somatostatin is believed to be a key player in a negative feedback loop that controls incretin release in the gut. The existence of the negative feedback loop on GLP-1 secretion is supported by further evidence in dogs and humans. Conscious dogs were orally given a DPP-4 inhibitor, which increased meal-induced active GLP-1 levels (101). However, the total GLP-1 levels in these dogs were reduced (101), presumably due to the inhibitory effect of elevated active GLP-1 on endogenous GLP-1 secretion. A similar result was observed in healthy human volunteers who received an oral dose of a different DPP-4 inhibitor (102). These data support the notion that GLP-1 can inhibit its own secretion *in vivo* as part of a negative feedback loop.

In addition to direct stimulation by nutrients, GLP-1 secretion is also indirectly regulated by GIP released in the proximal intestine in rodents. After a meal, nutrients are expected to reach the distal L cells and stimulate GLP-1 release via direct contact. However, this does not explain the biphasic pattern of GLP-1 secretion after a meal, including a 15–30 min rapid rise after oral ingestion followed by a second minor peak at 90–120 min (15, 103). Since all the initial findings indicate that L cells are located in the distal intestine (ileum, colon, rectum), the rapid early rise of GLP-1 after food ingestion within 5–15 min is faster than the time required for unabsorbed nutrients to reach the L cells in the distal intestine. A proposed mechanism is the existence of a neuroendocrine loop that regulates GLP-1 secretion distally once the ingested nutrients reach the proximal intestine (duodenum). This regulatory mechanism is referred to as proximal–distal neuroendocrine loop or duodeno-ileal endocrine loop. Since high GIP levels can stimulate GLP-1 secretion (104, 105), it is possible that nutrient entry into the duodenum stimulates GIP release, which in turn stimulates GLP-1 secretion in the distal intestine even before the nutrients arrive. This notion is supported by several studies. First, intraarterial infusion of GIP into perfused rat colon strongly stimulated GLP-1 secretion (106). In another study, the flow of nutrients to the distal intestine was restrained in rats to prevent direct interaction of the luminal content with the distal L cells (107). Next, when fat or glucose was placed in the duodenal lumen of these animals, GLP-1 release was induced at a level comparable to that by directly placing nutrients into the ileum (107). In the meantime, a rapid rise in GIP was also observed (107). This finding suggests that GIP released from the proximal intestine may mediate the early secretion of GLP-1 in the distal intestine. The vagus nerve appears to play an important role in this regulation because bilateral subdiaphragmatic vagotomy abolished the GLP-1 secretion by fat placed into the duodenum (108). Further, GLP-1 secretion stimulated by physiological concentrations of infused GIP was completely abrogated with selective hepatic branch

vagotomy (108). These data suggest that the vagus nerve mediates the GIP-stimulated GLP-1 response in the distal intestine in rats.

The GIP-mediated regulation of GLP-1 release has not been validated in humans. Although there is an early rise of GLP-1 after oral ingestion in humans (15, 103), GIP does not play a role in mediating this response. Intraduodenal infusion of a small amount of glucose produced a rapid and short-lasting GLP-1 response but the GIP level did not change (20), suggesting that the GLP-1 response to the duodenal glucose infusion is not mediated by GIP. In a separate study, synthetic GIP was infused into both type 2 diabetic patients and normal subjects. The exogenously administered GIP increased insulin secretion but had no effect on circulating GLP-1 level in normal subjects (109, 110). A further study was carried out in patients with upper and lower gut resections (jejunal or ileal small intestinal resections and colectomy), and it was found that a clear and early (peak at 15–30 min) GLP-1 response after food ingestion was observed in the patients with gut resection as well as controls (111). These studies demonstrate that the early GLP-1 response to food ingestion is not mediated by GIP in humans. One proposed explanation is that the early rise in GLP-1 is also a direct effect of nutrients on L cells because in contrast to previous reports that L cells are primarily located in the distally lower jejunum, ileum, colon, and rectum (1), GLP-1 positive cells were also found in human duodenum in recent studies (75, 76). These data suggest that the early rise in GLP-1 in humans after a meal could be ascribed to GLP-1 secretion from L cells in the upper gut.

## DISORDERS IN INCRETIN RESPONSE IN TYPE 2 DIABETES

Meal- or oral glucose-induced GLP-1 response is decreased in type 2 diabetic patients as well as subjects with impaired glucose tolerance (IGT) (112, 113), but the GLP-1 response in IGT subjects trends higher than that in type 2 diabetics (112). This impairment could at least in part contribute to the disease pathogenesis. If this is one of the major causes of the defective glucose homeostasis in type 2 diabetes, administration of exogenous GLP-1 is expected to help normalize glucose control. It is encouraging that despite reduced GLP-1 secretion, type 2 diabetics still respond to GLP-1 infusion with augmented insulin release and improved glucose tolerance (109, 114). However, there are individual variations in response to exogenous GLP-1 administration among type 2 diabetics; glucose elimination is faster and lower glycemia was achieved in patients with lower baseline fasting plasma glucose (114). This finding suggests that GLP-1 treatment becomes less effective as the disease progresses. In contrast to GLP-1, GIP has diminished incretin effect in type 2 diabetic patients, suggesting that the GIP response is largely lost in the disease state (109). The underlying mechanism behind this observation is not clear. Based on these data, only GLP-1 is expected to have potential therapeutic value in treating type 2 diabetes.

## OTHER GI PEPTIDE HORMONES OR NEUROTRANSMITTERS

In addition to the incretins, other peptide hormones and neurotransmitters are also involved in the regulation of gastric emptying, food intake, and energy metabolism.

There are many such peptides and some are yet to be assigned exact functional roles. They are secreted by different types of enteroendocrine cells distributed in different segments across the GI luminal surface. These cells sense luminal contents through direct interaction and secrete peptide hormones with regulatory effects.

PYY and CCK are both involved in the regulation of food intake and gastric emptying. PYY-immunoreactive L cells are found in the distal small intestine, the colon, and the rectum (115). There are two forms of PYY, PYY(1–36) and PYY(3–36), in human blood (116), with PYY(3–36) derived from PYY(1–36) through DPP-4 proteolytic cleavage. Unlike GLP-1, both forms of PYY are bioactive. PYY(3–36) is the major form in human colonic mucosa. The plasma PYY level increases several fold after meal ingestion in humans. Compared to equivalent calories of protein and carbohydrate diets, fat is a more potent stimulus of PYY secretion (117). PYY can inhibit gastric acid and pepsin secretion and delay intestinal transit time (117), suggesting that PYY is a negative regulator of energy intake in response to food ingestion. PYY can interact with a family of $G_i$-coupled GPCRs, including Y1R, Y2R, Y4R, Y5R, and Y6R. Peripheral injection of PYY(3–36) was shown to inhibit food intake and reduce body weight in rats (118). PYY(3–36) also inhibited food intake in mice but not in Y2R-null mice (118), suggesting that the anorectic effects are mediated by Y2R. Consistent with the findings in animals, PYY(3–36) infusion significantly reduced appetite and food intake in human subjects of normal weight (118). Further, the circulating levels of PYY were significantly lower in obese subjects compared to lean controls, and like its effect in lean subjects, PYY infusion reduced food intake in obese individuals (119). These findings demonstrate that PYY is an anorectic agent and could be used to treat obesity. However, in contrast to peripheral administration, central administration of PYY increased food intake (120). Moreover, the anorectic effects of peripheral PYY(3–36) administration could not be reproduced by some research groups (121), although others have been successful in replicating the original findings (122, 123). These discrepancies remain to be resolved with further studies.

Similarly, CCK is another gut peptide involved in the regulation of food intake and related physiological activities. CCK is expressed as a 115-amino acid peptide in cells and undergoes posttranslational proteolytic processing to generate CCK-58 (124, 125), the main circulating form. CCK is secreted by both I cells in the proximal intestine and L cells in the distal intestine (5). CCK is also found in the brain (5). Further proteolytic cleavage of CCK-58 generates smaller but still biologically active CCKs, including CCK-39, CCK-33, CCK-22, CCK-12, and CCK-8 (126). CCK is secreted and released into the blood circulation upon food ingestion and induces satiety. Two CCK receptors mediate the CCK function: CCK-1 receptor, primarily expressed in the GI tract, and CCK-2 receptor, mainly expressed in the brain. CCK-1 receptor is also expressed in the hindbrain and hypothalamus. Part of the CCK action in the brain is mediated by suppressing the expression of orexins A and B, two peptides produced in the lateral hypothalamic areas that stimulate food intake (127). The suppression of food intake by CCK was demonstrated in animal models as well as humans. Rats deficient in CCK-1 receptor had increased meal size and developed obesity (128), suggesting that the satiation signal is mediated by CCK-1 receptor. CCK administration also decreased food intake in humans by

shortening meals (129). The anorectic effects of CCK are weak because rats deficient in CCK-1 receptor developed only mild obesity (128), and CCK-1 receptor-null mice did not develop obesity (130). In addition, the anorectic effects were rapidly lost during repeated CCK administration (131), suggesting that behavioral tolerance may have developed under such a condition. These data question the suitability of CCK as an anti-obesity therapy.

Other important GI peptides include oxyntomodulin, GLP-2, and ghrelin. Like GLP-1, oxyntomodulin and GLP-2 are proglucagon-derived peptides secreted from L cells (84, 85). Oxyntomodulin has been demonstrated to reduce food intake and body weight gain in rodents (132–134) and humans (135, 136). Interestingly, oxyntomodulin also increases energy expenditure in both animals and humans (133, 137). These effects are presumably mediated by GLP-1 receptor, although oxyntomodulin binds to it less avidly than GLP-1 (132). Oxyntomodulin also binds to glucagon receptor as its N-terminus contains the full glucagon sequence (138). However, it has a lower affinity than glucagon itself (138). The dual activation of both GLP-1 and glucagon receptors by oxyntomodulin might be a better explanation for the effects on food intake, body weight gain, and energy expenditure. Two independent studies demonstrated that dual activation of both GLP-1 and glucagon receptors with oxyntomodulin- or glucagon-derived peptides reduced food intake, body weight gain, body fat, hepatic steatosis, and blood glucose, and improved insulin sensitivity and lipid metabolism (138, 139). Although also derived from the proglucagon polypeptide and secreted from L cells, GLP-2 has no incretin effect. Rather, it is an intestinal growth factor. GLP-2 stimulates crypt cell proliferation and bowel growth in an ErbB-dependent manner (140, 141). GLP-2 also increases intestinal lipid absorption through activation of CD36 (142), thereby mediating a key function in response to food intake. Ghrelin is secreted from the stomach (6, 143) and is the endogenous ligand of the growth hormone (GH) secretagogues receptor (143). There are acylated and unacylated forms of ghrelin and the acylation is essential for the activity (143). Unlike the incretins or PYY, it increases food intake and is involved in meal initiation marked by a pre-meal surge (144). Ghrelin is likely involved in the long-term regulation of body weight (145). Interestingly, ghrelin improved cardiac functions in rats with heart failure (146), suggesting that there may be a role of ghrelin in regulating cardiovascular function.

## THE PHYSIOLOGICAL IMPORTANCE OF THE GUT: LESSONS LEARNED FROM GASTRIC BYPASS

The metabolic role of the gut is further implicated in the fascinating findings from bariatric surgery, which produces dramatic and durable weight loss (147). Among many different types of bariatric surgical operations employed to treat severe obesity (147), the most commonly performed are laparoscopic adjustable gastric banding (LAGB), gastric bypass, and biliopancreatic bypass (147). Gastric bypass (or Roux-en-Y gastric bypass, RYGB) involves surgical reduction of the size of stomach and bypassing a portion of the proximal small intestine (Figure 1.3). The portion

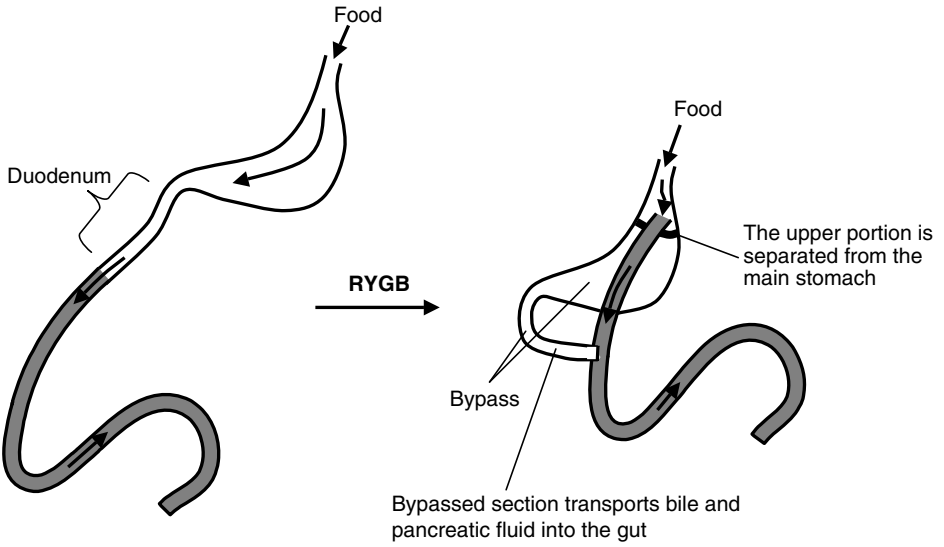


**Figure 1.3** Illustration of Roux-en-Y gastric bypass.

bypassed is connected to the distal small intestine to allow the passage of pancreatic fluids and bile into the gut (Figure 1.3). This procedure causes dramatic weight loss and has been the most effective treatment of severe obesity. In a series of 608 patients with 95% follow-up for at least 16 years, the mean weight loss was 106 lb (148). Surprisingly, more than 80% of the patients with type 2 diabetes developed complete remission of the disease after the surgery (148, 149). Weight loss does not fully explain the remission of type 2 diabetes after gastric bypass because within days after surgery the hyperglycemia and hyperinsulinemia were totally normalized (148). Although the mechanisms behind the antidiabetic effect are not entirely clear, increased insulin secretion and improved β-cell function are likely involved. Late-onset hyperinsulinemic hypoglycemia has been observed in patients after the surgery (150–152), and some may even require partial or total pancreatectomy to prevent recurrent hypoglycemia (150, 152). This phenomenon underscores the robust improvement of pancreatic function achieved by RYGB.

GLP-1 and PYY are two important gut hormones that are believed to mediate the more robust beneficial effects of RYGB compared to LAGB, a procedure that restricts food intake by banding the stomach but does not involve the bypass of the proximal intestine. The metabolic effects of LAGB are therefore results of reduced food intake and weight loss. The average reduction in body weight after LAGB is 28% compared to 40% after RYGB, and the remission of type 2 diabetes occurs in 48% relative to 84% in RYGB (153, 154). One of the key differences between these two different operations is the greater GLP-1 and PYY response post meal after RYGB surgery (155), suggesting that these peptide hormones may play an important role in promoting weight loss and improved insulin sensitivity. As mentioned above, RYGB results in improved insulin sensitivity before weight loss in the short term. This effect

seems to persist even in the long run in a weight loss independent manner, although weight loss itself can lead to improved insulin sensitivity. When compared with a weight-matched group, the patients who underwent RYGB had lower fasting insulin and better insulin sensitivity (156), suggesting that in addition to weight loss something else leads to further improved insulin sensitivity in RYGB patients. In addition to suppressing appetite and weight loss after RYGB (157), the increased postprandial GLP-1 response could further improve insulin sensitivity by increasing β-cell mass and improving β-cell function. In fact, there is sustained elevation of GLP-1 secretion post meal in RYGB patients compared to normal controls (158). This may be counterintuitive because L cells are also found in human duodenum (75) and nutrient bypass of the proximal intestine is expected to cause reduction in GLP-1 release. It could be that this is a small loss relative to the robust increase in GLP-1 secretion by the distal intestine so that the total GLP-1 secretion is still elevated after RYGB.

Two hypotheses have been proposed to explain the weight loss independent effect in RYGB based on the roles of the foregut and the hindgut. The hindgut hypothesis proposes that the beneficial effects result from the expedited delivery of nutrients to the distal small intestine and enhancement of physiologic signals that improve glucose homeostasis (159); the foregut hypothesis holds that the weight loss independent effect depends on the exclusion of the duodenum and proximal jejunum from nutrient passage, therefore preventing the secretion of a physiologic signal that promotes insulin resistance (159). Using nonobese diabetic Goto-Kakizaki (GK) rats, Rubino et al. demonstrated that duodenal–jejunal bypass (DJB), a stomach-preserving RYGB, improved oral glucose tolerance compared to a pair-fed sham-operated group (159). However, restoration of duodenal nutrient passage in the DJB rats reestablished impaired glucose tolerance (159), suggesting that the weight loss independent metabolic benefits in the DJB rats were likely to be driven by the nutrient bypass of the foregut. Why does the duodenal nutrient passage have a negative effect? These researchers proposed that a physiologic signal induced by duodenal nutrient passage might play a role. This negative signal could be an anti-incretin factor, which might be secreted from the proximal intestine in response to nutrient passage and stimulate insulin resistance (159). The anti-incretin factor may interfere with the incretin secretion and/or actions and ultimately inhibit insulin action (159). One of the possibilities is that the anti-incretin inhibits GLP-1 secretion and after nutrient bypass of the proximal intestine the suppression is relieved leading to elevated GLP-1 secretion. Although this hypothesis is consistent with the improved β-cell function in RYGB patients, it remains to be validated by identification of a factor with anti-incretin effect. While the anti-incretin concept helps explain the weight loss independent effects in RYGB patients, Rubino's data do not exclude the involvement of the hindgut in the improvement of metabolic effects. In fact, a study in mouse models indicates that there is increased gluconeogenesis in the distal intestine post DJB but not gastric banding (160), and the increased local glucose concentration is detected by a GLUT2-dependent hepatoportal sensor, which leads to reduced food intake and body weight and improved insulin sensitivity (160). Thus, it seems that different sections of the small intestine

play important roles via distinct mechanisms to achieve beneficial metabolic effects in RYGB.

## SUMMARY

In addition to its role in food intake and nutrient absorption, gut is also an endocrine organ for secreted GI peptides. The release of these peptides in response to food intake is mediated by the direct contact of macronutrients with enteroendocrine cells on the luminal side distributed throughout the GI tract. These GI peptides regulate a variety of physiological actions in response to food intake, including the feedback response to suppress food intake and the feedforward response for nutrient assimilation. The incretin GLP-1 plays important roles in both regulatory pathways. Different sets of GI peptides are stimulated in response to specific types of macronutrients. There are several potential nutrient-sensing mechanisms mediated by taste receptors, $K_{ATP}$ channels, glucose transporters, and GPCRs. Further studies are required to clarify the relative contributions of these pathways. The robust metabolic benefits associated with RYGB suggest that changes in the secretion profiles of GI peptides may be beneficial, although the exact mechanism is still elusive. Further studies in gut biology will likely shed new light on the metabolic functions of GI peptides.

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